University of Southampton
Centre for Life Sciences

The effect of the aphid sex pheromone on the aphid *Myzus persicae* and its parasitoid *Aphidius colemani*

by

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UNIVERSITY OF Southampton

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“You can’t build a peaceful world on empty stomachs and human misery”

Norman E. Borlaug (1914-2009)

ABSTRACT

UNIVERSITY OF SOUTHAMPTON

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THE EFFECT OF THE APHID SEX PHEROMONE ON THE APHID *MYZUS PERSICAE* AND ITS PARASITOID *APHIDIUS COLEMANI*

Aphids remain an enormous threat to the sustainability of crops in glasshouse and field environments around the world. It is known that the aphid sex pheromone is used as a kairomone by its natural enemies, such as parasitoids. The focus of this research was how the aphid sex pheromone component, (4a*S*,7*S*,7a*R*)-nepetalactone, affects a host, its parasitoid and the host-parasitoid interaction in a tritrophic system. A model system of Chinese cabbage *Brassica rapa* sp. *Pekinensis* Cv. Wong bok, the peach-potato aphid *Myzus persicae* and the generalist parasitoid *Aphidius colemani* is applied with a particular emphasis on understanding parasitoid foraging and how it may be affected, and potentially manipulated, by nepetalactone.

Firstly, it was demonstrated that asexual *M. persicae* are capable of detecting the sex pheromone components, despite their components having no previously known ecological function in parthenogenetic populations. Although it was found that they avoid the odour in high concentrations, it was concluded that performance on an individual or population level were unlikely to be affected. The ability of the parasitoid *A. colemani* to detect nepetalactone was confirmed at the electrophysiological level. Nepetalactone did not elicit any behavioural response when presented in isolation but was found to increase retention of the parasitoid within a patch if other host cues were also present. It was found that *Nepeta cataria* oil, from which nepetalactone can be isolated, increased the success of parasitoid oviposition in the host. To enhance parasitoid foraging, it was investigated whether learning was possible with nepetalactone; an odour already known to elicit an innate response. Learning through emergence conditioning was ineffective in altering parasitoid behaviour; however, ovipositional experience did induce a change in foraging patterns. This change in foraging pattern did not translate to more effective host location when tested in the laboratory, which led the research towards experimentation in a more complex spatial-temporal environment. Nepetalactone, or the learning of nepetalactone, were not found to have an effect on parasitoid success at this scale. It was found that the introduction of parasitoids into a glasshouse environment reduced aphid population growth at a rate disproportionate to the rate of mummification. This highlighted the importance of indirect consequences of parasitoid visitation on aphid population control. In a separate assay it was identified that aphid population size affects plant fitness, such that smaller aphid populations result in greater plant fitness, thus demonstrating benefits of parasitoids in biological control which are often overlooked.

This work provided a greater insight into the role of nepetalactone in a tritrophic system and how odours may be used by parasitoids during foraging. Finally, the key findings of this study are discussed and the possible direction of future work. A new interpretation of parasitoid foraging is discussed, by the integration of information provided by this study and knowledge generated by previous work.

Contents

List of figures

List of tables

Declaration of Authorship

I, Gabriel Mandela Fernández-Grandon declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

"The effect of the aphid sex pheromone on the aphid Myzus persicae and its parasitoid Aphidius colemani**"**

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;

2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;

3. Where I have consulted the published work of others, this is always clearly attributed;

4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;

5. I have acknowledged all main sources of help;

6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;

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On a personal level I also owe a great deal to a great many. Some of these are the cast of Boldrewood/B85 and the SCR who have provided relief over breakfast, lunch, dinner and nights out. Prammy P., Maaike B., Naimh O. and Yasser A[5] Ibrahim for the games nights. All the Lads from back home who ensured I had a warm welcome on the rare chance I had to visit. Specifically I have to mention Michael R. Burns and Ruairidh A. Falconer who have never failed to lift my spirits. Thanks to the basketball teams I have played for/coached: The Ravens, Eagles, Supersonics and Swifts that have kept me fit and happy. My many thanks to Coach Tomasz Sosna for giving so much of his time.

Gracias a mis padres, que me han dado tanto. La educación comenzó 100 años antes de que yo naciera. Ustedes han luchado para que nosotros podamos aprender y vivir una vida rica, gracias Modge y Dodge. Gracias a mi familia cerca y lejos. Lalo, Bruni and Lia thank you, you have always looked after me well and expected little in return. Lastly, but far from least, I would like to thank Kirstin J. Williamson for continuing to love and support me. I look forward to the future as we tackle the world together.

[1] *rb > c*

1 General Introduction

1.1 Aphids

1.1.1 Lifecycle

Aphids are small soft bodied hemipterans. With around 4400 extant species (Resh & Cardé, 2009) aphids are a large and ecologically well distributed group. All but two families of the aphid families are found in the Aphidoidea superfamily (Phylloxeridae and Adelgidae can be found in the Phyllxeroidea superfamily). Aphids use their stylet to pierce plant tissues and enter the phloem where they obtain the sugar-rich sap. Whilst the majority of aphid species are autoecious (completing their life cycle on one or more species of closely related plants) some of those that are hetereocious (spending different stages of their lifecycle on unrelated hosts) can be highly polyphagous; feeding on a wide variety of plants. An example is *Myzus persicae* which is capable of feeding on, and spreading viruses between, over 30 different plant families (van Emden *et al.*, 1969).

Aphids go through four instars of development before becoming adults. Adults which form wings (the buds of which will be seen in earlier instars) are known as alatae though the majority will remain wingless, known as apterae. Production of alatae provide aphids access to more distant resources and areas subject to less predation, however, the morphological change also displays trade-offs with the reproductive ability of the individual (Dixon *et al.*, 1993), as is generally seen for dimorphic insects (Guerra, 2011).

The majority of aphids studied alternate between sexual and asexual generations (Figure 1.1). Many prominent aphid pests follow a heteroecious feeding pattern: feeding on crop plants during the year and utilising shrubs or trees to overwinter. In holocyclic lifecycles the aphid will produce a sexual form with overwintering eggs. Less common are anholocyclic lifecycles which remain asexual throughout the year. The majority of pest aphid populations are asexual for most of the year but pass through a sexual phase (Powell & Pickett, 2003).

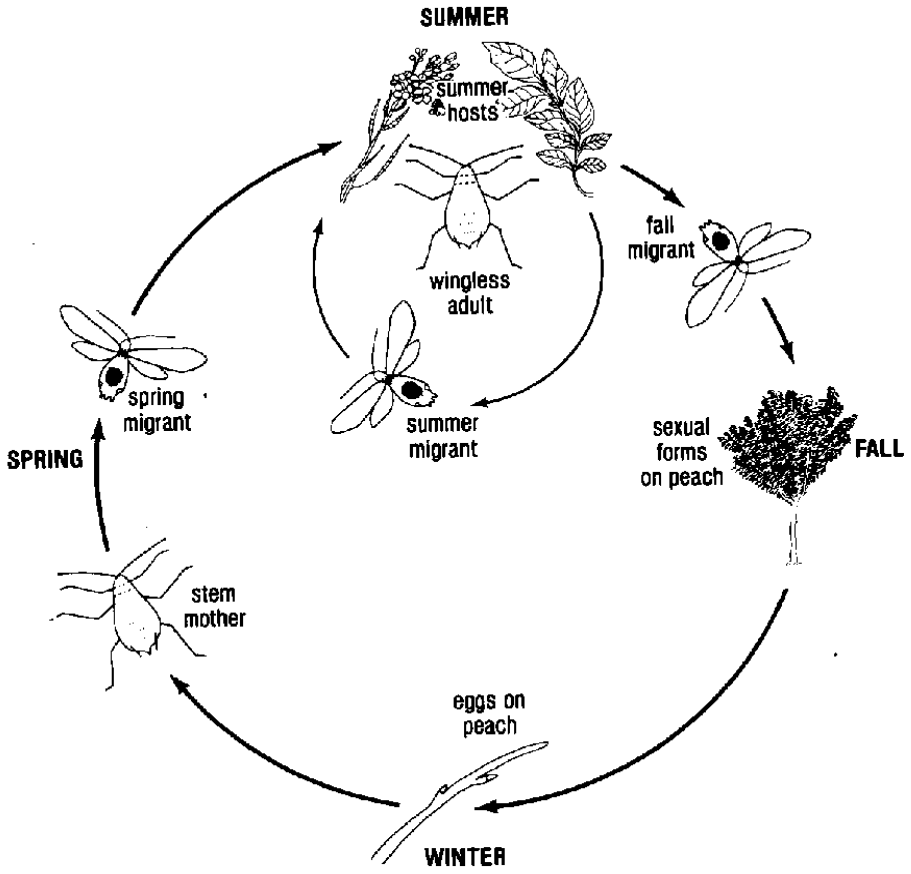


Figure 1.1 Typical lifecycle of a peach-potato aphid.

Illustration of *M. persicae* lifecyle when peaches are present from Radcliffe *et al.* (1993).

The parthenogenetic reproduction seen during asexual generations produces a 'telescoping of generations' with the unborn offspring starting to develop their offspring allowing for exceedingly rapid population growth (Blackman & Eastop, 1984). Through this extraordinary reproductive strategy aphid populations are capable of exponential growth (Lin & Ives, 2003), an attribute that adds to difficulty in creating effective pest management strategies.

1.1.2 Economic and Ecological Impact

Aphids are prevalent pests in agricultural systems around the world. This is due to direct damage caused by feeding on the plant phloem, a process which removes many of the plants essential nutrients, but also their efficacy as vectors for a range of plant viruses (Lushai & Loxdale, 2004, Martinez-Torres *et al.*, 1999). Populations of aphids have proven difficult to control because of their rapid rate of reproduction, adaptation to insecticides and polyphagous nature which allows persistence even when the host plant is protected. In the UK aphids are responsible for over half of all plant virus transmission which, along with direct feeding damage, leads to losses exceeding £100

million per annum (Barker *et al.*, 2003). Traditionally large quantities of pesticides have been applied to reduce aphid populations but this has often proven to be ineffective (Kim *et al.*, 2001) and in some cases has even aided the growth of aphid populations (Chen *et al.*, 1991). Furthermore, the frequent application of pesticides has led to selection for resistant strains (Wang *et al.*, 2007) which has reduced their efficacy (Foster *et al.*, 1998, Foster *et al.*, 2002). Fears raised by the possible effects of pesticide residues (Nasreddine & Parent-Massin, 2002) are reflected in increased consumer distrust of chemical application (Atkinson *et al.*, 2003) and stricter government regulations (Europa, 2008) which have meant that persistent high level use of pesticides is no longer an option for European growers. Among the most devastating pests to agriculture globally is the peach-potato aphid, *Myzus persicae*.

1.1.3 The peach-potato aphid *Myzus persicae*

Myzus persicae are wide spread heteroecious aphids normally found in temperate climates. Asexual generations are produced during the year; as the temperature decreases sexual morphs are produced and, after mating, the oviparous females lay overwintering eggs (Figure 1.1). This typical *M. persicae* life cycle is mediated by changes in temperature and photoperiod throughout the year (Trionnaire *et al.*, 2008). It is often seen in temperate regions, such as those seen across much of the UK, *M. persicae* will exclusively reproduce asexually, producing only females in each generation. It was established by Horsfall (1924) that *M. persicae* go through four instars and live to approximately 23 days, though subsequent studies (MacGillivray & Anderson, 1958) have described five instars and a longevity of around 41 days, the latter of which is supported by personal observations.

M. persicae are highly polyphagous, capable of feeding on plants in over 30 different families (van Emden *et al.*, 1969) and able to act as a vector for over 100 plant viruses (Kennedy *et al.*, 1962). As a consequence of the strong selection pressures applied to *M. persicae* feeding on crops they exhibit some of the most highly adapted mechanisms of insecticide resistance seen in aphids (Fenton *et al.*, 2004).

1.2 Parasitoids

1.2.1 Abundance and Diversity

There are currently around 68,000 described species of parasitic wasps, or parasitoids, meaning that they alone account for 4% of all known metazoan species (Godfray, 1994). Of this substantial number the majority (around 50,000) are Hymenoptera (Gaston, 1991). They are often considered an intermediate between predators and true parasites, because, like many true parasites they often require only one host to

complete development, however, like predators their feeding also ensures the death of their host. A parasitoid differs from predators and from true parasites in that the death of the host is a necessary part of the lifecycle; this is what defines parasitoids. The word was originally coined by Reuter (1913) to describe an organism which develops as a larva inside other insects and eventually leads to the host death. They have been of great interest to biologists for their curious lifecycles, display of complex learning and the practical application they have in pest population control.

1.2.2 Aphid Parasitoid Lifecycle

Parasitoids display a vast array of complex lifecycles which are intimately related to those of their hosts. For aphid parasitoids the lifecycle commences with the female locating a viable host and ovipositing (Figure 1.2). Aphid parasitoids are generally found to prefer juveniles, producing their largest offspring if ovipostion occurs in hosts at the 3rd instar of development (Sequeira & Mackauer, 1992a).

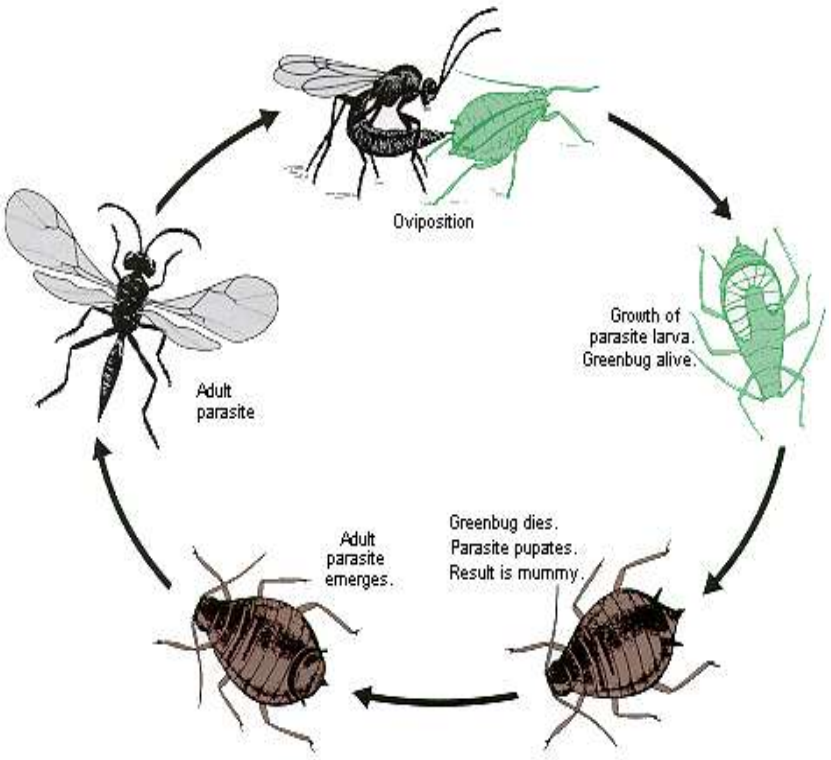


Figure 1.2 Typical aphid parasitoid lifecycle.

The parasitoid illustrated here is *Lysiphlebus testaceipes* (taken from Hoffmann & Frodsham, 1993).

Following oviposition, the parasitoid larva will complete development inside the host utilising it as a source of energy and protection. In many parasitoids superparasitism may occur in which several eggs may develop within the same host, however, aphid species only contain the resources for the successful development of a single larva.

During the early stages of parasitoid larval development the aphid host may employ chemical means to supress the larva's growth. The immune response is often sufficient to entirely prevent parasitoid development, however, if it fails to prevent parasitoid development the larva will absorb all the nutrients required for its growth and begin form a silk cocoon within the host to complete pupal growth. During this stage of development parasitoids are themselves vulnerable to parasitism. In natural environments, and those produced by glasshouses, this form of secondary parasitism, or hyperparasitism, frequently occurs and can provide difficulty in implementing sustainable parasitoid-based biological control solutions. If the primary parasitism proceeds without interference the host aphid's body will swell, with the cuticle becoming hardened displaying the bronze/gold colour characteristic of an aphid 'mummy' (Figure 1.3).

Figure 1.3 Picture an aphid mummy.

Image showing the circle from where the parasitoid has emerged from the host. Courtesy of the Abbot Lab, University of Vanderbilt.

Once the pupa is fully matured it will chew a circle out of the upper rear of the mummy (Figure 1.3) through which it will escape, emerging fully developed and ready to mate or locate a host. Males tend to have a shorter gestation time, most likely due to their smaller body size (Sequeira & Mackauer, 1992a). Once emerged, males will often remain in the patch ready to mate with emerging females. As is typical of many hymenopterans, aphid parasitoids are haplodiploid meaning that fertilized eggs laid by the female will develop into females and the unfertilised eggs will develop into males (Hickman *et al.*, 2004). This allows mated females control over the sex of the egg that

is deposited in the host. It is normally found that females are laid in higher quality host (Charnov, 1982) though can be related to other factors such as the age of the mother (He & Wang, 2008).

1.2.3 Aphid Parasitoid *Aphidius colemani*

Aphidius colemani is a generalist parasitoid (Hymenoptera:Braconidae) that most likely originated from Northern India or Pakistan though is now found Australia, Europe and across North and South America (Starý, 1975). *A. colemani* has been reared on dozens of different aphid species, however, due to a lack of interbreeding between populations and slight morphological variations between them it has been suggested that *Aphidius colemani* is actually a collection of sibling species (Messing & Rabasse, 1995). If this is the case then *A. colemani* may not be as generalist as previously believed since not all the studies were conducted with the same populations. *A. colemani* are commercially produced and available as a control agent in the UK, primarily for *M. persicae* and *Aphis gosspyii* aphid populations (Grasswitz & Resse, 1998). Amongst insects Hymenoptera display a high aptitude for learning and strong reliance on olfaction, making them ideal model specimens for study. In addition *A. colemani* are prevalent in biological control programmes and widely commercially available providing a potential application for knowledge gained from this study. The knowledge gained in laboratory assays regarding foraging will be tested in a glasshouse environment (Ch.5) and it is, therefore, pragmatic that widely used biological control agent is used.

1.2.4 Parasitoids in Biological Control

Biological control, or biocontrol, is the use of living organisms to control pest populations. The introduction of such methods is believed to have started over 1600 years ago in ancient China where the arboreal ant *Oecophylla smaragdina* was first used to protect crops (Wei *et al.*, 2005). The first known successful introduction of a natural enemy for biological control in modern times is believed to have occurred in the eighteenth century (DeBach & Rosen, 1991). Parasitoids have been a prominent feature in integrated pest management since the early 19th century and have proven an integral component (Orr & Suh, 1998) with 66% of successful control plans involving a species of hymenopteran parasitoid (Thacker, 2002). Parasitoids are effective in biological control programmes because they are often capable of attacking a large number of hosts each day and many species can be reared on a large scale. In aphid biological control, unsuccessful applications of parasitoids have been ascribed to a lack of synchrony with the host (Powell & Pickett, 2003), poor quality of the parasitoids (Fernandez & Nentwig, 1997) or failure in the initial stages of foraging (Lewis *et al.*,

1990). A potential solution to problems in synchrony and foraging may be the application of semiochemicals.

1.3 Semiochemicals

A semiochemical is any chemical used by organisms that conveys information concerning the organism itself or the environment (Law & Regnier, 1971). The term comes from the Greek routes of semeion (σημειου) meaning signal and was suggested as a simple term covering both inter- and intra-specific chemical communication (Law & Regnier, 1971). The term 'pheromone' was introduced prior to semiochemical (in 1959) to distinguish "substances that are secreted by an animal to the outside and cause a specific reaction in a receiving individual of the same species" (Karlson & Butenandt, 1959). The word is offered as an alternative to previously used 'ectohormones' and is derived from the Greek root with pherein meaning to carry and horman to excite (Karlson & Butenandt, 1959). Although a pheromone in its true sense refers to intraspecific communication it is often used to encompass the range of olfactory signals produced by an organism. Semiochemicals are most commonly divided by the cost/benefit relationship that exists between the signaller and the receiver. A kairomone is a semiochemical received by an individual of another species to their benefit. A synomone is a semiochemical received by a member of another species which benefits both the signaller and the receiver, though this is often at the expense of another species. An allomone is a semiochemical received by a member of another species that will elicit behaviour which benefits the signaller.

It is recognised that all these interpretations of pheromone communication are subjective. To take the example of the aphid sex pheromone: it is produced by a calling female aphid to attract male conspecifics, in this sense it is a classic pheromone. However, the reception of the signal by a parasitoid using the aphid as a host makes it a kairomone. In addition the odour may be used by a hyperparasitoid, which will attack the parasitoids, benefiting the aphids and could therefore be viewed as a synomone. It is also recognised that in this sense the term signaller can be misleading. 'Signaller' normally implies an individual instigating communication, however, in many cases the signal is produced passively with negative consequences to the individual e.g. waste produced by the 'signaller' is a necessary product of obtaining a balance of nutrients and energy but when received by a natural enemy can be a kairomone to aid in the location of a host. The terms ascribed to semiochemical subcategories can be useful in describing an olfactory interaction, though it is important to recognise that the complexity of multitrophic systems means that each odour may be involved in a range of interactions, whether this is the intention of the signaller or not.

1.3.1 Discovery

The first observation of pheromones is credited to Jean-Henri Fabré at the end of the 19th century. The discovery was made when Fabré noted that a virgin female emperor moth he had left covered in his study had 'attracted forty lovers eager to do homage to the maiden' (Fabré & Miall, 1911). He went on to find that it was still attracting males so long as the covering was not airtight, and from this deduced that a chemical component must be involved. The first full identification of such a pheromone only came in the late 1950s when Butenandt's research group dissected 500,000 silk moths in order to remove the 12mg of fluid from the hormone glands needed to analyse and identify the chemical constituents of a pheromone for the first time (Butenandt *et al.*, 1959). Since then, the techniques for identifying semiochemicals have advanced enough that often only one individual is needed. As a result a wide variety of semiochemicals have been characterised, including those of several aphid species.

1.3.2 Identification of an Aphid Sex Pheromone

During sexual phases of the aphid lifecycle female aphids will produce sex pheromone to attract or 'call' males. The presence of an aphid sex pheromone was first demonstrated by the attraction of male aphids in an olfactometer (Pettersson, 1970), and later through chemical analysis (Dawson *et al.*, 1987). Oviparous females emit the pheromone from glands on the metathoracic tibia (Marsh, 1975). When calling, the female typically raises her hind legs and waves them in the air allowing for a greater exposure of the odour emitting glands (Pettersson, 1970). The odour is then received by male aphids via the second rhinaria of the antennae (Pickett *et al.*, 1992).

Using gas chromatography techniques Dawson *et al* (1987) were able to analyse extracts from excised legs of the vetch aphid (*Megoura viciae*) to find two active ingredients: (+)-(4a*S*,7*S*,7a*R*)-nepetalactone and (-)-(1*R*,4a*S*,7*S*,7a*R*)-nepetalactol. When tested individually neither of these isomers were found to elicit a strong response in males. The two combined did show a response equal to that of the original extract, demonstrating that the blend of the two components was important to aphid response.

It has been found that oviparous females do produce trace amounts of sex pheromone when not calling (Dawson *et al.*, 1990) but it is not known whether the amount produced would be sufficient to attract males. The sex pheromone production of the female is not consistent throughout the adult's life, and also varies with day length (Goldansaz & McNeil, 2003). It has been shown for the greenbug aphid *Schizaphis graminum* productivity is greatest between 6-8 days of the adult stadium (Eisenbach &

Mittler, 1980) and for the rosy apple aphid, *Dysaphis plantaginea*, pheromone production is greatest on the 8th day of the adult stadium (Stewart-Jones *et al.*, 2007). The quantity of pheromone that aphids emit varies widely by species with the vetch aphid, *Megoura viciae*, producing around 100-150 ng per ovipara per day (Hardie et al., 1990) to the rosy apple aphid, *Dysaphis plantaginea*, producing only 60-90 ng per ovipara per day (Stewart-Jones *et al.*, 2007).

An understanding of the parasitoid response to semiochemicals may assist biological control programmes in overcoming some of the challenges faced by foraging parasitoids. A promising application of semiochemical technology may be through genetically modified plants (Poppy & Powell, 2004), which have already shown capable of expressing the aphid alarm pheromone and had success in attracting parasitoids (Beale *et al.*, 2006). The promising results of such work has encouraged further study in the form of full-scale field trials (Pickett *et al.*, 2012). However, the use of genetically modified plants is also facing strong public opposition or unease (Grant, 2003) and it is likely any introduction of semiochemical technology in pest control in the near future will rely on pre-existing methods of application.

1.3.3 Ubiquity of Nepetalactone in Aphid Species

The two active components of the aphid sex pheromone were found to be the monoterpenoid isomers (4a*S*, 7*S*, 7a*R*)-nepetalactone and (1*R*, 4a*S*, 7*S*, 7a*R*)-nepetalactol (Figure 1.4) (Dawson *et al.*, 1987). Neither component was found to be attractive to male vetch aphids, *Megoura viciae*, when presented alone; however, a combination of the two compounds resulted in a behavioural response equal to that elicited by the leg extract. Since this discovery it has been noted that nepetalactone and nepetalactone are ubiquitous components of aphid sex pheromones (Dawson *et al.*, 1990). They appear as primary constituents in the pheromones of all aphids, with the only exceptions observed to date being the damson-hop aphid (*Phorodon humuli*) which utilises two different nepetalactol diastereoisomers (Campbell *et al.*, 1990) and possibly the additional compound (1*S*, 2*R*, 3*S*)-dolichodial produced by the rosy apple aphid, *Dysaphis plantaginea* (Dewhirst *et al.*, 2008).

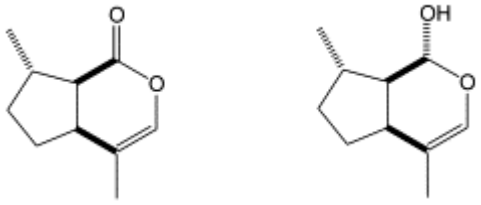


Figure 1.4 The two ubiquitous aphid sex pheromone components.

Left: (4aS, 7S, 7aR)-nepetalactone, Right: (1R, 4aS, 7S, 7aR)-nepetalactol (taken from Birkett & Pickett, 2003).

With two active compounds comprising the majority of aphid sex pheromones it is understood that the males differentiate between species by the ratio of -lactol to -lactone. The blend of many aphid sex pheromones have been described (Table 1.1), although it is observed that the ratio of compounds that the female produces can vary during her adult phase (Hardie, 1990).

Table 1.1 Ratios of nepetalactol to nepetalactone found in entrainments collected from oviparae of different aphid species (taken from Stewart-Jones et al., 2007).

Common name	Species name	Ratio (ol:one)	Reference
Cabbage aphid	*Brevicoryne brassicae*	0:1	(Gabryś *et al.*, 1997)
Black-berry cereal aphid	*Sitobion fragariae*	0:1	(Hardie *et al.*, 1992)
Grain aphid	*Sitobion avenae*	(trace) 0:1	(Lilley *et al.*, 1994a)
Spiraea aphid	*Aphis spiraecola*	1:2[a]	(Jeon *et al.*, 2003)
Peach aphid	*Tuberocephalus momonis*	1:4	(Boo *et al.*, 2000)
Vetch aphid	*Megoura viciae*	1:5–1:12 (age effect) 1:6	(Hardie, 1990)
			(Dawson *et al.*, 1990)
Spiraea aphid	*Aphis spiraecola*	1:6–1:8 (age effect)[b]	(Jeon et al., 2003)
Black bean aphid	*Aphis fabae*	1:29	(Dawson et al., 1990)
Pea aphid	*Acyrthosiphon pisum*	1:1	(Dawson et al., 1990)
Peach-potato aphid	*Myzus persicae*	1.5:1	(Dawson et al., 1990)
Rosy apple aphid	*Dysaphis plantaginea*	3.7:1 3.7:1–3.3:1 (age effect)	(Stewart-Jones *et al.*, 2007)
Potato aphid	*Macrosiphum euphorbiae*	4:1–2:1 (age effect)	(Goldansaz & McNeil, 2003)
Greenbug	*Schizaphis graminum*	8:1	(Dawson *et al.*, 1988)
Currant aphids	*Cryptomyzus* spp.	30:1	(Guldemond *et al.*, 1993)
Bird cherry-oat aphid	*Rhopalosiphum padi*	1:0	(Hardie *et al.*, 1994c)
Damson hop aphid	*Phorodon humuli*	1*:0	(Campbell et al., 1990)

[a] Field-collected oviparae. [b] Laboratory-reared oviparae

Using *M. viciae* it has been shown that the males are responsive to sex pheromone blends associated with other aphid species but had the strongest response to the ratio represented by their own species (Hardie, 1990).

1.3.4 Commercial production of aphid sex pheromone

Behavioural bioassays conducted by Hardie *et al* (1997) confirmed that the enantiomeric purity of the compounds needed to be high in order for them to be biologically active. This discovery made the previous methods of nepetalactone/lactol

from citronellol extraction unfeasible (Dawson *et al.*, 1996). The relatively small quantity of pheromone produced by the aphids themselves meant that any production on a commercial scale would require an alternative source. Male aphids of *M. viciae*, *Aphis fabae* and *Acyrthosiphon pisum* respond to synthetic sex pheromone in the laboratory (Hardie, 1990) and *Sitobion fragariae* males have shown attraction to synthetic sex pheromone in the field (Hardie *et al.*, 1992) along with a range of parasitoid species (Hardie *et al.*, 1991). While synthesis of the compounds from raw materials is possible, and ecologically applicable, it is too costly to be performed on large scale (Birkett & Pickett, 2003). A steam distillation system was developed using the catmint plant, *Nepeta cataria*, which has allowed low cost and high yield production of the sex pheromone components. The essential oil is extracted from the fresh plant via steam distillation with the addition of cyclohexane to collect volatile oil. The steam and cyclohexane are entered into a condenser where the cyclohexane fraction and the water are separated. Vacuum distillation is performed on the cyclohexane fraction to remove the volatile oil (Birkett & Pickett, 2003). To slow the rate of release, and improve commercial potential, the extract can be infused into a polymer strip of ethylene vinyl acetate, polyvinyl acetate or polyvinyl chloride. The inexpensive mass production of nepetalactone has opened-up opportunities for commercial application of the pheromone in biological control programmes. If parasitoid foraging is to be enhanced through the use of nepetalactone it is first necessary that the processes involved in parasitoid host selection and the role that odours play is understood.

1.4 Parasitoid Host Selection

1.4.1 Stages of Host Selection

Divisions of the stages of host selection by a hierarchy of cues, or the order in which they are encountered, are common in the literature (van Alphen & Vet, 1986, Wellings, 1991). One of the most widely adopted categorisations, and that most applicable to this study, is that defined by Vinson (1976). Vinson describes five distinct stages to the host selection process. Although chemicals play a vital role in all stages of host selection it is speculated that odour is most prominent in the initial three stages, which will be the focus of this study. Experiments in this study are designed to address foraging issues associated with these initial three stages although a review is given below of each stage to provide understanding of the complete process. It is also noted that visual and vibrational cues can be important in host location (Fischer *et al.*, 2001 respectively, Michaud & Mackauer, 1995), though it is odour that is most extensively covered in the literature and will be discussed here. It is recognised that these stimuli may be used by the parasitoid in conjunction (Wäckers & Lewis, 1994) but appears that

odour is the most important cue in long range foraging. All statements made regarding host selection in the following sections are supported directly by Vinson (1976) unless an alternative reference is provided. Division of host selection into five stages is applicable to all foraging parasitoids; however the focus of this text is the application of the theory to aphid parasitoids. Therefore, unless otherwise stated, the context of explaining host selection is provided with relation to aphid parasitoids.

1.4.2 Host Habitat Location

To initiate the host selection process it is necessary for the parasitoid to locate the habitat of its host. Whilst the ideal chemical signal for a parasitoid is one that relates directly to the presence of a suitable host, these are not always readily available. Any cue released by the host that is easily detectable to a parasitoid from a distance will be selected against. For this reason, few signals of this nature exist or are broadcast over a large distance and instead parasitoids resort to utilising other cues from the environment which may relate to the presence of the host. Plants that are preferred by hosts tend also to be preferred by parasitoids. Due to the greater volume of odour produced, plant volatiles are much more readily available than pheromones produced by the host and are, therefore, frequently the main cue used by a foraging parasitoid at long distance. Plants subjected to damage by the host will often produce a greater volatile output (Kessler & Baldwin, 2001) with an altered ratio of compounds (Paré & Tumlinson, 1999). Plants releasing volatiles indicating a level of stress are selected by the parasitoid over a healthy one because it is more likely to have feeding hosts (Du *et al.*, 1998), and preference for a plant will be greater still if the preferred host is present (De Moraes *et al.*, 1998). The cause of the volatile output differences are not fully understood, though it is believed that in aphids components of the saliva elicit the distinct volatile complex. The role of these salivary proteins in aphid feeding is not completely understood either but it is hypothesised that that they play an important role or would have been selected against considering the disadvantage of being more easily located by parasitoids (Powell & Pickett, 2003). Since aphids are commonly found in sporadically located but densely populated patches it would be expected that the parasitoids would be adapted to respond to signals released upon aggregation of aphids to increase the chance of encountering a patch rich in hosts. Indeed *Aphidius ervi* have been seen to have a threshold of 40 aphids before significant levels of response are seen (Powell *et al.*, 1998). Similarly *Cotesia vestalis*, a parasitoid of *Plutella xylostella* was shown to display a density dependent response to the odour of feeding caterpillars (Girling *et al.*, 2011).

1.4.3 Host Location

When a parasitoid locates a patch containing the host, it encounters an additional range of odours, some of which are produced directly by the host. At this stage in location, aphid-associated cues such as exuviae and honeydew can be utilised by the parasitoid or any of the semiochemicals produced by the host e.g. alarm pheromones that are often employed by aphids to signal the presence of an enemy. The alarm pheromone, (E)-β-farnesene, is secreted by the aphids through the cornicles causing conspecifics to scatter or seek other parts of the plant. This is a highly informative signal because it demonstrates the presence of an aphid colony, although only one that is already making attempts to escape the area. By utilising host waste produce or their pheromones as kairomones parasitoids are able to gain more reliable information as to the presence of the host.

1.4.4 Host Acceptance

When a host is successfully encountered and available, the parasitoid may still select not to oviposit in the individual, this stage of selection is the host acceptance. The basis for host acceptance includes shape, size, movement and sound of the host, but again odour is recognised as having an essential role. The presence of the host odour is sufficient to elicit the parasitoid piercing with its ovipositor (Picard, 1922) but it appears other factors are necessary to induce the eggs to be released. These factors could be olfactory stimuli or tactile chemical communication but this remains unknown, and is likely to be a combination of these factors (Battaglia *et al.*, 1993). Acceptance levels by the parasitoid to certain hosts vary based on size of the host or age, which is often correlated. It has been shown that aphids of different ages produce differing levels of pheromones, and it is therefore likely that certain chemical cues have diminished in adults that would otherwise induce oviposition. It is also possible that the preferences of the parasitoid could be learnt relative to previous success and may therefore vary depending on their environment or life history. Another factor in host acceptance is the pheromones left by previous parasitisation. Upon contacting the host a parasitoid may leave a chemical marker, intentially or not, indicating that the host has already been parasitized, or visited. This avoids the parasitoid wasting more eggs in the same host and creating competition between its offspring but it also reduces possible resource competition with other parasitoids. It is speculated here that if the host marker odour is strong enough it may play a role in the previous stages in host selection as it signals that viable hosts were available and therefore the environment is conducive to the feeding of the host species and the presence of a host colony.

1.4.5 Host Suitability

The initial three stages of host selection contribute to aspects of the foraging process. The location of an appropriate host is vital, but alone it does not guarantee reproductive success for the parasitoid. Host suitability relates to the ability of the parasitoid larva to develop inside the host. If superparasitism has occurred then competition may take place within the host for resources or more direct conflict may arise between the larvae. Toxins may also be released to eliminate a competitor or alter the physiological state of the host, some of which will be induced by the mother during oviposition. Another barrier to successful development is toxins which may already be carried within the host or diseases that it may carry which the parasitoid is susceptible to. Due to disease or predation the host may die before the parasitoid larva is able to complete the necessary stages of development. This is one of the reasons that the use of pesticides and parasitoids in pest control would have to be carefully controlled; ensuring that hosts are not made unviable due to the levels of pesticides in their body which would also eliminate the parasitoid population.

1.4.6 Host Regulation

At the final stage it is necessary for the parasitoid to regulate the host's development to permit its own survival. Host regulation is very closely linked to host suitability in many aspects. This stage can be viewed as the processes carried out by the parasitoid to make the host an adequate source of food and shelter. One of the first and most significant steps in host regulation for many species of parasitoid is the paralysation of the host. This can be permanent or transient and allows control over the host body. The dose of venom must be carefully controlled to ensure the beneficial effect for the parasitoid without killing the host. A parasitoid may also regulate growth rate, food consumption, development, morphology, behaviour, respiration, biochemical and physiological activities within the host (Vinson & Iwantsch, 1980). The regulation of the host may be necessary to ensure the survival of the host until the parasitoid has reached the required stage of development. It follows through kin selection theory that the premature death of a parasitized aphid will enhance the survival of its genes and therefore be selected for. It is recognised that regulation is caused by the larva whilst within the host but also much of it is initiated by the parasitoid mother during oviposition.

1.5 The Reliability-Detectability Trade-off

The stages of host selection provide a framework to interpret the process undertaken by a parasitoid. However, it does not fully address some of the challenges faced at each stage or why host selection does occur in these stages. For the purpose of this study it is important to understand why parasitoids adopt the strategies observed

during foraging and how they utilise the odours available. The dependency on habitat and host associated cues in the early stages of host selection requires that the parasitoid evaluate the accuracy of the signal in conveying information regarding the host's location. A notable challenge that arises in signal detection is that the value of such a signal is often indirectly related to its availability. This phenomenon is known as the reliability-detectability problem.

1.5.1 Theory and Hypotheses

To optimise foraging potential within a multitrophic context it is necessary that parasitoids evaluate the validity of the chemical information available, and respond accordingly. It is recognised that the most reliable cues for a parasitoid to respond to are those directly emitted by a viable host. However, selection pressures exist to ensure that cues emitted from the hosts remain discrete to avoid such detection by predators or parasitoids. Selection pressure on the parasitoid to find a host is also strong because failing to find a suitable host will mean a failure to produce offspring. Within a multitrophic system there may also exist organisms which suffer no deleterious effect as a consequence of detection, or may even benefit from it, such as the plants being attacked by herbivores (van Loon *et al.*, 2000). From this arises the reliability-detectability trade-off (Vet & Dicke, 1992) which represents the challenge faced by a parasitoid in an environment where cues of greater detectability are less reliable, and those highest in reliability remain more discrete. Reliability here is defined as odours more accurately indicating the presence of a viable host, while detectability is a measure of how available the odour is to detection.

It is hypothesised that three strategies may be employed by the parasitoid to overcome the reliability-detectability problem:

1. The use of more conspicuous chemical cues from individuals of the host species from a different stage of development (infochemical detour).
2. Responding more strongly to stimuli released by the host's interaction with its food plant.
3. Learning to associate easily detected stimuli to less readily detected but more reliable signals.

1.5.2 Infochemical Detour

An infochemical detour is the use of more conspicuous chemicals released by the host species though not necessarily at a stage viable for parasitisation. In aphidiine parasitoids the use of the aphid sex pheromone to locate viable hosts (Hardie *et al.*, 1991) could be an infochemical detour: using the pheromone produced by adult females (Hardie, 1990) to locate the juveniles which are used as hosts, a phenomenon

that can also be seen in other host-parasitoid models (Wiskerke *et al.*, 1993). Infochemicals can also be in the form of waste material produced by the host such as; aphid exuviae for *Aphidius ervi* (Battaglia *et al.*, 2000), aphid honeydew for *Aphidius rhopalosiphi* (Budenberg, 1990) or host faecal matter for the lepidopteron parasitoid *Microplitis croceipes* (Lewis & Tumlinson, 1988).

As a result of low mobility and a high reproductive rate, juvenile aphids are often found alongside adults. This increases the consistency with which parasitoids find viable hosts using an infochemical detour.

1.5.3 Host/Food Source Interaction

In 1937 Laing noted that a braconid wasp could respond to cues from their host's food source (Laing, 1937). In this early case it was uninfested meat that elicited a response from the parasitoid *Alysia manducator* whose host, the *Calliphora* maggots, normally feed on meat. This represents the initial stage of host selection, habitat location. In the case of *Alysia Manducator* exposed meat is reliable as the abundance of *Calliphora* species provide a high rate of infestation for any meat exposed to the environment. In a similar fashion aphid parasitoids show a response to plant volatiles (Elzen *et al.*, 1983), however, due to the abundance of such volatiles within the environment the odours do not infer the same reliability. To increase the reliability of response the parasitoids respond more strongly to damaged plants (Guerrieri *et al.*, 1993). It is common for parasitoids to utilise plant volatiles and, if the theory that modern entomophagous parasitoids evolved from wasps parasitizing plants (Malyshev, 1968) is correct, it would imply that the use of plant volatiles in foraging predates even the use of an insect host. A response is seen most strongly to infested plants, less so to artificially damaged plants and rarely to completely healthy plants (Dicke *et al.*, 1999, Girling *et al.*, 2006, Turlings *et al.*, 1995). The reason appears to be the increased release of volatiles emitted by the plant when damaged (Rose *et al.*, 1996, Turlings *et al.*, 1990) but also the qualitative differences in volatile emission between naturally damaged, artificially damaged and intact plants (De Moraes *et al.*, 1998, Rose *et al.*, 1996). These volatiles are increased enormously following herbivore attack (Elzen *et al.*, 1986) because releasing such compounds may in fact increase the plants' fitness if they can attract more parasitoids to attack the herbivores grazing on them (Kessler & Baldwin, 2001, van Loon *et al.*, 2000).

The relationship of herbivore damage and parasitoid response is in concurrence with the reliability-detectability model in that parasitoids are seen to respond to cues that more closely represent the presence of a suitable host. A further refinement of host habitat detection is the ability of the parasitoid to associate the feeding of suitable hosts on the plant as opposed to that of other herbivores. The ability to distinguish

specifically the desired host species' feeding from that of other plant volatiles could greatly increase the reliability of the cue and decrease foraging time. Indeed this has been observed (De Moraes *et al.*, 1998) and it is believed to be achieved by the use of cues from salivary proteins (Powell & Pickett, 2003 the former suggesting aphid saliva as an elicitor; the latter demonstrating a response from caterpillar saliva, Turlings *et al.*, 1990). For this reason response to a plant previously infested with the host aphid remains more attractive (Du *et al.*, 1998).

It is also implied by the greater detectability and quantity of volatile emissions that the parasitoid would use them at a longer range and focus more on semiochemical releases from the host itself during closer-range foraging; evidence for this has been seen in trials with *A. ervi* (Du *et al.*, 1997).

1.5.4 Learning

To overcome the challenge of foraging for a discrete host, a parasitoid may learn cues associated with successful host location. Several modes of learning may be employed in foraging and any other challenges faced in the parasitoid life. To understand the different forms of learning, and the benefits they infer to the parasitoid, all the aspects of learning relevant to parasitoid foraging are reviewed below. Using the reliability-detectability framework also provides insights as to how various chemical cues may be learnt, and how susceptible they will be to learning. Odours which are highly reliable and subject to little variation are more likely to be innate in the parasitoid, conversely, odours which will vary with the environment may retain a more plastic response. This is demonstrated by the innate response of Aphidiine parasitoids to the aphid sex pheromone (Poppy *et al.*, 1997) and the particular plants of their host (Storeck *et al.*, 2000). The latter of which can be subject to some variability (as host will frequently be associated with other plants), and will be readily replaced by learning of chemical signals relevant to the existing enrivonment (Storeck *et al.*, 2000).

1.6 Learning

Learning is defined by Dukas (2008) as 'the acquisition of neuronal representations of new information' and is closely associated with memory, which is the retention of such information over time. More is now being realised about the rapidity with which parasitoids can learn and the prominence of learning in adapting their foraging behaviour. It was previously believed that insects were largely innate creatures and would not be capable of learning and retaining the new information. Recent studies have shown that hymenopterans are capable of complex levels of learning to entirely novel environments (Giurfa, 2003, Laska *et al.*, 1999, Olson *et al.*, 2003) often using

an olfactory sense considerably greater than electronic olfactometers (Rains *et al.*, 2004).

1.6.1 Maternal influence

Prior to the emergence of the wasp it is likely it will already have acquired some information about its environment, or at least that of its mother's. It has been demonstrated in *Aphidius colemani* that a maternal cue is passed onto the offspring during oviposition (Douloumpaka & van Emden, 2003). This cue is viewed as relatively weak as it is often overwritten by experiences during emergence or experience gained by the parasitoid as an adult.

1.6.2 Emergence conditioning

Emergence conditioning is the learning of the chemical environment that occurs in a parasitoid as it emerges from its pupal cocoon. Many parasitoid behaviours previously thought to be innate are now understood to be learnt upon emergence (van Emden *et al.*, 2008, van Emden *et al.*, 1996). The emergence conditioning theory suggests that the parasitoid obtains experience of the volatiles present in the environment by making contact with those that have adhered to the mummy surface. The contact - and learning - is made as the parasitoid cuts its way out of the mummy. Emergence conditioning was first conclusively shown by the removal of the lepidopteron parasitoid *Microplitis demolitor* from the cocoon it had created (Hérard *et al.*, 1988). Once removed from the cocoon it was shown that *M. demolitor* had lost the host plant odour preferences which are normally exhibited. In 1996 (van Emden *et al.*) this was shown to be true of the aphid parasitoid *Aphidius rhopalosiphi* when excised from the mummies of *Metopolophium dirhodum*. It has since been shown, using the same aphid parasitoid/host system, that the parasitoid does not only learn the host plant odour but is able to pick-up subtle cues about the presence of other plants within the environment (van Emden *et al.*, 2002). Further evidence for this is that *Aphidius colemani* raised on *Myzus persicae* on an artificial diet show no preference for a host plant and will show preference to the host plant of mummy exuviae they are exposed to if removed from their own mummy case before emergence (van Emden *et al.*, 2008). Emergence conditioning is likely to be selected for in an environment where more than one host is used by the parasitoid and/or the host feeds on more than one species of plant. The emergence of the parasitoid is evidence of the success of its mother, by following the same odours the newly emerged parasitoid is, therefore, more likely to achieve success finding a host in this environment.

1.6.3 Associative learning

Associative learning is the association of different stimuli through experience. Associative learning takes place when a parasitoid encounters a reward or aversive stimuli alongside an odour. Following this encounter, or repeats of the same experience, the parasitoid will learn to associate that odour with reward or the aversive experience. The ability to associate odours in this fashion can be greatly advantageous to an individual as it allows it to adapt to an unpredictable environment. It is hypothesised that individuals living in a more variable environment will have a greater ability in associative learning and a reduced reliance on innate responses (Vet & Dicke, 1992). Following this theory a generalist parasitoid will show a greater reliance on associative learning as the host present in the environment is subject to change, in addition to the hosts' food source.

In aphid parasitoids one of the strongest rewards that can be provided is access to a viable host. It has been shown that an odour learnt following ovipositional experience can take precedence over previously learnt cues (Storeck *et al.*, 2000). Although encountering a host may be the optimal reward for a parasitoid, related stimuli are also sufficient in eliciting a learning experience, as has been shown with aphid honeydew and exuviae (Bouchard & Cloutier, 1984, Du *et al.*, 1997), or with the lepidopteran parasitoid *Microplitis croceipes* using its hosts' faecal matter (Lewis & Tumlinson, 1988). The relative strength of the learning experience is related to the value of the reward with which it is associated (Battaglia *et al.*, 2000), therefore, cues that are more directly related to a food or host source will be more effective in reinforcing learnt behaviours. The associative learning effectively collates information from the hosts' environment, that may be unreliable on its own, to create a more consistently dependable cue.

It has been shown that parasitoids have an increased responsiveness to odours which are likely to occur in their environment, odours which are likely to already exist in the parasitoid's chemical catalogue (Turlings *et al.*, 1993). This makes the learning of 'novel' odours such as chocolate and vanilla compounds (Takasu & Lewis, 1993) easier than truly novel odours such as octanal (Olson *et al.*, 2003), as the former are derived from plant sources. Associative learning is essential to parasitoid success as it allows plasticity and adaptability to conditions that may vary between generations.

1.6.4 Alpha conditioning

Alpha conditioning is the process by which the response observed in a parasitoid to a cue may be enhanced through learning. It is understood that a parasitoid's weaker responses, those that relate to odours which vary within the environment, will be

subject to greater levels of enhancement. This is notable with odours such as plant volatiles; it is beneficial to the wasp to respond to plant volatiles innately, however, the volatiles that it responds to may not be available in a given environment. Therefore a rewarded encounter with the volatile will provide alpha conditioning to the odour and may mean this odour takes precedence over other plant volatiles. At present it is not known if alpha conditioning can operate on already strong innate cues. It has been hypothesised that those odours which offer highly reliable information regarding the host may already elicit an optimal response and will not be subject to alpha conditioning (Glinwood *et al.*, 1999a).

1.6.5 Success motivated retention time

Success motivated retention time is the theory that if an organism has success in the environment (finding a suitable host or food source) it will remain there for a longer period. The theory can be extended to different levels of 'success' e.g. if a parasitoid encounters an odour associated strongly with a host it may extend the length of time it will spend foraging in that patch, extending its 'give-up time'. Give-up time was originally described by Croze (1970), for carrion crows as the time from finding the last item until 'flying or walking well away'. This principal can be extended to any circumstance where an organism is foraging a resource patch but is capable of leaving to search for another resource patch. In the lepidopteron parasitoid *Venturia canescens* it has been demonstrated that the greater ovipositional success it has in an area the longer it will stay foraging (Bernstein & Driessen, 1996), the patch will also be revisited more frequently which is probably due to host markers which are left at the site. It is hypothesised that aphidiine parasitoids will commit more time foraging a patch if they have already had success in this area, though, in a natural environment other factors such as the possible markers of other parasitoids, health of the plant and proximity of other aphid colonies might be a factor. At present it is still not established if the reward of energy or a host is required for the parasitoid to learn the environment is favourable or if the encounter of semiochemicals relating to the host is sufficient.

1.7 Parasitoid response to nepetalactone

In 1991 *Praon* species were used to show that aphid parasitoids could be attracted to the aphid sex pheromone in field trials (Hardie *et al.*, 1991). The synthetic form of the pheromone was used in this initial study but it has since been shown in *Aphidius ervi* and *Praon volucre* that the level of attraction did not vary significantly between synthetic and naturally extracted nepetalactone (Glinwood *et al.*, 1999b) which is the sex pheromone component shown to elicit the greatest response (Hardie *et al.*, 1991).

Various studies since have confirmed that aphid parasitoids are capable of detecting the pheromone components and may utilise it in foraging as a kairomone (Table 1.2).

Table 1.2 Parasitoids which have exhibited a response to aphid sex pheromones (taken from Powell & Pickett, 2003). * This study came out after the original publication and has been added by the author

Parasitoid	Pest aphid hosts	Evidence of response
Aphidius rhopalosiphi	Cereal aphids	Field experiments Laboratory bioassays Electrophysiology
Aphidius ervi	Pea aphid Cereal aphids Glasshouse aphids	Field experiments Laboratory bioassays Electrophysiology
Aphidius eadyi	Pea aphid	Field experiments Laboratory bioassays
Aphidius matricariae	Glasshouse aphids	Electrophysiology
Diaeretiella rapae	Brassica aphids Russian wheat aphid	Field experiments Laboratory bioassays Electrophysiology
Praon volucre	Wide range of hosts	Field experiments Laboratory bioassays Electrophysiology
Ephedrus plagiator	Wide range of hosts	Laboratory bioassays
Aphidius gifuensis	Wide range of hosts	Electrophysiology*
Aphidius colemani	Wide range of hosts	Laboratory bioassays*

Aphidius ervi, which had no previous encounter of aphid sex pheromone and reared on asexual aphid populations, demonstrated a positive response upon encountering the sex pheromone, confirming a genetic basis for the behavioural response (Poppy *et al.*, 1997). Despite being an innate response, if no reward is given during learning there is evidence that the parasitoid may habituate to the pheromone (Glinwood *et al.*, 1999a) showing the flexibility in their learning. While the adaptability of learnt response opens up opportunities to 'programme' the parasitoid to a specific host/plant complex it also emphasises the complexity of introducing a semiochemical into the ecosystem.

Kairomones can often be enhanced by other cues present in the environment, such as plant volatiles, with which a synergy is observed in predators and parasitoids of other insect orders (Light *et al.*, 1993 for Lepidoptera, Ruther *et al.*, 2000 for Coleoptera) and more recently for those of aphids (Pope *et al.*, 2007).

The exact response of aphid parasitoids to aphid sex pheromone components remains unclear. The five basic responses to semiochemicals can be defined as: attractant, repellent, stimulant, arrestant and deterrent. It was initially assumed that an attraction was responsible for increased catches in field trials (Hardie *et al.*, 1991), however, subsequent studies have often failed to show such a clear attraction to the odour. In a square olfactometer Ameixa and Kindlmann (2011) demonstrated increased visitation of *Aphidius colemani* to *Nepeta cataria* essential oil, but not the isolated nepetalactone compound. Large scale field trials have also shown a benefit to the introduction of nepetalactone, but it was suggested that such a benefit was seen through altered spatial distribution and not direct attraction of the wasps (Powell *et al.*, 2004). Work by Glinwood *et al* (1998) in which nepetalactone did increase parasitisation rates of *Aphidius rhopalosiphi* and *Praon Volucre* suggested that the foraging response of the two may be altered by the pheromone in different ways. The generalist *Praon volucre* was found attack more in greater proximity to the pheromone lures, however, *A. rhopalosiphi*, a specialist on cereal aphids, showed an increased attack spanning a greater distance. This may be explained by an arrestant behaviour elicited by nepetalactone and affecting the parasitoids at different thresholds. A greater response threshold for the generalist may be necessary because of the decreased specificity required in host selection, however, a specialist may be move heavily involving blend and ratio to encounter a host and place less importance on overall concentration. This example reflects well the current understanding of the aphid sex pheromone as a kairomone for foraging parasitoids; although there is demonstrable evidence of a response by the parasitoids, the exact nature of that response remains unclear.

1.8 Thesis aims and hypotheses

To understand the impact that nepetalactone will have on a tritrophic system it is necessary to study the ecology and interactions of the organisms at each level. To achieve a comprehensive understanding of the tritrophic system; electrophysiological, physiological and behavioural response of the host and parasitoid were studied. It is currently unknown if virginoparae *M. persicae* are capable of detecting the sex pheromone or display any behavioural response to the odour. Although it is known that various parasitoids utilise the aphid sex pheromone, this work addresses the exact behavioural changes induced by nepetalactone in the initial three stages of host selection (Ch. 1.4.1). Developing on the current knowledge of foraging strategies, odour reception and learning involved in host-parasitoid interactions an investigation is made into methods of enhancing the efficacy of parasitoid host location. Laboratory assays are used to provide an understanding of the specific behaviour elicited by nepetalactone. This knowledge of how nepetalactone affects parasitoids and their host was then applied in the glasshouse making it possible to assess the true implications of nepetalactone on the tritrophic system.

1.8.1 Overall aim of research

To establish how the aphid sex pheromone affects a host, its parasitoid, and the host-parasitoid interaction in a tritrophic system.

1.8.2 Individual chapter key hypotheses

Chapter 2 focuses on the effect that nepetalactone has on virginoparous *M. persicae* that do not encounter the sex pheromone in their natural environment. Although virginoparae are not exposed to nepetalactone in a natural setting, the implementation of nepetalactone within a glasshouse environment may impact their ecology.

Key hypotheses

1. *M. persicae* virginoparae are capable of detecting the aphid sex pheromone.
2. Virginoparae avoid nepetalactone as a means of avoiding conspecifics.
3. An avoidance of nepetalactone will negatively affect aphid performance on an individual and population level.

Chapter 3 aims to ascertain the behavioural response of *A. colemani* to the sex pheromone. Parasitoids used for study in this chapter are naïve in the sense that they have had no previous encounter with the sex pheromone. The absence of experience of nepetalactone reflects the condition that parasitoids are normally introduced to biological control programmes.

Key hypotheses

1. *A. colemani* are capable of detecting the sex pheromone components.
2. A synergy exists between nepetalactone and host-associated volatiles.
3. *A. colemani* will show greater ovipositional success with nepetalactone present.

Chapter 4 utilised learning in an attempt to manipulate the parasitoid response to nepetalactone. Emergence conditioning and ovipositional experience are used in attempts to achieve alpha-conditioning with what is considered an already strong innate response.

Key hypotheses

1. Emergence conditioning with nepetalactone increases parasitoid foraging efficiency during a subsequent encounter.
2. Ovipositional experience with nepetalactone will lead to increased foraging efficiency when the odour is encountered.

Chapter 5 applies knowledge gained from the previous chapters to a glasshouse environment. Synergy of nepetalactone and host related cues and learning are applied in attempts to enhance parasitoid foraging. The direct impact of the nepetalactone on the aphid populations is also considered through measures of the aphid population growth and dispersal. Using this larger spatial-temporal scale it is possible to test findings from laboratory trials in an environment more accurately reflecting that of typical biological control system.

Key hypotheses

1. Parasitism will benefit plant growth.
2. Nepetalactone will increase the rate of parasitism in the glasshouse.
3. Parasitoids trained to the host-plant complex will be more efficient in foraging.

Chapter 6 summarises the results from each chapter and describes the significance of these findings in explaining chemical ecology in a tritrophic system. Discussing work from this and previous studies, a theory is synthesised of how parasitoids utilise nepetalactone in host foraging. An understanding of how aphids and parasitoids respond to nepetalactone provides a basis to discuss the impact it may have on the ecology of the tritrophic system. Finally future work in this field is outlined and a discussion of the potential of this work in an applied context is provided.

2 The effect of nepetalactone on Myzus persicae virginoparae host choice and population growth

This chapter is currently under review to be published in the Bulletin of Entomological Research as: 'Do asexual morphs of the peach-potato aphid, *Myzus persicae*, utilise the aphid sex pheromone? Behavioural and electrophysiological responses of *Myzus persicae* virginoparae to (4a*S*,7*S*,7a*R*)-nepetalactone and its effect on aphid performance' *Fernández-Grandon, G.M, Woodcock C.M. and Poppy, G.M.*

2.1 Introduction

Utilising natural enemies to control aphids is an essential part of integrated pest management strategies. Without control from natural enemies or a limitation of resources, aphid populations will grow at an exponential rate (Costamagna *et al.*, 2007, Lin & Ives, 2003). To limit such growth, it is necessary for natural enemies to locate the host early on in the season or infestation, before the population is too large to be reduced effectively by biological control alone. Optimal foraging theory predicts, and experimental evidence demonstrates, that certain patches in the field will be preferentially visited by parasitoids (Wajnberg, 2006). The parasitoid is only able to select foraging patches based on the information it has available, information which it acquires largely through olfaction.

Organisms utilise a wide range of cues within the environment, regardless of whether or not they are the intended receiver (Rutledge, 1996, Wyatt, 2003). Most commonly, these are chemical signals which are exploited due to their abundance within, and passive movement through, the environment. An odour which is used by conspecifics as a cue to aggregate may equally be viewed as a sex pheromone (Wertheim *et al.*, 2005), or as a range of kairomonal cues for other species (Ruther *et al.*, 2002). The potential multifunctionality of an odour signal is frequently overlooked by researchers once a role for the odour within the environment has been ascribed. In many situations, it is likely that production of a semiochemical initially served a distinct function, such as aggregation, alarm response or mating. However, once the pheromone is present within the environment, the opportunity is there for natural enemies, allies or prey to evolve a distinct response to it.

Parasitoids have been the subject of a considerable amount of study in chemical ecology (Godfray, 1994). This is for both practical and applied reasons. The acuity with which they can detect and learn odours make them ideal candidates for theoretical studies and their widespread use in control programmes can provide a direct application for such work. Many of the cues that parasitoids use in foraging

have been identified (Poppy *et al.*, 1997, Rehman & Powell, 2010), aphid sex pheromone being one. Methods are already being investigated to incorporate aphid-associated cues such as their alarm pheromone, (*E*-β-farnesene), into biological control programmes (Beale *et al.*, 2006, Yu *et al.*, 2012) and it is likely that the aphid sex pheromone could serve a similar role in enhancing parasitism. In addition to attracting male aphids, the synthetically produced pheromone has been shown to attract female parasitoids within the field (Hardie *et al.*, 1991) either as an isolated compound mix (Glinwood *et al.*, 1998) or as individual components (Hardie *et al.*, 1994a), and this attraction leads to increased rates of parasitism of various aphid hosts (Glinwood *et al.*, 1998). Parasitoid species have been shown to respond innately to the aphid sex pheromone (Poppy *et al.*, 1997, Powell & Pickett, 2003). An innate response could confer an advantage in manipulating foraging behaviour over relying on plant volatiles or host-related cues that often require learning experiences to achieve an optimal response. Learning of host/plant complexes can be particularly problematic for commercially reared biological control agents that are unlikely to be maintained on the same complex they will encounter in the glasshouse. Having an odour present that parasitoids respond to innately may override, or compensate for, learned preferences of the parasitoid. The potential for aphid sex pheromone components within biological control programmes has not been overlooked, although an optimum strategy for its incorporation is still to be achieved. However, any beneficial results of introducing the pheromone in biological control may be negated if they lead to increased fitness of aphid populations.

During the reproductive phase of the aphid life cycle, sexual (oviparous) females release a sex pheromone to attract mates (Hardie *et al.*, 1992, Pettersson, 1970). Populations which tend to be most problematic in the glasshouse are asexual morphs (virginoparae) which produce no sex pheromone (Hardie *et al.*, 1990). Because the sex pheromone is not produced in their natural environment, it is not known how its components may affect virginoparous aphids. If virginoparae are capable of detecting aphid sex pheromone components, this may cause them to be dispersed by, attracted to, or even to alter their rate of reproduction, any of which may lead to an overall increase in aphid damage. The aphid sex pheromone is comprised predominantly of two cyclopentanoids that are well described: (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol and (4a*S*,7*S*,7a*R*)-nepetalactone, and in some cases may involve a third component (1*S*,2*R*,3*S*)-dolichodial (Dewhirst *et al.*, 2008). A study is conducted into the impact that nepetalactone may have on virginoparous morphs of one of the most prevalent aphid pests, the peach-potato aphid, *Myzus persicae* by assessing short-term implications that may be observed through behavioural changes and long-term effects that may be manifested as changes in the aphids' performance throughout its life or over generations.

2.2 Materials and methods

2.2.1 Aphid and plant cultures

Myzus persicae were maintained on Chinese cabbage plants, *Brassica rapa* sp. *Pekinensis* Cv. Wong bok in a controlled environment room at 20 ± 1°C and 16L : 8D. The aphids had no contact with the sex pheromone and were maintained as asexual morphs.

2.2.2 Y-tube olfactometry

A glass Y-tube olfactometer was employed to investigate the odour preference of aphids when no visual cues are present. The Y-tube and set-up were as described by Pope *et al.* (2011), except that an internal copper wire was connected in the middle of each arm, allowing the aphids to walk down the centre of the tube. After every five replicates, the Y-tube was turned over so that the treatments were now on the opposite sides; this was done to avoid any directional bias. When the Y-tube was turned over or treatments were changed, the internal copper wire was removed and cleaned with 70% ethanol. Airflow was adjusted through a flow meter to ensure that a consistent stream of 200 ml/min filtered air entered each arm of the Y-tube. Aphids were stored in individual glass vials for one hour before the experiment commenced to allow them to acclimatise to the environment. To start the bioassay, an aphid was placed at the base of the wire using a size 0 paintbrush. The aphid was then given five minutes to move towards the odour sources and make a choice. Following the criteria used in previous studies (Girling *et al.*, 2006, Pope *et al.*, 2008), a choice was determined as the aphid spending more than 30s in the upper half of one of the arms.

2.2.3 Effect of nepetalactone on aphid performance

The mean relative growth rate (MRGR) was evaluated using batches of aphids. Due to the light weight of *M. persicae* during their first instar, the weight of the individual cannot be easily obtained. For this reason, the group weight of a batch of 10 was used. Eighty one-day-old aphids were separated into 8 batches. Each batch was then weighed using a 5-point balance (Ohous corporation, Nänikon, Switzerland) and placed on a three-week old Chinese cabbage plant, giving a total of eight plants, each infested with 10 *M. persicae*. Four of the plants were placed in a large ventilated Perspex cage containing nepetalactone, and the other four plants were placed in a cage containing hexane. Hexane was administered by placing a clear 5 ml vial (Chromacol, UK) containing 200 µl of hexane in the centre of the cage. The same was done in the treatment cage, with the vial containing 200 µl of 10 mg/ml nepetalactone in hexane.

Both cages were kept in a controlled environment (20 ± 3°C, 16:8 light:dark) with a regular airflow.

The data provided by these experiments were then input into the MRGR formula (taken from Leather & Dixon, 1984).

$$\text{MRGR (mg, mg}^{-1}\text{, day}^{-1}) = \frac{\ln W_2 \text{ (ug)} - \ln W_1 \text{ (ug)}}{t_2 - t_1}$$

Where W1 represents the weight at birth and W2 represents the adult weight. The time of birth is shown as t1 and t2 is the time of maturity measured in days. The mean was taken from the four samples run simultaneously and contributed to an N of one, therefore, the complete experiment allowed 4 pseudo replicates within each real replicate for each generation.

2.2.4 Effect of nepetalactone on aphid population growth

The intrinsic rate of natural increase (r_m) was calculated for *M. persicae* virginoparae. A population of *M. persicae* was exposed to nepetalactone, or hexane in the control, for four generations prior to the commencement of the study. One-day-old virginoparae were separated into eight batches of 10, four batches in the control group and four in the treatment. The aphids were then placed on three-week old Chinese cabbage plants. Exposure to the nepetalactone and hexane was conducted in the same way as the MRGR trials: a vial containing 200 µl of 10 mg/ml solution was placed in the centre of the cage containing the plants and 200 µl of hexane was used as the control sample.

The position of the treatment and control samples within the room was switched between each trial. Each day, the adults were moved onto a new plant and the offspring that they had produced in the 24 hour period were counted. This was done for the number of days that it took the adult to reach maturity, *i.e.* if it took the aphid 7 days to develop from first instar to a reproductive state, the r_m counts would take place for 7 days after the birth of the first offspring. Data collected this way could be input into the r_m formula (taken from Wyatt & White, 1977).

$$r_m = 0.74 (\ln F_D/D)$$

Where F_D is the number of offspring produced in the same number of days it took for the aphid to reach maturity.

2.2.5 Electrophysiology

Electroantennogram (EAG) recordings from asexual *M. persicae* were made using Ag-AgCl glass electrodes filled with saline solution (composition as in Maddrell, 1969 but without glucose). The head of an apterous virginopara was excised and placed within the indifferent electrode, with the antennae protruding. Both antennae, after the tips had been removed, were inserted into the recording electrode, whilst ensuring that the primary and secondary rhinaria remained exposed. The signals were passed through a high impedance amplifier (UN-06, Syntech, the Netherlands) and analysed using a customized software package (Syntech). The delivery system, which employed filter paper in a disposable Pasteur pipette cartridge, has been described previously (Wadhams *et al.*, 1982). The stimulus (2 sec duration) was delivered into a purified air stream (1 l/min) flowing continuously over the preparation.

Samples (10 µl) of the nepetalactone and nepetalactol, diluted in hexane (50 µg/ml, 1 mg/ml and 10 mg/ml), were applied to filter paper strips and the solvent was allowed to evaporate (30s) before the strip was placed in the cartridge. The control stimulus was hexane. Fresh cartridges were prepared immediately prior to each stimulation. EAG responses to the two sex pheromone components were normalised to the response to the aphid alarm pheromone, (*E*)-β-farnesene (1 mg/ml), applied at the beginning and end of each run (N = 5).

2.2.6 Cleaning of Perspex and glassware

Volatiles may adhere to glass and Perspex surfaces used in the experiments; to ensure that these do not persist and affect the results of subsequent experiments, it was necessary to clean all equipment thoroughly. Following use in experiments, all glassware was cleaned in a solution of Decon 90 (Decon Laboratories Limited, UK), rinsed and washed with acetone then distilled water before being placed in the oven at 200°C for a minimum of 3 hours, following the method used by Pope et al. (2011). All Perspex equipment was washed in Decon 90, rinsed, washed in a 70% ethanol solution and rinsed in distilled water before being left in a ventilated space to air dry.

2.2.7 Statistical analysis

Aphid preference. The significance of odour preferences evaluated in a Y-tube olfactometer was evaluated using a heterogeneity G test. This provided values on the preference within each trial and confirmed that the preferences did not vary significantly between sampling days.

Aphid performance. A two-way repeated measures ANOVA was conducted to analyse differences in the mean relative growth rate between treatments and generation.

Repeated measure analysis was necessary as the aphid generations are not independent variables. The analysis was conducted as part of a general linear model on SPSS (IBM© SPSS© Statistics v 20).

Population growth. The natural rate of increase of *M. persicae* was compared using a one-way ANOVA on Minitab (Minitab ® 16).

Electrophysiology. Responses of aphid antennae to treatments were compared with the response to the hexane control using a Mann-Whitney rank sum test on Minitab (Minitab ® 16).

2.3 Results

2.3.1 Aphid preference

Olfactometry work on virginoparae *Aphis fabae* have shown them to respond positively to host plant volatiles (Nottingham *et al*., 1991); and a similar response was expected for *M. persicae* when presented with a viable host. In a Y-tube olfactometer, *M. persicae* virginoparae preferred the arm containing Chinese cabbage odour (G_1 = 19.27, P < 0.001) (Figure 2.1a) to clean air, which demonstrates the utility of the bioassay in distinguishing aphid odour preferences. Right and left arms of the olfactometer were equally attractive in the absence of odour stimuli (G_1 = 2.19, P = 0.139), confirming that there was no directional bias shown by the aphids and that preferences seen will be to the odours presented.

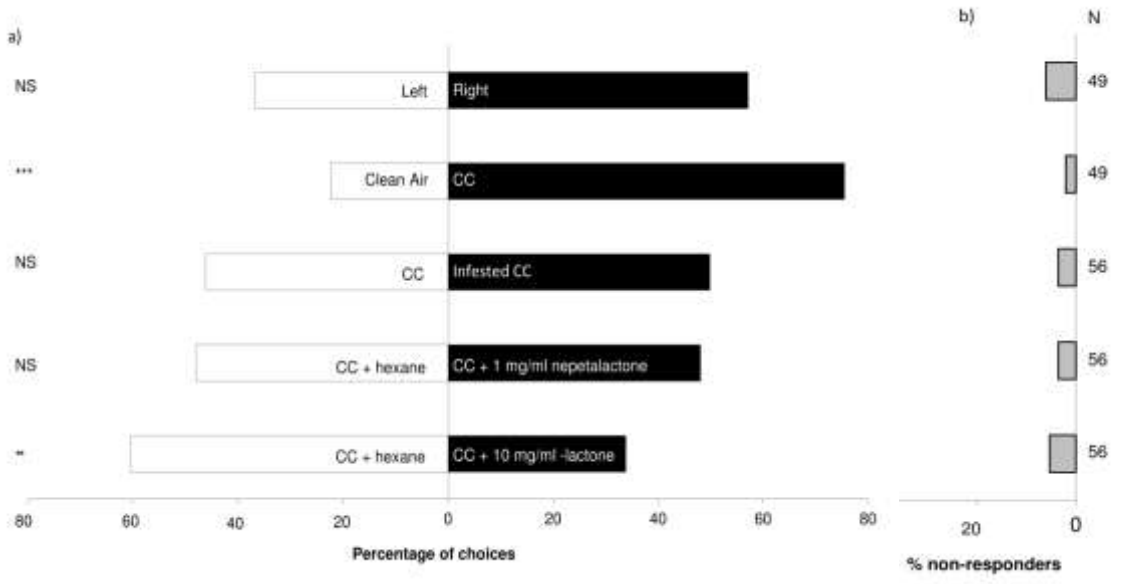


Figure 2.1 Preference of Myzus persicae virginoparae

Aphid preference was determined using a linear wire Y-tube olfactometer. *a)* Volatiles used in the series of dual-choice assays where CC is Chinese cabbage. ** P < 0.01,*** P < 0.001, NS = Not significant. *b)* Percentage of aphids that failed to choose an arm within the 5 minute period or dropped from the internal wire more than 3 times during the assay.

Significantly more aphids chose the arm containing Chinese cabbage + hexane than the Chinese cabbage + 10 mg/ml nepetalactone (G_1 = 4.3, P = 0.038). This result confirms that nepetalactone was responsible for any deterrent effect, as all other conditions of the arms remained equal. If the avoidance of the nepetalactone at a high concentration was to avoid aphid populations, the response would be expected to reflect the response of the aphid when given a choice between an infested and non-infested plant, which it did not. *M. persicae* virginoparae showed equal levels of attraction to the infested and non-infested plant (G_1 = 0.07, P = 0.785). It was noted that the concentration of nepetalactone was a factor in the deterrent behaviour observed and with 1 mg/ml alongside the plant, it remains equally as attractive as the control plant (G_1 = 0, P = 1). The low number of non-responders across all treatment groups suggests that none were repellent to the aphids (Figure 2.1b), as non-attractive odour choices in a Y-tube tend to be reflected in poor rates of overall response (Wang *et al.*, 2010).

2.3.2 Aphid performance

The long term study of nepetalactone on aphid MRGR did not distinguish any growth rate differences between virginoparae subjected to nepetalactone and those exposed only to the hexane control ($F_{1,3}$ = 0.001, P = 0.976) (Figure 2.2). Physiological effects that were not immediate may have been observed as a difference across generations, but MRGR did not change throughout the generations exposed to nepetalactone ($F_{3,9}$ = 0.56, P = 0.654). No difference in growth rate was seen as a result of the interaction between treatment and generation ($F_{3,9}$ < 0.001, P = 0.728)

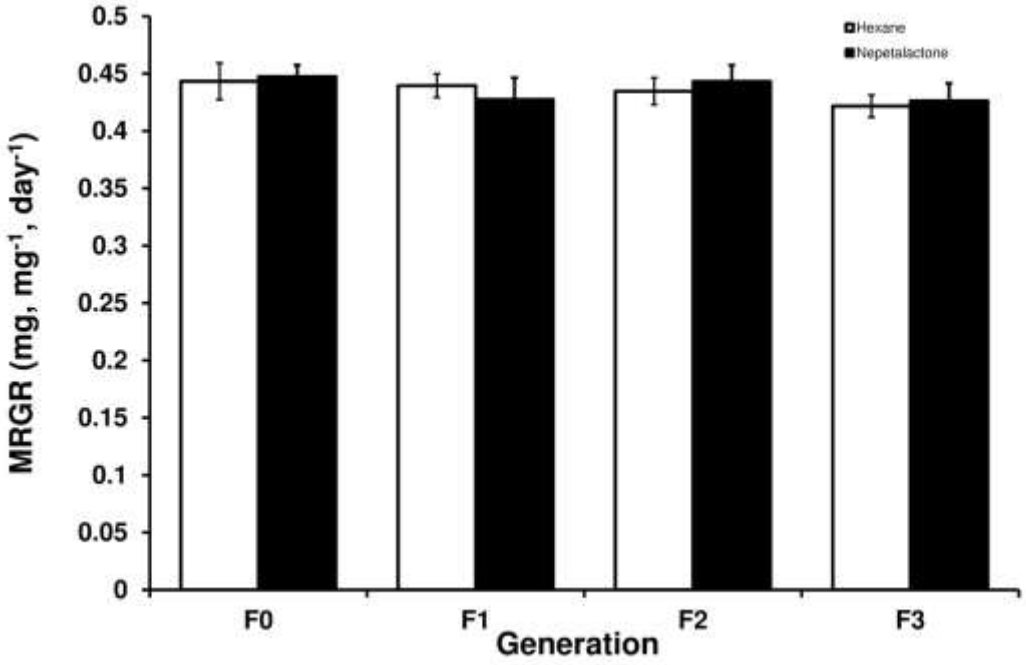


Figure 2.2 MRGR of M. persicae virginoparae.

Treatments reflect environments containing either nepetalactone or hexane. No significant difference ($F_{1,3}$ = 0.001, P = 0.976) was seen between nepetalactone and hexane treatments in any of the generations, between any of the generations ($F_{3,9}$ = 0.56, P = 0.654), or as a result of their interaction ($F_{3,9}$ < 0.001, P = 0.728).

2.3.3 Population growth

Nepetalactone did not affect the natural rate of increase for *M. persicae* virginoparae, although there was a strong trend representing a higher r_m in the control group ($F_{1,7}$ = 5.42, P = 0.059) (Figure 2.3). The mean natural rate of increase for *M. persicae* virginoparae exposed to nepetalactone was 1.229 compared to a mean of 1.309 in the hexane control group, which relates to a mean production of 5.34 and 5.93 offspring per aphid per day, respectively.

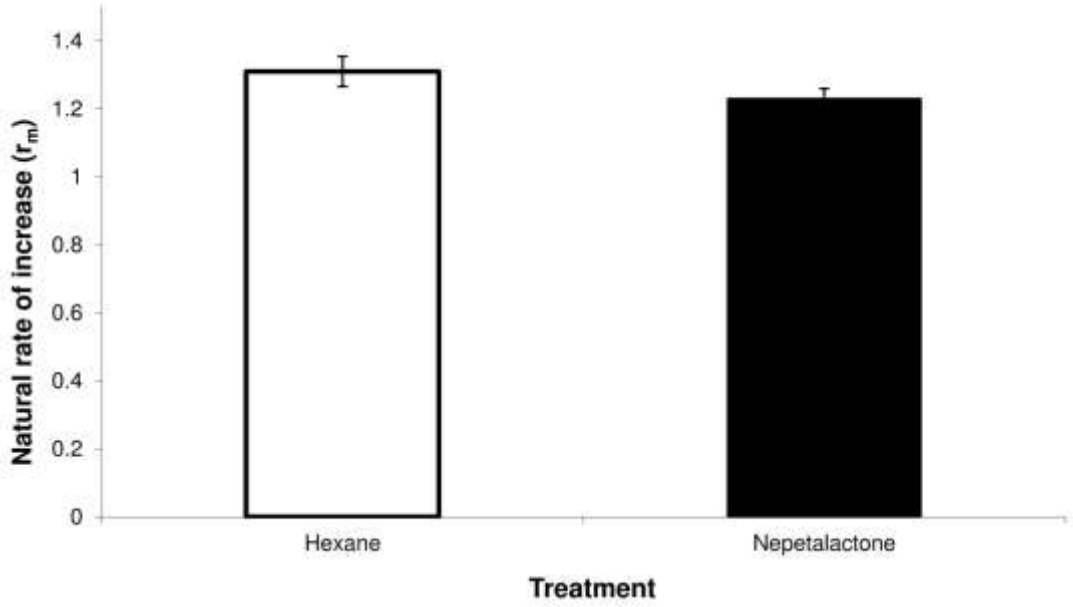


Figure 2.3 Natural rate of increase of Myzus persicae virginoparae.

M. persicae were maintained on Chinese cabbage when exposed to a treatment of either nepetalactone or hexane. Nepetalactone was applied as 200 µl of 10 mg/ml dissolved in hexane; hexane alone was used as the control. No difference was found between treatments ($F_{1, 7} = 5.42$, $P = 0.059$).

2.3.4 Electrophysiological response

M. persicae virginoparae showed a greater EAG response to (4a*S*,7*S*,7a*R*)-nepetalactone and (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol at concentrations of 1 mg/ml (d.f. 4, $P < 0.05$) and 10 mg/ml (d.f. 4, $P < 0.05$) compared to the hexane control (Figure 2.4).

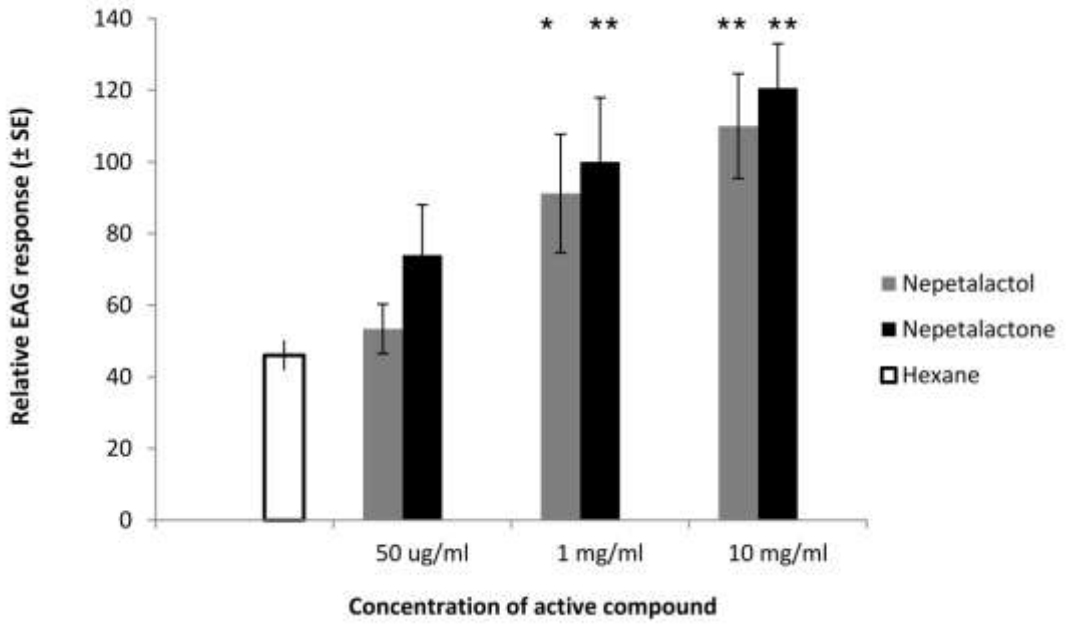


Figure 2.4 Electrophysiological responses of Myzus persicae virginoparae.

Electoanntenograph recordings are shown for (1*R*,4a*S*,7*S*,7a*R*)–nepetalactol and (4a*S*,7*S*,7a*R*)-nepetalactone at 3 concentrations. *P < 0.05. Responses are expressed as a percentage where (*E*)-β-farnesene at 1 mg/ml = 100% (measurements taken at beginning and end of each trial).

2.4 Discussion

Y-tube olfactometry demonstrated no turning bias, which has been shown to affect insect choice in other species (for individual specific turning bias in ladybirds: Girling *et al.*, 2007, for general turning bias in waterbugs: Kight *et al.*, 2008). The aphids' avoidance of nepetalactone in high concentrations could be an avoidance of competition. The virginoparae may avoid high doses of nepetalactone, utilising it as an indicator of the presence of a large grouping of aphids which would offer competition for resources. The competition for the plants as a resource was relatively weak as apterae were unlikely to leave the plant, and competition remains similar throughout the plant. Furthermore, it is unlikely for virginoparae that the nepetalactone would be a reliable cue, due to the scarcity with which they would encounter it. For an aphid avoiding a population of conspecifics, the stress volatiles produced by the plant, induced by aphid feeding, would be reliable and readily detectable cues (in the sense defined by Vet & Dicke, 1992); however, the aphids showed no such avoidance behaviour when presented with the odours of a stressed plant. The increased movement of aphids caused by their avoidance of nepetalactone could reduce the time available for feeding as they try to move away from the odour source. The reduced feeding time would result in a decrease in available energy, which

would in turn be reflected in reduced growth rate or fecundity. The fact that aphid performance was not altered may be indicative that plant odour alone was preferable, and not that the nepetalactone was deterrent. It is interesting to note that the environment containing nepetalactone was not sufficiently unfavourable to the virginoparae to cause an increase in the production of alatae, as is often observed in less favourable environments (Mcvean & Dixon, 2001, Sutherland & Mittler, 1971). This may be an encouraging result for biocontrol programmes, because an increase in alatae could accelerate the spread of the population and also improve their ability to escape natural enemies within the area, though this would trade-off with a decreased individual fitness (Peters & Barbosa, 1977). The r_m of *M. persicae* was not significantly different between treatments of hexane and nepetalactone ($P = 0.059$) though it was recognized that if sample error had been reduced or the sample size increased marginally ($N = 4$) it may result in a lower rate of growth for nepetalactone. If a difference was observed it would represent an r_m of 0.08 greater in the hexane group over the nepetalactone treatment. The difference initially appears small though, from the means provided, it would represent 3.5 more offspring per aphid for the six days after becoming a fully developed adult; scaled-up to account for the number of reproductive females within an aphid population this could represent a substantial difference in real terms.

At concentrations of 1 mg/ml or above, both the nepetalactol and nepetalactone elicited a significant electrophysiological response from the antennae of virginoparous *M. persicae*. To the authors' knowledge, this is the first time that a response of *M. persicae* virginoparae to the aphid sex pheromone has been demonstrated. Studies have demonstrated that male *M. persicae* are attracted to the sex pheromone components (Geng *et al.*, 1997), as are males of other aphid species (Boo *et al.*, 2000). Studies involving field trapping suggest that they use it to locate sexual females (Hardie *et al.*, 1992). Work by Campbell et al. (2003) using single cell recording confirmed that gynoparae of the damson-hop aphid, *Phorodon humuli*, responded electrophysiologically to nepetalactone, as do the *P. humuli* spring migrants and the alatae virginoparae of *Sitobion avenae* (Woodcock, unpublished data, 1992-1994). Why they would detect the odour is not fully understood, but it is suggested that it may be used to discriminate plants already colonized by sexual females of other species. It is possible that the gynoparae have a greater selection pressure to avoid colonies of sexual females, as it is their offspring that will compete to attract mates. However, for the virginoparae, this is not going to have an immediate effect on their offspring and, in non-temperate regions (where they will remain perennially in an asexual cycle), will never affect them. Both viginoparous and gynoparous females (alatae and apterae) of the black bean aphid, *Aphis fabae*, have demonstrated an electrophysiological response to nepetalactone and nepetalactol, with a greater

response seen in the males (Hardie *et al.*, 1994d). An explanation may be that the response elicited is, in fact, an evolutionary relic. The morphology of the antennae of male and female aphids in various species show many similarities (Hardie *et al.*, 1994d) and it is possible that the associated cost of the sensory cells has not been great enough for other evolutionary pressures to 'weed-out' the ability in parthenogenetic clones. If the ability to detect sex pheromone components holds no additional benefit for asexual females, it is hypothesized that the response would be weaker than in male *M. persicae* (although this would be relative to the time over which the asexual forms evolved) and that the strength of response would diminish over evolutionary time. A comparison between the females within more recently formed species and those closer to the ancestral forms may offer some support for this theory.

Another hypothesis follows the evolution of nepetalactone as a sex pheromone. It is likely that pathenogenesis predates the radiation of extant aphid species. This is evidenced by the prevalence of an asexual cycle observed in all aphid species (Moran, 1992). Current research also suggests that the same sex pheromone components are used by many pest aphids, with the exception so far of *Phorodon humuli* (Campbell *et al.*, 1990) and possibly *Dysaphis plantaginea* (Dewhirst *et al.*, 2008), and it is primarily the ratio of compounds distinguishes the pheromone blends for different species (Stewart-Jones *et al.*, 2007). It may be that the response to nepetalactone has an additional role to its function in mating. By detecting the compound, aphids may have controlled spatial distribution, avoiding dense populations. Conversely, males, after leaving a population, would benefit from encountering one of these large populations as there would be more potential mates present. Therefore, while females may detect the pheromone, it would be expected to be at a lower sensitivity: sensitive enough to avoid competition with a large population and the increased predation it attracts, but not so sensitive that they disperse too distantly and lose the benefits of attracting males searching for a mate. Following this rationale, virginoparae may exhibit greater levels of avoidance as they reap less reward from encountering males in the environment, but have the same disadvantages of competition and predation in a large group.

It is known that various natural enemies are capable of detecting the aphid sex pheromone, for example lacewings (Boo *et al.*, 1998), ladybirds (Leroy *et al.*, 2011) and parasitoids (Glinwood *et al.*, 1999a, Powell *et al.*, 1998). The avoidance of a large population is likely to reduce the susceptibility to attack from these natural predators. A "selfish herd" (Hamilton, 1971) may exist when the predator has an increased handling time, but with the high fecundity and rate of attack in parasitoids, greater distribution will benefit the hosts. A dense population is particularly susceptible to parasitoid attack because those remaining are in immediate danger from the

emergence of the next generation of parasitoids amongst them. It is hypothesised that the detection of the pheromone has adapted to a greater extent than has the volume of pheromone produced by the aphid. A greater limitation is observed on the output of sex pheromone; increasing the quantity will advertise more readily to natural enemies, though with limited benefit to the signaller as passive movement of the odour signal reduces its reliability.

The high concentration of nepetalactone used in the trials may have confused the aphids' sensory detectors by producing a different level of odour quality (Baker *et al.*, 1988), causing the level of deterrence observed. If high concentration like this were introduced in the field it may result in a reduced ability of the aphids to effectively utilise other odours in the environment, reducing their ability to find host plants and respond to other pheromones produced by conspecifics. In sexual populations, the presence of high levels of pheromone may hinder the ability of males to locate calling females, leading to a reduction in the population, a method of control which has already seen success primarily for moth species (for review see Carde & Minks, 1995). With new technologies increasing the efficacy of mating disruption (Nansen *et al.*, 2007) pest sex pheromones may see greater application in the field. With the current understanding of *M. persicae*, it was not possible to conclude whether virginoparae actively try to avoid other aphid populations in the natural environment.

If nepetalactone lures are to be introduced into the field it is likely that there would be no immediate effect on behaviour or growth of virginoparae *M. persicae* populations. A very high concentration may influence aspects of the aphids' performance, though it is unlikely that such levels of the compounds would ever be applied. It remains unclear as to why *M. persicae* virgonparae were capable of detecting the aphid sex pheromone compounds, though an understanding of why could provide insight into the evolutionary history of aphids.

Our research demonstrates the need for future studies to broaden their focus and consider the impact of pheromones in a multitrophic context. The results are promising for the application of aphid sex pheromone technologies in agricultural practices, though it is recognised that experiments need to be trialled out of the laboratory to better reflect real world situations.

3 Detection and response of Aphidius colemani to the aphid sex pheromone nepetalactone in a laboratory environment

3.1 Introduction

The stages of host selection in parasitoid foraging are most clearly described by Vinson (1976) and therefore this framework will be applied throughout this study. Vinson describes the complete process of parasitism as consisting of five stages in each of which chemicals play a role: host habitat location, host location, host acceptance, host suitability and host regulation. This chapter focus on the first three stages of the process; host habitat location, host location, in which odour perception is critical, and host acceptance in which the role of ambient odours is unclear. The location of the host habitat is normally achieved through the reception of volatile odours produced by the plant, which are found in to be altered following herbivore feeding (De Moraes *et al.*, 1998, Liu *et al.*, 2009). Location of the host itself often relies on more conspicuous odours such as frass, exuviae or pheromones used in conspecific communication, such as the aphid sex pheromone. It has previously been hypothesised that the aphid sex pheromone component nepetalactone does not actively attract the parasitoids to an area but causes arrestant behaviour once the area is entered (Glinwood *et al.*, 1998). This may explain responses previously observed in field trials (Glinwood *et al.*, 1998) and how the presence of nepetalactone increases parasitism rates; it is likely that if a parasitoid is to spend a longer time within an area it will also encounter, and consequently parasitise, more hosts. Enhancing the efficacy of biocontrol through nepetalactone may be achieved, not through the specific attraction of parasitoids or an increase in their rate of attack, but through a greater retention within a specific area.

Aphidius colemani is a generalist parasitoid able to successfully develop in dozens of different species of aphid (Messing & Rabasse, 1995). The species is found across tropical regions but is believed to have originated in northern India or Pakistan (Starý, 1975). There is evidence that parasitoids of many aphidiine species are capable of detecting the aphid sex pheromone (see Ch. 1.7) and that introduction of sex pheromone components can lead to increased rates of parasitism (Glinwood *et al.*, 1998, Hardie *et al.*, 1994a, Powell *et al.*, 2004). To the author's knowledge there is only one published study concerning the response of *Aphidius colemani* to the aphid sex pheromone (Ameixa & KindImann, 2011); despite it being a commonly used and economically important biological control agent. Ameixa and Kindlemann (2011)

showed that in a laboratory environment females will choose a *Nepeta cataria* essential oil solution more frequently than they will (4aS,7S,7aR)- nepetalactone, E-β-farnesene or a hexane control. This demonstrates that extract from the catnip oil elicits a response in the parasitoids and may therefore be utilised to manipulate their behaviour. There are few studies of any aphidiine parasitoids that demonstrate how their behaviour is altered to achieve the higher rate of parasitism seen in previous field trials (Glinwood *et al.*, 1998, Powell *et al.*, 2004). *A. colemani* is employed in glasshouses throughout the UK, primarily to control populations of *Aphis gossypii* and *Myzus persicae*. Therefore the study of this species will provide information that is not only of scientific and ecological interest, but may help optimise the foraging efficiency of parasitoids in biological control systems.

In this chapter the response of *A. colemani* females to the aphid sex pheromone was studied through electrophysiology, single attack and retention time assays. Parasitoids used in this chapter have no prior experience with the aphid sex pheromone, and in this regard they are described as naïve. The ability of the parasitoid to detect the pheromone on an electrophysiological level is determined using electroantennography (EAG). EAG gives an indication of the parasitoid's capability in detecting the pheromone but does not provide any information as to how it will respond upon detection. To understand the role that nepetalactone may play in the foraging process, the effect it has on attraction and retention of the wasp is assessed. It is recognised that in a natural environment nepetalactone does not occur in isolation, but is normally found in association with other host and plant volatiles. These additional odours may be necessary to stimulate responses from the wasps, or capable of enhancing the response. Synergies that may exist with plant or host related odours are investigated through a study of the wasps' retention time. In addition to the host habitat location and host location stages of foraging (Vinson, 1976) it is also speculated that the parasitoid may utilise the odours, such as the sex pheromone, during the host acceptance stage. To evaluate difference in host acceptance, the speed at which a host is accepted and the rate at which it lays eggs in viable hosts is assessed using a single-attack assay.

3.2 Materials and methods

3.2.1 Culturing

Aphidius colemani used in the electronantennography were laboratory-reared on asexual populations of *Myzus persicae* maintained on Chinese cabbage (see Ch. 2.2.1 for details of rearing). Parasitoid and aphid cultures were maintained in separate controlled environments each regulated at 20 ± 1°C with a L:D 16:8 photoperiod.

Aphids used in parasitoid rearing were exposed to *A. colemani* for a 24 h period whilst on Chinese cabbage. Mummies that had formed were then removed from the plant using soft forceps (Bioquip, Warrington, UK) 10 days following exposure.
The *A. colemani* used in all other assays were provided by Koppert (Koppert B.V., Netherlands) from a different host/plant rearing background. The use of parasitoids from a different rearing background for assays provides a more accurate reflection of the biological control system whereby the parasitoid is rarely reared on the intended host but is still likely to respond to the volatiles broadly associated with their hosts' environments e.g. plant stress volatiles. Electrophysiology work with *A. colemani* reared on *Myzus persicae* were comparable because there was no host contact, or cues associated specifically with the host used in the assays; the *M. persicae* used were entirely asexual so none of the parasitoids used have made contact with the sex pheromone and were thus naïve. All parasitoids used in experiments were 2-4 day old females that had been allowed to mate and were satiated with 20% honey solution.

3.2.2 Electrophysiology

Electroantennography (EAG) recordings were taken from adult female *Aphidius colemani* to determine the insect's electrophysiological response to aphid sex pheromones components. Parasitoids were kept in a 1.5 ml microtube (Eppendorf, UK) which were placed in ice following the methods used in Bruce *et al.* (2008). Following the chilling, the wasps had their heads excised and the tips of the antennae removed. The base of the head was placed in an Ag-AgCL glass electrode filled with saline solution (as used by Wadhams *et al.*, 1982). The tip of the electrode was broken to allow a tight fit when the base of the head was placed inside. The circuit was completed by connection of the antennae to the recording electrode, leaving most of the antennae open and therefore available for exposure to the prepared treatments. The odours were introduced via a continuous purified airstream which passed over the antennae (1 litre/min). The compounds were delivered using a Pasteur pipette cartridge, as previously described by Wadhams et al. (1982). Hexane (99%, Fluka Analytical, UK), which was the solvent for all the other compounds, was used a negative control and the plant volatile linalool was used as a positive control as it has been shown to be attractive to the closely related species *Aphidius ervi* (Du *et al.*, 1998). It was hypothesised that the linalool will elicit a response weaker than the sex pheromone components because sex pheromones provide a signal of greater reliability for the parasitoid (Vet & Dicke, 1992) and it has been demonstrated in the aphid parasitoid *Lysiphlebia japonica* that aphid sex pheromones elicited a relatively strong electrophysiological response compared to plant volatiles (Hou & Yan, 1995). The linalool was dissolved in hexane to a concentration of 1 mg/ml, 10 µl of which was used. The two components of the aphid sex pheromone, (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol

and (4a*S*,7*S*,7a*R*)-nepetalactone, were tested at concentrations of 50 µl/ml, 1 mg/ml and 10 mg/ml. The response of the antennae progressively diminishes following the removal of the head; to account for the disparity in recordings, the response taken to hexane at the start and end of each repeat were used to calculate the degeneration of the response and thus 'standardise' the series, as done by Park *et al.* (2001). The control reading taken at the end can be calculated as a percentage of the initial reading, providing the total rate of signal deterioration. Using this total rate it was possible to estimate the loss over the period as a constant and apply this percentage to the readings obtained. Each individual was tested within a 25 minute period to ensure that the antennae were still responsive over the complete treatment set. The antennae were not exposed to the same odour consecutively to avoid habituation and they were also given odours in increasing concentrations to avoid overwhelming the antennae with a strong initial stimulus. The compounds were introduced in the following order linalool 1 mg/ml, hexane, -lactol 50 µg/ml, -lactone µg/ml,-lactol 1 mg/ml, -lactone 1 mg/ml, -lactol 10 mg/ml, -lactone 10 mg/ml, linalool 1 mg/ml and hexane. Difference in the antennal responses were analysed with a Mann-Whitney rank sum test using Minitab software (Minitab 16, 2010). 5 individuals were tested, each representing an independent replicate.

3.2.3 Square assay

A square olfactometer was employed to assess the effect of odour on the retention and attraction of the parasitoid. A 24 cm x 24 cm glass arena was used with divisions into 16 squares (as used by Ameixa & Kindlmann, 2011) (Figure 3.1). Divisions were drawn onto the base of the olfactometer and offered no physical boundary for the parasitoids. The arena was lit from below by two fluorescent lights with a prismatic filter providing 1.7 kilolux of light, to diffuse the source further a sheet of tracing paper covered the arena base. Treatments tested in the assay were placed in the corner squares of the arena. A glass lid covered the arena preventing the escape of the parasitoids and allowing air to be drawn out the centre at 200 ml/min. Compounds were dissolved in a solution of 1 mg/ml and applied in doses of 2 µl to a 2 cm x 1 cm piece of filter paper for use in the assay. Corner treatments were 6-methyl-5-hepten-2-one, nepetalactone, 6-methyl-5-hepten-2-one with nepetalactone (a 1:1 ratio) and no treatment. 6-methyl-5-hepten-2-one was selected because it is a common host induced plant volatile (HIPV) and the closely related parasitoid species *Aphidius ervi* has been shown to respond innately to this odour in a positive manner (Poppy *et al.*, 1997). Using a soft brush, a female *A. colemani* was placed in the centre and given 10 minutes to move freely within the arena; the time spent in each square within the arena was recorded. After every 3 repeats, the surrounding equipment was rotated to avoid any bias in light or direction. The positioning of the treatments was changed after

every 9 repeats. All parasitoids were mated females aged 2-4 days old. Time spent in each square and each quadrant of the assay was determined using a 1—sample T-test comparing it to an expected value of 0.0625 or 0.25 respectively, representing the time that would be spent in these areas if movement was random. A total of 73 replicates were completed, with each representing an individual female.

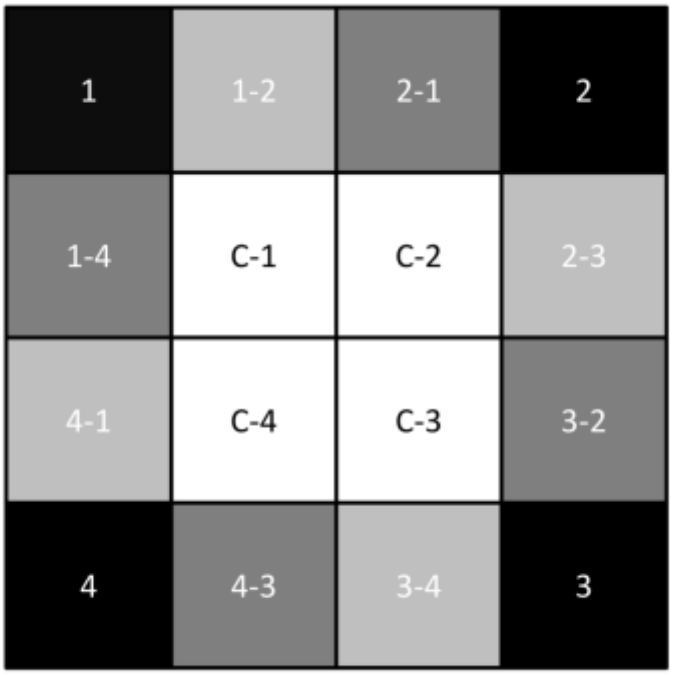


Figure 3.1 Layout of the square assay arena

Base of the olfactometer is shown modelled after Ameixa and Kindlmann (2011). 1,2,3 and 4 represent treatment squares, numbers separated by hyphens represent transition squares between these and C followed by a hyphen denotes centre squares.

3.2.4 On-leaf retention time

In a natural environment specific chemical compounds are not found in isolation and normally form only a part of the total blend an insect will detect at a given time. It is likely that the response of the parasitoid will depend on a combination of these odours. The on-leaf retention time assay allows an evaluation of potential synergies between plant volatiles, aphid associated odours and nepetalactone.

Leaves were excised from three-week-old Chinese cabbages. Each leaf was placed in a Blackman box measuring 7.5 x 4.4 x 2.2 cm which was rested in a shallow basin of water, allowing the leaf moisture (for full description see Blackman, 1971).
Treatments in the first set of the retention time assay were: an empty arena (which functioned as a negative control), a leaf, a leaf alongside nepetalactone, a previously infested leaf and a previously infested leaf alongside nepetalactone. The second set

used treatments of: an empty arena, a leaf alongside a high concentration of nepetalactone, a systemically damaged leaf alongside nepetalactone, aphid related cues alongside nepetalactone and a previously infested leaf alongside nepetalactone. Treatments using nepetalactone had it introduced via a 10 µl microcapillary (Drummond Scientific Co., USA) affixed to the side of the Blackman box. The standard concentration of nepetalactone used was 1 mg/ml and the high concentration was 10 mg/ml. Those described as previously infested had 10 aphids (3rd instar to adult) placed on the leaf using a size 0 paintbrush and allowed to feed. After 16 h, when the experiment was to be conducted, the aphids were gently removed from the leaves but any exuviae was allowed to remain. The treatment involving aphid related cues was achieved by allowing the aphids to feed on the leaf for 3 days, after which time they were removed (though honeydew and exuviae remained) and the leaf was allowed 24 h with no aphid attack to allow volatile production to diminish (Du *et al.*, 1998, Guerrieri *et al.*, 1997). The systemically damaged plants were created by allowing 20 aphids, contained within a clip cage, to feed on a leaf on the plant for 72h. A separate leaf from the same plant was then used in the assay.

To commence the assay a naïve female *Aphidius colemani* was placed on the centre of the leaf surface, or empty arena, and its behaviour observed. In set 1 the parasitoid was allowed 15 minutes before the assay was completed, in set 2 each parasitoid was allowed 20 minutes. The difference in time was used to assist in differentiating the extent of retention in already favourable conditions. The assay was stopped if the parasitoid walked or flew out of the Blackman box. The time of leaving the leaf or, when no leaf was present, the central area was also recorded.

Data were analysed from both sets of experiments using one-way ANOVAs with posthoc Tukey tests on Minitab 16 statistical software (© 2010 Minitab Inc). Thirty five replicates were completed for each treatment, with each individual parasitoid and leaf representing an independent replicate.

3.2.5 Single-attack assay

The likelihood of *A. colemani* successfully ovipositing in a host may be related to the reliability of the odour signals in the environment. It has been noted in previous experiments that *A. colemani* make ovipositional attempts at many non-hosts targets including leaf material or aphid exuviae (personal observation). Non-host attacks have been noted in other parasitoid species and are often stimulated by visual and odour cues (Battaglia *et al.*, 1993). Since parasitoids do not inject an egg following every ovipositional 'stab', or attack, an evolutionary strategy may favour the avoidance of egg-laying in non-viable targets, even if the ovipositor has made contact with the

target. Mediation of ovipositional behaviour may be reliant on the odour cues received by the wasp during insertion of the ovipositor and matching the stimuli received to recent host encounters.

Aphid sex pheromone components present in the *Nepeta cataria* essential oil (Bristol Botanics, UK), 19% (4*aS*,7*S*,7*aR*)-nepetalactone, were used to test if a strong stimulus will increase the rate of successful egg deposition in potential *M. persicae* hosts. *Nepeta cataria* essential oil was used in these trials as opposed to the isolated nepetalactone compound; this was partly a result of an error at when the study commenced, however, previous work has shown that *Nepeta cataria* essential oil is attractive to *Aphidius colemani* (Ameixa & Kindlmann, 2011) and may alter behaviour in a similar way to the sex pheromone. It was hypothesised that experience may be effective in increasing the rate of egg deposition by the wasp and speed at which it attacks as it attempts to reduce handling time when it approaches a host colony. Experience and *N. cataria* oil treatments were tested against a control baseline of a wasp with no additional stimuli.

Female wasps were removed from cultures and stored in individual 'caps' before the experiment commenced. The caps were constructed by removing the tapered end of a microtube using a sharp scalpel. The tip of the microtube was also removed in the same fashion to produce a plastic funnel 1 cm in height. The narrow end of the cap was sealed using cotton wool soaked in 20% honey solution (Figure 3.2). This allowed the parasitoid to feed while it acclimated to the environment and be satiated for the commencement of the assay, however, it was noted that if the honey soaked bud was present during the assay the wasp would rarely stop feeding to make oviposition attacks, for this reason the honey-soaked bud was replaced by clean cotton wool during the assay.

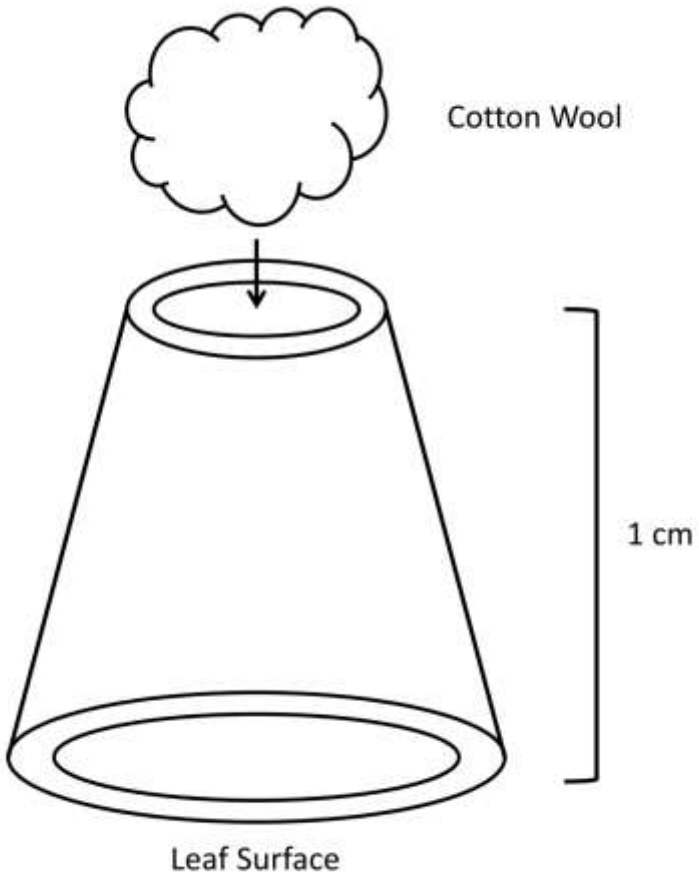


Figure 3.2 Diagram of single-**attack assay 'cap' for parasitoids.**

Parasitoids would be kept in the container cap prior to the assay with 20% honey solution cotton wool which was replaced with clean cotton wool when the assay commenced.

Parasitoids given experience were placed on a 7 cm Petri dish containing a leaf infested with aphids for 30 minutes during which time they could freely attack the aphids present. To commence the assay a small leaf (taken from Chinese cabbage in the 3rd week of growth) with a single adult *M. persicae* was placed in a Blackman box (Blackman, 1971) upon the laboratory bench. A cap containing one female was placed over the aphid and allowed 10 minutes to find and attack the aphid. The assay was ended immediately after an attack was made by removing the parasitoid. The aphid was kept in the Blackman box where it was monitored for 14 days. Offspring produced by the female were counted and removed every second day; this also provided an opportunity for the leaf to be changed if any loss of turgidity was noted. At the end of the 14 days a total offspring count could be attained for each aphid and whether or not mummification had occurred.

3.3 Results

3.3.1 Electroantennography

The antennae of *Aphidius colemani* females were shown to have a significant response to the nepetalactone isomer in concentrations of 1 mg/ml (T_4 = 4.09, P = 0.026) and 10 mg/ml (T_4 = 12.6, P < 0.001) (Figure 3.3) when compared to the hexane control. Nepetalactol was also shown to elicit a greater response in *A. colemani* than the hexane control when presented at a concentration of 10 mg/ml (T_4 = 3.72, P = 0.02). The plant volatile linalool was shown to elicit a significant response at the concentration of 1 mg/ml (T_8 = 2.9, P = 0.02). The response to linalool, unlike the response to sex pheromone components, may not be innate as the parasitoid may potentially have learnt the signal through emergence from the host mummy.

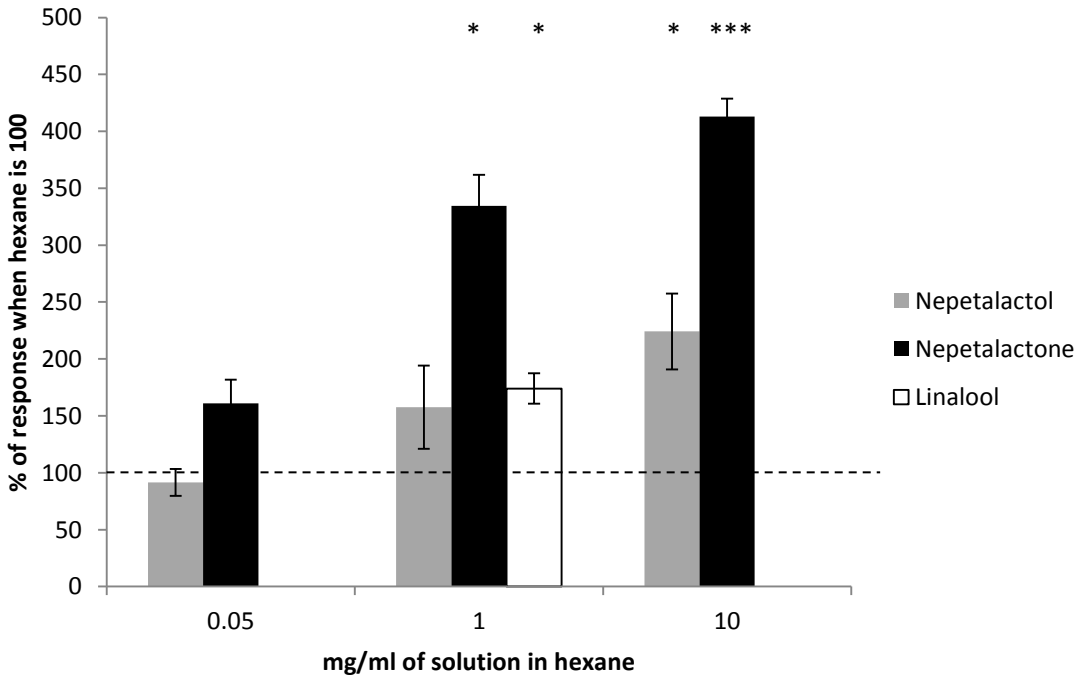


Figure 3.3 Aphidius colemani EAG response.

A. colemani response to aphid sex pheromone components and the plant volatile, linalool, when readings are standardised to initial and final hexane reading to account for signal deterioration across the antennae. The dashed line displays the hexane response. * P < 0.05, *** P < 0.001

3.3.2 Square assay

Parasitoids were found to spend less time in the squares of the arena containing either 6-methyl-5-hepten-2-one (T_{72} = -2.83, P = 0.006) or Nep. + 6-methyl-5-hepten-2-

one (T_{72} = -3.35, P = 0.001) (Figure 3.4). None of the other squares were visited more or less frequently than would be expected if the parasitoid was moving at random. The squares adjacent to the treatment (the 'transition' squares) could be combined to form one treatment quadrant. It would be expected that the parasitoid would spend 25% of its time in any given quadrant if moving at random. No quadrant was visited for more or less time than would be expected (n = 73, P > 0.05) (Figure 3.4). This implies that any deterrent effect that was observed between the squares was fairly limited in effective range. With no additional odour or visual stimuli *A. colemani* was not attracted to, or retained longer in the presence of, either the plant volatile 6-methyl-5-hepten-2-one or the aphid sex pheromone component nepetalactone.

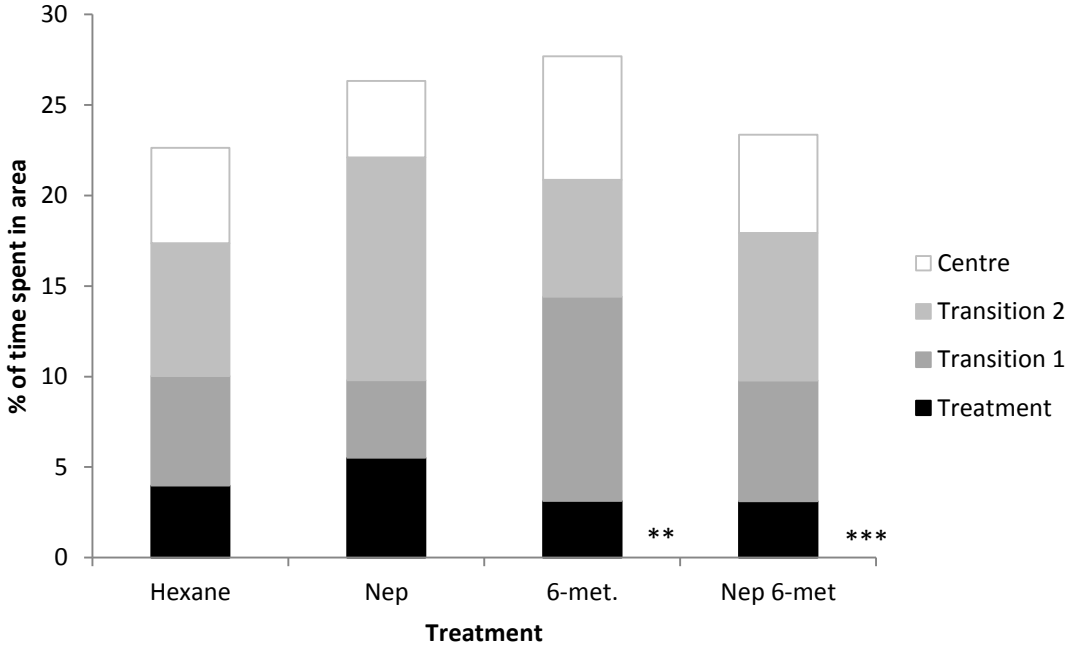


Figure 3.4 Time spent by Aphidius colemani in each area of the square olfactometer.

Treatment squares were those in the corner containing chemical compounds, transition squares were those adjacent to the treatment and the centre square was that diagonally linked to the treatment square (see figure Figure 3.1 for complete layout). Time spent did not vary between quadrants, however, within quadrants less time was spent in treatments squares containing 6-methyl-2-hepten-1-one or nepetalactone + 6-methyl-2-hepten-1-one. ** < 0.01, *** < 0.001.

3.3.3 On-leaf retention time

A one-way ANOVA revealed that differences existed between the retention time of the parasitoid within the arena ($F_{4, 159}$ = 15.55, P < 0.001). A Tukey posthoc test revealed that parasitoids stayed longer in the assay containing a leaf than if no leaf was present (Figure 3.5). No difference was observed between those containing the leaf, leaf and nepetalactone or a previously infested leaf. Parasitoids were retained on the leaf

significantly longer in the treatment containing the infested leaf alongside nepetalactone.

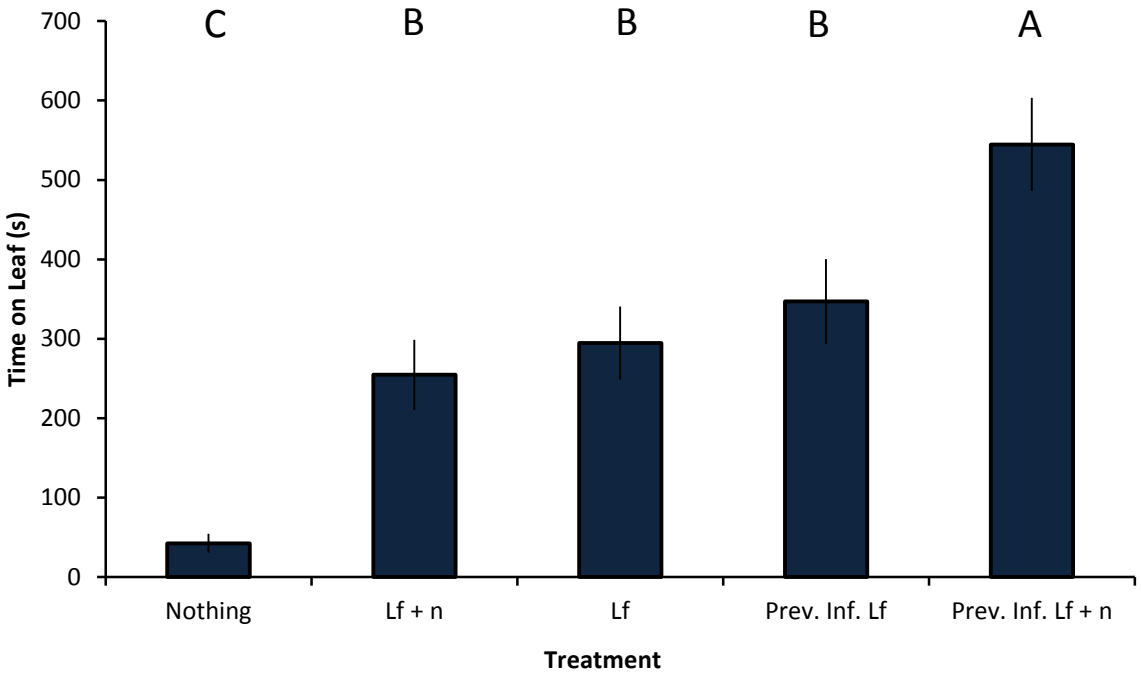


Figure 3.5 On-leaf retention time set 1

Differences in the retention time of the parasitoid within the arena for set 1. Where treatments are Nothing = Empty arena, Lf + n = nepetalactone alongside intact leaf, Lf = leaf, Prev. Inf. Lf = previously infested leaf and Prev. Inf. Lf + n = nepetalactone alongside a previously infested leaf. Those not sharing a letter are significantly different ($F_{4,159}$= 15.55, P < 0.001) as obtained by a one-way ANOVA with a posthoc Tukey test.

The first set of the on-leaf retention time assay revealed nepetalactone and the volatiles associated with aphid feeding may have a synergistic effect on parasitoid retention time. To determine where this synergy exists it was necessary to evaluate the sources of the volatile output alongside the nepetalactone.

In the second set of on-leaf retention time assays it was confirmed, using a one-way ANOVA, that the treatments led to differences in the retention time of the parasitoids ($F_{4,160}$= 21.42, P < 0.001) (Figure 3.6). Using a Tukey posthoc test it was seen that the control of no leaf showed a significantly lower parasitoid retention time than any other treatment containing the leaf (Figure 3.6). The treatment of a high concentration of nepetalactone (10 mg/ml) alongside the leaf resulted in the same retention time of the systemically damaged leaf with nepetalactone (1 mg/ml). The treatments of nepetalactone alongside aphid related cues and nepetalactone alongside a previously infested leaf elicited the greatest retention response in the parasitoids.

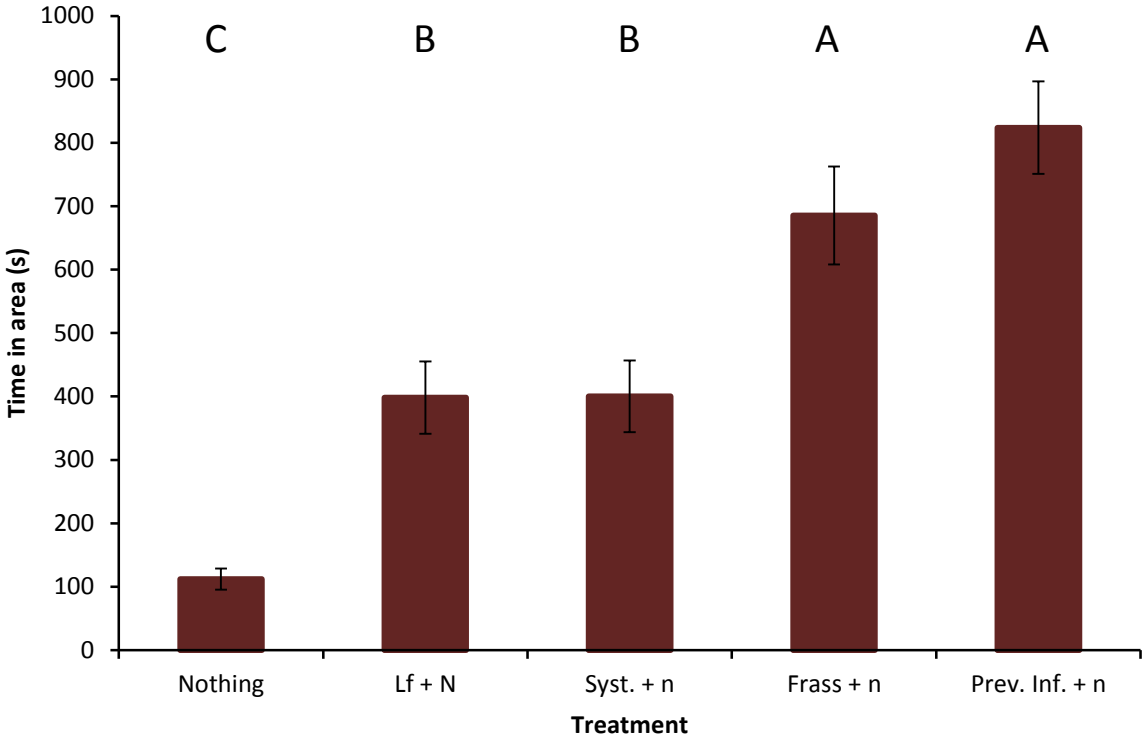


Figure 3.6 On-leaf retention time set 2

Differences in the retention time of the parasitoid within the arena for set two of the on-leaf assays. Where treatments are Nothing = Empty arena, Lf + N = 10 mg/ml nepetalactone alongside intact leaf, Lf + n = 1 mg/ml nepetalactone alongside systemically damaged leaf, Frass + n = 1 mg/ml nepetalctone alongside a leaf containing aphid related cues and Prev. Inf. Lf + n = 1 mg/ml nepetalactone alongside a previously infested leaf. Those not sharing a letter are significantly different ($F_{4,160} = 21.42$, $P < 0.001$) as obtained by a one-way ANOVA with a posthoc Tukey test.

3.3.4 Single-attack assay

Control groups were omitted from analyses of speed of attack and mummy formation for the reason that no attacks were involved. Speed of making the first attack was different between treatments ($F_{2,57} = 17.86$, $P < 0.001$) (Figure 3.7). A post-hoc Tukey test confirmed that each treatment differed significantly from the others, with experienced parasitoids showing the quickest rate of attack (22.25 ± 3s), followed by the *Nepeta* oil treatment (136.4 ± 36s) and naïve parasitoids without any additional stimuli took the longest to attack at 250.56 ± 30s.

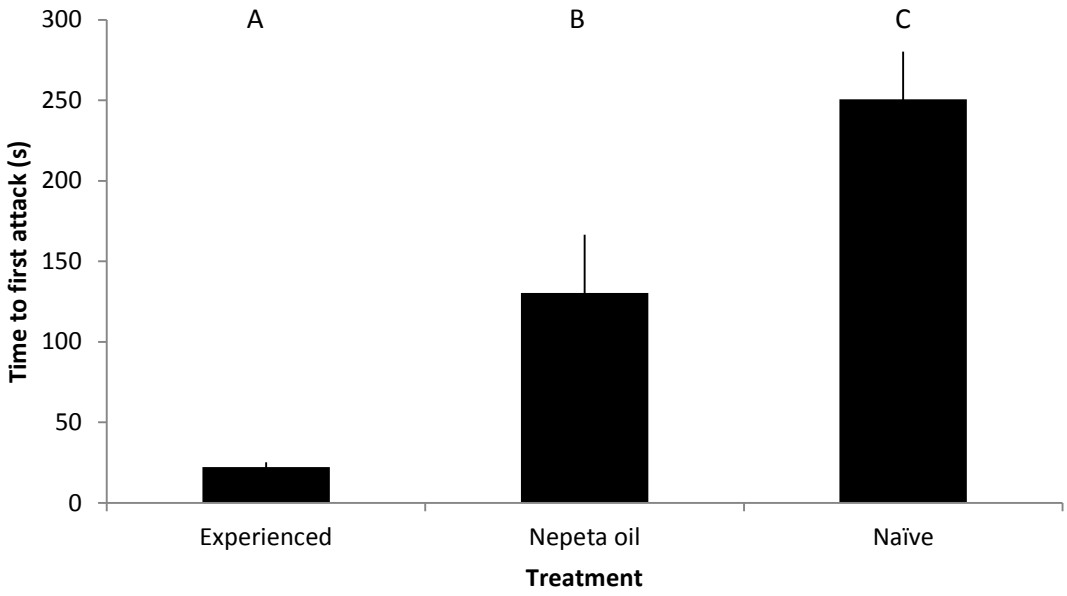


Figure 3.7 Time to first attack

Time of first attack for *A. colemani* females on their host *M. persicae* given treatments of: experience, *Nepeta cataria* essential oil or naïve without additional stimuli. Those not sharing a letter are significantly different ($F_{2,57}$= 17.86, P < 0.001).

The fecundity of the aphids was found to be different between treatments ($F_{3,76}$ = 34.34, P < 0.001). A Tukey post-hoc test shows the control group to have significantly higher aphid fecundity than all other treatments (35 ± 1.34 offspring) followed by naïve parasitoids (26 ± 1.82). Experienced parasitoids (14.9 ± 2.58 offspring) and those exposed to *Nepeta cataria* essential oil (7.65 ± 2.5) were both significantly lower than the other treatments but not from each other.

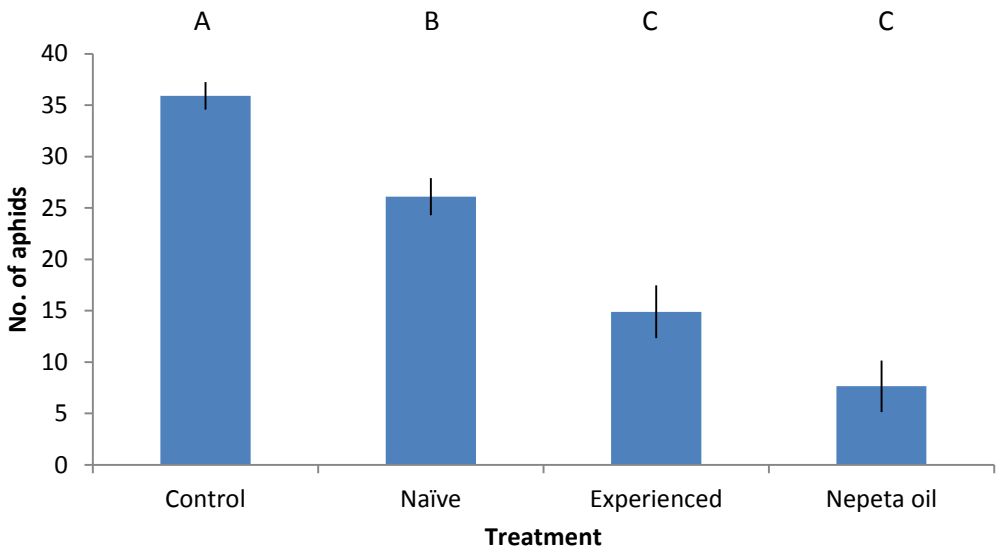


Figure 3.8 Offspring after 14 days

Mean number of *M. persicae* offspring found after 14 days as a result of treatment to the parasitoid, with the exception of the control group which were not exposed. Those not sharing a letter are significantly different ($F_{3,76} = 34.34$, $P < 0.001$).

Development of the parasitoid pupae within the host aphid can reduce the reproductive potential of that individual (Campbell & Mackauer, 1975). It is likely that the aphid count would be strongly negatively correlated to the number of individuals successfully parasitized, although the count of total aphids produced compared to treatment also provides a more ecologically relevant insight to the effect of odour and experience on aphid control. It was found that development of the parasitoid larvae in the host, leading to mummification, did reduce the fecundity of the aphids ($F_{1,78} = 219.15$, $P < 0.001$)(Figure 3.9).

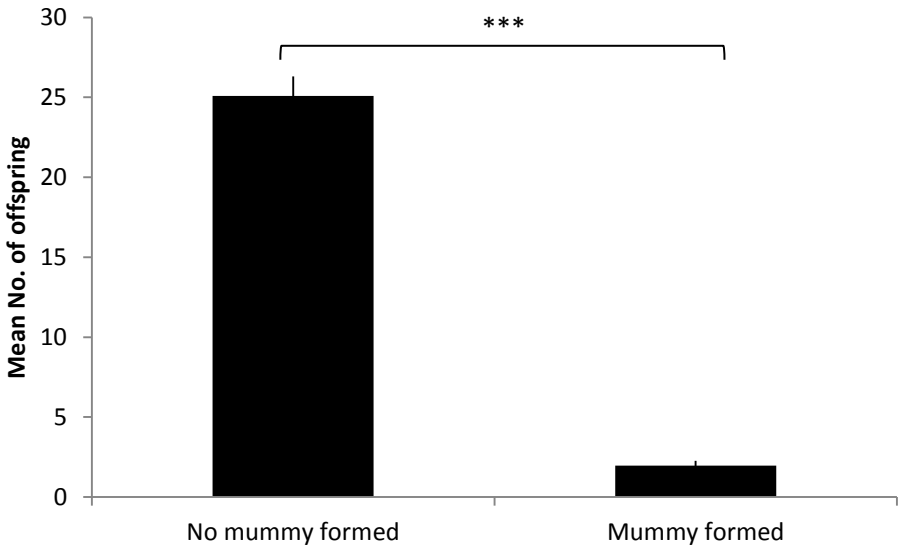


Figure 3.9 Fecundity following parasitism

Effect of successful development of the parasitoid host, as shown by formation of the mummy. *** $P < 0.001$

Although the formation of the larval parasitoid was responsible for a reduction in the aphid fecundity, it was not the only factor. A comparison of the control group and individuals from the treatment groups that had not resulted in mummy formation demonstrate that ovipositional attack alone causes a reduction in aphid fecundity ($F_{1,55} = 31.41$, $P < 0.001$)(Figure 3.10).

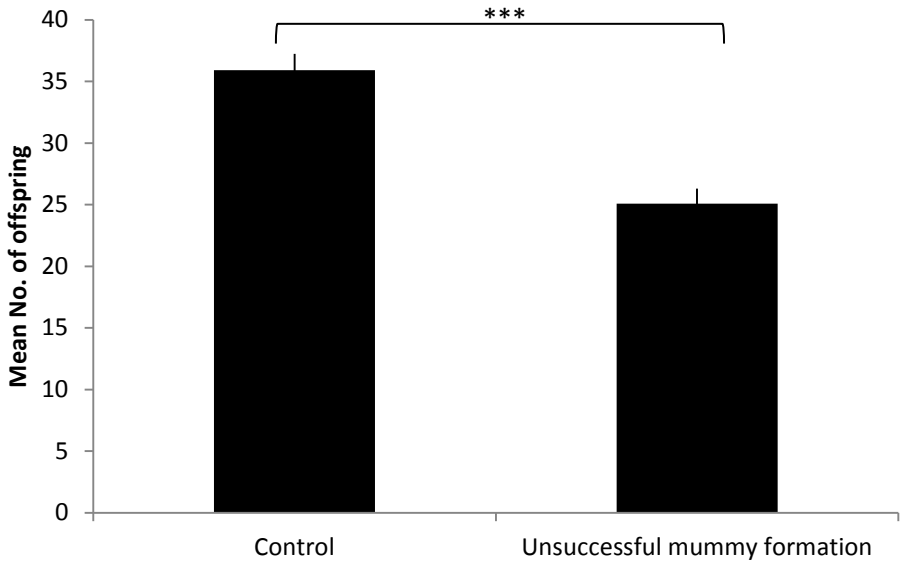


Figure 3.10 Effect of parasitoid attack on fecundity

Number of offspring produced by aphids in the control group compared to those in attacked without successful mummy formation. ***P<0.001

A significant difference in the rate of mummy formation was observed between treatments (χ^2 = 12.87, N = 23, P < 0.01). Parasitoids exposed to *Nepeta cataria* essential oil during oviposition showed the highest rate of successful oviposition and naïve parasitoids had the lowest success rate. No mummies were found to develop in the control group, confirming that no parasitoid contamination occurred in the laboratory.

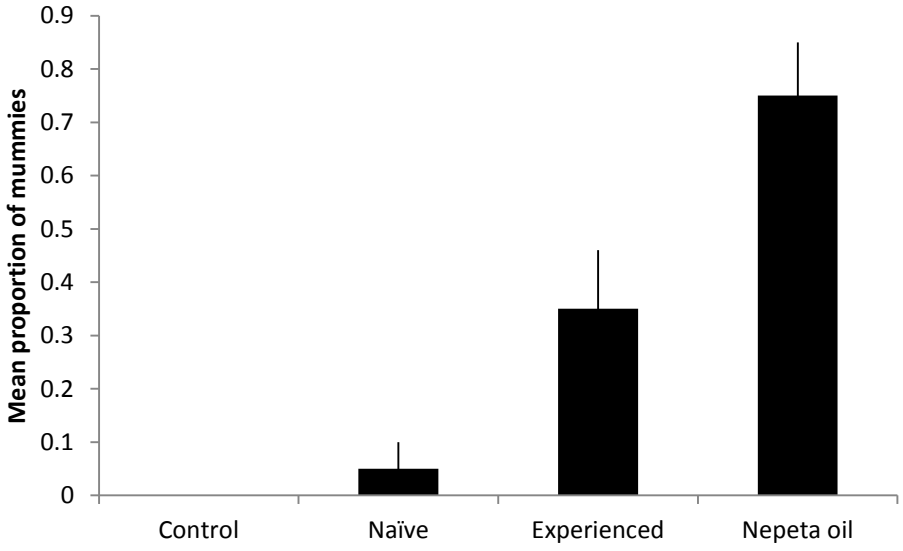


Figure 3.11 Success of attack

Proportion of attacked aphids resulting in the successful formation of a mummy. Control group experienced no attacks and the lack of mummy development demonstrates no contamination took place after the experiment.

3.4 Discussion

It is recognised that the complete process of host selection by a parasitoid (as defined by Vinson's [1976] five stages) utilises chemical cues at every stage. There is strong evidence to suggest that the aphid sex pheromone is one of these cues utilised in the early stages of host selection (Gabryś *et al.*, 1997, Glinwood *et al.*, 1999a, Glinwood *et al.*, 1999b, Glinwood *et al.*, 1998, Hardie *et al.*, 1994a, Hardie *et al.*, 1991, Hardie *et al.*, 1997, Powell *et al.*, 2004), and that the presence of the pheromone may increase parasitism rate (Glinwood *et al.*, 1998, Powell *et al.*, 2004), though the exact response of generalist parasitoids to the odour remains unclear. To clarify the role that the aphid sex pheromone may play in foraging it was first necessary to confirm that the generalist *Aphidius colemani* was capable of detecting the odour.

A.colemani shows an electrophysiological response to the aphid sex pheromone components (1R,4aS,7S,7aR)-nepetalactol and (4aS,7S,7aR)-nepetalactone. At a dose of 50 ug/ml the nepetalactol and nepetalactone show no significant difference to the hexane control. With 10 µl of the solution on filter paper there was an estimated 500 ng of the active compound present, though the exact concentration reaching the antennae was unknown. It has been shown previously that the aphidiine parasitoid *Aphidius ervi* only shows a flight response after a minimum of 40 aphid hosts have been feeding for 72 h on the host plant (Guerrieri *et al.*, 1999). Considering the importance of the plant volatile output to parasitoids' in host location (for overview see Turlings & Benrey, 1998) it is hypothesised that a similar volatile threshold may exist for the parasitoid in detection, or response to, aphid sex pheromone, though with a different quantitative value. The threshold may be lower than that of plant volatile emissions due to the greater 'reliability' of sex pheromone components (Vet & Dicke, 1992) and the importance of finding an overwintering host. The response elicited by nepetalactone on parasitoid behaviour may have selected for the threshold at which it detects the compound; parasitoids with a higher threshold before the odour is detected will only see behavioural changes as they are closer to the host and not waste time searching nearby empty patches where the compounds have diffused into. Linalool is common plant volatile component that has been shown to be released in greater quantities following herbivore damage (Loughrin *et al.*, 1994). Linalool, present at 1 mg/ml gives some context as to relative importance of these volatiles, though it is recognised that ascribing ecological significance to electrophyiological results alone should be done with caution.

A greater response was seen to the nepetalactone at 1 mg/ml than the hexane control confirming that the compound was detected by *Aphidius colemani* antennae beyond

the solvent baseline. When introduced at a concentration of 10 mg/ml the response to the nepetalactol and nepetalactone becomes more notable. It is consistent with our understanding of *Aphidius colemani* that it would respond strongly to nepetalactone as it is known to parasitise dozens of aphid species (Messing & Rabasse, 1995) which have it as a common element (Stewart-Jones *et al.*, 2007). It is understood that it would not be of value for a generalist to be finely tuned to specific sex pheromone ratios but rather respond to the predominate compounds present.

Nepetalactone alone is likely to be effective as a cue since it present in the majority of aphid sex pheromone blends known. A parasitoid that attacks a greater range of aphid hosts may rely less on blend specificity though would maintain- or have an amplified response to- a single common component of the hosts' volatile output. Conversely, more specialist parasitoids may show greater specificity to the particular sex pheromone blend ratio of its host to reduce energy spent foraging for nonviable hosts. The most obvious response of a parasitoid to an odour prominent in host habitat location or host location would be an attraction, movement up the concentration gradient, or an arrestant effect where the wasps retained in an area where it perceives the odour, or a threshold concentration of the odour in the environment. The square assay employed by Amiexa and Kindlmann (2011) was seen as a suitable olfactometer to explore these behaviours. Without other odours present nepetalactone and/or the plant volatile 6-methyl-5-hepten-2-one did not retain the parasitoid for a longer time within an area. Similarly Amiexa & Kindlmann, did not find *A. colemani* to be retained any longer in squares containing nepetalactone, nor did they find them to visit the nepetalactone treated square more frequently than any other. Different treatments used in the Ameixa & Kindlmann work mean that the results of the study were not comparable; particularly the inclusion of *Nepeta cataria* essential oil in their study which did increase the number of visits made, number of first choices made and mean time spent in treated squares. Attraction of various parasitoids to nepetalactone has been reported in several studies (Gabryś *et al.*, 1997, Glinwood *et al.*, 1999a, Glinwood *et al.*, 1999b, Hardie *et al.*, 1991) though absence of such attraction in this study may be due to the size of the assay arena. Over a long distance a cue may elicit an oriented flight, however, in closer proximity a walking search pattern may be deployed. The wasps did spend less time in squares containing nepetalactone and 6-methyl-5-hepten-2-one or 6-methyl-5-hepten-2-one alone which may be related to an altered foraging pattern employed by the parasitoid. The generalist wasp may reach its response threshold to these odours in the olfactometer before entering the square that contained them; any arrestant effect would be more likely to be observed in the transition squares surrounding these treatments, or farther from the source. Behavioural alterations away from the source may include retention

or may be a stimulation of foraging behaviours, either of which would require a separate assay to distinguish.

It may also be the case that when the odour is presented in isolation it is not reliable enough to elicit such a response. The reliability of an odour signal to indicate a viable host may be drastically increased by the presence of associated volatiles, as it is unlikely that in a natural environment any of these cues would appear in isolation. If a female aphid is calling males by producing the sex pheromone, she will be doing so from a plant and therefore it is likely that the same pressures that would select for a response the sex pheromone would also utilise the HIPVs concurrently. It would be anticipated that the synergy between plant volatiles and the sex pheromone would also be observed in the sexual communication of aphids, however, this would be difficult to distinguish as both male and female aphids respond to plant volatiles as a food source (Hardie *et al.*, 1994b). Following the reliability-detectability theory, increasing the specificity of odours involved in eliciting a response will increase the reliability of such an odour, it is presumably for this reason that such a wide range of plant volatile-pheromone synergies are observed (for review see Reddy & Guerrero, 2004). It is, therefore, likely that at the next trophic level the parasitoids utilising such odours as kairomones would still rely on synergy with available plant volatiles.

Introduction of nepetalactone alongside a leaf did not increase the retention time of the parasitoid any more than a leaf alone, but an aphid-damaged leaf with nepetalactone retained the wasps longer than a damaged leaf alone. This demonstrates that a synergistic interaction was occurring with the aphid damaged leaf and the nepetalactone, however, the aspect of the aphid infested leaf involved was not immediately clear. The second set of the on-leaf retention assay clarifies that odours directly associated with the aphid host were most important to the retention of the parasitoid. This is directly in accordance with the reliability-detectibility theory which would predict that the parasitoid may utilise the plant-related volatiles over a greater distance (due to their abundance) but for more thorough searching of a patch would rely on cues more directly related to the host, which more reliably indicate the presence of a viable host. An understanding of this phenomenon also demonstrates that the effect of nepetalactone may be more complex than initially speculated and rely, not solely on the odour of nepetalactone, but the context in which it is available.

Oriented flights towards nepetalactone alongside the host/plant complex, observed in other species such as *Aphidius ervi* (Glinwood *et al.*, 1999a), may be due to a synergy with the VOCs rather than the aphid related cues. It is feasible that parasitoids, and other foragers, may have evolved patterns of foraging that alternate in relation to the reliability of the odours present; while more abundant, though less reliable, odours

might elicit a flight response, as observed with *Cotesia glomerata* (Connor *et al.*, 2007), the more reliable odours, which are more scarcely encountered, may elicit a greater retention within an area or searching within a patch, as seen with the tachinid fly *Exorista japonica* in the presence of host frass (Tanaka *et al.*, 2001). More reliable odours by their nature indicate a greater proximity to the host, less reliable odours may provide information of a suitable environment but not where the host is within that environment. In effect, it may be described that less reliable odours play a more prominent role in the first stage of host selection: host habitat location, whilst more reliable odours are important in the second stage: host location. A complete absence of habitat or host related odours may lead to the notable movement patterns covering large distances, and may explain movement patterns often interpreted as levy flight patterns (Mandelbrot, 1983).

Although the focus of this study is olfactory cues, it is recognised that the reliability of such odour signals may be dependent on additional vibrational or visual information about the environment, of which visual cues may have played a role in the on-leaf retention time assay.

The on-leaf retention assay was able to establish that a synergy existed with nepetalactone and cues relating to aphid feeding. The retention response is likely to enhance foraging during the host habitat location stage, but it is also important to establish the role it may play in host location and host acceptance. The single-attack assay was devised to investigate the importance of nepetalactone at these stages of the foraging process.

When females are observed in the laboratory they frequently make ovipositional attempts at non-host targets. These attacks can be induced by objects that bare a visual (personal observation) or odour resemblance to the host (Battaglia *et al.*, 1993). In addition, many more ovipositional stabs are made when hosts are encountered than will lead to the depositing of an egg. In many parasitoid species ovipositional stabs resulting in the deposit of an egg can be distinguished from those that do not through observation, though this cannot be seen in *A. colemani*. It is assumed that the parasitoid assesses the viability of the target to avoid laying eggs in non-host tissue which would be unable to develop. The principle factor in the host acceptance stage of parasitism is expected to be kairomones that the parasitoid receives upon contact with the host but some of the additional elements in the decision to oviposit may be the cues from the surrounding environment. If the parasitoid recognises aspects of the environment as relating to the host it may be more likely to oviposit an egg because the increased likelihood that the target is a viable host. Experience on the host/plant complex will also produce a similar result as the parasitoid has learnt that the

environment contains the host and any subsequent encounters were more likely to be viable hosts. The rate of host acceptance by *A. colemani* appears to have been enhanced both by the reliability of the environment (presence of pheromone-containing *Nepeta cataria* essential oil) and through experience of the environment. Experience of the plant/host complex increases the speed of attack for *A. colemani* wasps when a host was available. Attacks made following experience with the plant/host complex were also more likely to result in the development of parasitoid larvae. The single-attack assay continues to emulate the environment from which the experienced parasitoids were removed because the wasp was immediately presented with a host and may continue as though foraging within the same patch it left. Therefore, if the parasitoid has already assessed the value of the hosts in the previous encounters it will reduce the time taken to attack (by reducing or eliminating this evaluation process), and increase the eggs deposited in an attempt expend its eggload.

The *N. cataria* oil was also effective in decreasing the time taken to attack when compared to naïve wasps, suggesting that in a close proximity the sex pheromone odour may lead to improved host location, or that it is sufficient in decreasing handling time. It was ostensibly a consequence of the increased success rate that a reduction in the aphid fecundity was observed but it was interesting to see that the unsuccessful parasitism also appears to reduce fecundity of the aphids. The reduced fecundity following unsuccessful attack is indicative of the trade-offs which may exist following parasitoid attack. The production of alarm pheromone by the aphid, the time spent disrupted from feeding or the healing of the oviposition wound may have reduced the energy that the aphid had available for reproduction. The most energetically expensive consequence of parasitoid attack for the aphid is likely to be the deposit of an egg. It would be hypothesised that the cost of mounting an immune response to the developing larvae within the aphid host is what leads to the decrease in fecundity, however, the variation between treatments negates this possibility as it is improbable that ambient odour would affect the aphid immune response, and in such a drastic fashion.

It was proven that parasitoids with no previous experience of the aphid sex pheromone were capable of detection, and that interaction of the odour with associated volatiles is able to alter the parasitoids' behaviour causing increased retention within an area, more rapid attack and greater success in the attack of hosts. These results give insight into how *A. colemani* utilises the odour during host selection but may also demonstrate the potential of application of aphid sex pheromone components in biological control. If parasitoids were only retained by nepetalactone when it was introduced alongside odours relating to aphid damage the pheromone would not draw parasitoids (or retain them) in areas that do not contain the aphid host but would only

enhance the foraging if a host was present. This would minimise the energy wasted by parasitoids and may allay some of the fears that introduction in the field would draw parasitoids out of the margins when there was no viable host present. The single-attack assay demonstrates that it was possible to increase the success rate of *A. colemani* by manipulating the ambient odour, and that this increased success rate translates directly into decreased reproductive potential for the aphids. As an indirect result of the work it was observed that the efficacy of the parasitoids may often be underestimated if judged only by the rates of parasitism, as unsuccessful parasitism was also capable of reducing the aphid fecundity. Although these appear to be very promising results for potential biological control systems without trials in a larger spatial environment, with more hosts present, it is difficult to determine the benefit aphid sex pheromone may have.

These experiments demonstrated that the behaviour of *A. colemani* naive to the aphid sex pheromone can be manipulated by the presence of the aphid sex pheromone. It remains to be seen if a highly reliable innate response such as nepetalactone is subject to learning plasticity.

4 Effect of learning on the behaviour of parasitoid Aphidius colemani to an already innate cue; nepetalactone

4.1 Introduction

Learning can be defined as the 'the acquisition of neuronal representations of new information' (Dukas, 2008). Insects rely extensively on learning to overcome many of the major challenges that they encounter during their life (Dukas, 2008). Innate responses can greatly increase the fitness of an insect by ensuring it responds to vital and recurrent stimuli within the environment but they do little to account for variation within an environment or the adaptation required for a new environment. The acquisition of new information will more accurately reflect the present environment of the organism over any from which it has evolved; allowing a more adaptive behavioural response.

For a successful parasitism to occur all five steps of the host selection process need to be completed (Vinson, 1976) and one way to overcome any obstacles in locating the host is through learning. Insects can utilise several different modes of learning, with evolution selecting for those which are best suited depending on the frequency of the stimuli and their importance within the environment. Forms of learning described below are done so with a particular focus on parasitoid learning of olfactory signals relating to foraging success, which are predominantly forms of associative learning. Although the focus is on parasitoid learning, these modes of behavioural adaptation are not exclusive to hymenoptera, or even arthopods, and are observed throughout the animal kingdom. By understanding and exploiting parasitoid learning it may be possible to increase the efficiency with which they forage within an area, providing benefits in biological control programmes.

Associative learning is the process by which an organism learns to relate either a positive or negative stimulus with an unrelated signal. Once the stimulus is learnt the subject is likely to show a more favourable or aversive response to it, respectively. The ease by which an individual learns may be dependent on the source of the new signal, capacity for learning in the individual, condition of the individual and the strength of reward/punishment associated with it. Individuals may be better predisposed to learn stimuli which relate to their environment over those which remain completely novel

(Meiners *et al.*, 2003). The capacity for learning varies greatly between organisms and, though it is frequently assumed otherwise due to their small brain size, it has been demonstrated that insects have a great capacity to learn, specifically the hymenoptera (Giurfa *et al.*, 2001, McCall & Kelly, 2002). The condition of the individual at any given time is also likely to affect its readiness in learning a new cue e.g. if the individual requires energy and, therefore, an immediate food source it is less likely to learn stimuli that are unrelated to energy acquisition. The strength of the reward or punishment may also have a significant effect on how the parasitoid will learn the cue. The level to which the individual responds to the stimulus may be directly correlated to the strength of the related reward/punishment or it may require a threshold before the individual will learn any response; it would be disadvantageous for an organism to learn to respond strongly to a cue which offers little reward as it may distract it from finding sources of higher reward (Budenberg, 1990). To provide context to the study, an overview is provided explaining the forms associative learning experienced by aphidiine parasitoids and principles relating to learning.

4.1.1 Maternal Influence

Experiments with *Aphidius colemani* demonstrate that a weak maternal cue is deposited with the egg during oviposition (Douloumpaka & van Emden, 2003), though the exact mechanism for this remains unknown. As a cue it is considered weak because it requires reinforcement by subsequent oviposition i.e. if a parasitoid emerged from plant A and then oviposited on an aphid on plant A, a mild preference would be seen in the next generation for plant A. However, if oviposition occurs on plant B, or any other plant, no preference is observed. Such maternal influences were not initially recognised because they are normally overridden by emergence conditioning.

4.1.2 Emergence conditioning

Volatile compounds adhere to the surface of the silk cocoon and mummy case during the mummification of the aphid host. After the completion of larval development the parasitoid escapes from the silk cocoon, and host mummy, by eating its way out. The process of consuming host material and the odours that have become absorbed in it from the environment provide a learning experience for the parasitoid. A parasitoid emerging from a mummy will have a preference for that host/plant complex to which the host belonged (Storeck *et al.*, 2000, van Emden *et al.*, 1996). If excised from the mummy before emergence the parasitoid will fail to learn a preference for a host/plant complex. Evolutionary benefits of emergence conditioning are clear, as the successful development of a parasitoid is evidence of a successful environment: by foraging in similar patches the parasitoid is more likely to find suitable hosts and experience

similar reproductive success. Fluctuation of host dynamics within a population mean that success in the present generation may rely on a different set of stimuli than the previous generation, which is where ovipositional experience may play a role.

4.1.3 Ovipositional experience

In this form of associative learning the parasitoid learns the stimuli from the environment to relate to the reward of ovipositing in a viable host. The high value of the reward (in this case a viable host) leads to a strong positive enforcement of odours within the environment. The strength of this learning experience is demonstrated by the speed with which novel odours can be learnt (Takasu & Lewis, 1993), though there is a greater proclivity for the parasitoids to respond to odours more closely related to the natural environment of their host(s). Odours related to a successful ovipositional experience are the most reliable that a parasitoid can receive and, therefore, elicit a learnt response which overrides previous experience. Increasing reliability of a learning experience results in a preference of the parasitoid for the associated stimuli which results in the formation of a hierarchy of learnt response.

4.1.4 Hierarchy of response

In the context of the parasitoid-host system, reliability refers to the accuracy of the information to represent an environment abundant with viable hosts. Environmental factors regarding the host are subject to change; a polyphagous aphid may be found on different plants throughout the seasons or the growth of new plants in the environment may produce a new volatile blend in the environment. Due to such variation, reliability here is broadly defined as the most recent environment in which viable hosts can be found. Emergence conditioning will overwrite or reinforce maternally received preferences though successful ovipositional experience will take precedent over previously learnt cues (Storeck *et al.*, 2000). The associative learning that occurs during the adult stage does not require oviposition experience but may be elicited with a rewarding cue associated to the host or host environment (Du *et al.*, 1997, Lewis & Tumlinson, 1988). An increase in the value of the reward will relate to a greater learnt response in the parasitoid (Battaglia *et al.*, 2000). For this reason reference is made to 'weaker' forms of learning as those which represent less reliability and can, therefore, be overwritten by 'stronger' forms of learning offering more reliable information about the location of hosts within the current environment.

4.1.5 Alpha conditioning and the aphid sex pheromone response

Stimuli that are closely associated, consistently available or that are of great importance to the fitness of the individual when they are present may have a genetic basis. The innate responses to such stimuli are sufficient to elicit learning of new

stimuli but whether or not genetically determined innate response can be altered through learning is still not established. Alpha conditioning is the enhancement of a pre-existing response through learning. It may be that the strength of important innate responses is at an optimal level and can be enhanced no further.

This chapter focuses on the capacity of *Aphidius colemani* to further associate reward with (4*aS*,7*S*,7*aR*)-nepetalactone, an odour it already responds to innately (Ch.3.3.1). Although it is known that parasitoids utilise the sex pheromone it is not clear how their behaviour is altered by the odour nor the effect that learning may have, if any. In this chapter research was conducted to determine the potential of emergence conditioning and associative learning to increase attraction or retention of parasitoids and if it can lead to an increased rate of attack or successful oviposition. Previous studies have only utilised the emergence conditioning and associative learning responses to investigate learning of plant volatiles and it is not currently known if innate cues directly related to hosts are subject to similar levels of manipulation. In addition, experiments are conducted to establish how these behavioural changes induced by the pheromone impact foraging on a localised scale and if it does enhance the foraging success of the wasps. The experiments in this chapter represent challenges faced in the initial three foraging stages defined by Vinson (1976): host habitat location, host location and host acceptance. The work aims to determine how emergence conditioning and associative learning with nepetalactone may play a part at these stages to optimise the parasitoids' foraging strategy.

4.2 Materials and methods

4.2.1 Parasitoid cultures

Aphidius colemani were provided by Koppert (Koppert B.V., Netherlands) and maintained on *Myzus persicae* on Chinese cabbage, *Brassica pekinensis* cv. Wong Bok, in a controlled environment (20 ± 1ºC with a L:D 16:8 photoperiod). Only females 2-4 days old were used in experiments. For use in the four-arm olfactometer, attack/success rate and motivation assays all parasitoids were reared through one generation on *M. persicae* on Chinese cabbage. For the experiments using emergence conditioning it was necessary to rear the parasitoids in the laboratory so that mummies could be provided with exposure to the odours for a sufficient period of time before emergence, something that could not be guaranteed with mummies provided by biological control agencies, as wasps frequently emerge within two days of arrival. Although results are not shown here, motivation assay was also run using *Aphidius matricariae*, and to eliminate temporal bias, both *A. colemani* and *A.*

matricariae were bred in the laboratory so they would be exactly the same age for experiments (for *A. matricariae* data see Appendix A).

4.2.2 Parasitoid experience

Experience was provided by allowing a parasitoid five minutes to attack freely on a leaf containing 10 aphids. Compounds used in the experienced treatments were added to filter paper which was placed below the leaf; 2 µl of the given compound was applied at a concentration of 1 mg/ml, all compounds used were dissolved in hexane (99%, Fluka Analytical, UK).

4.2.3 Four-arm olfactometry

The four-arm olfactometer can be used effectively to gauge if the pheromone alone retains an organism in an area for longer than it would without stimulus (Vet *et al.*, 1983). Four-arm olfactometer bioassays were carried out using 2-4 day old female parasitoids (see Ch. 4.2.1). Ten days after the aphids were exposed to parasitism, therefore 3-4 days prior to emergence, the mummies were removed. The mummies were divided into small plastic containers which had their base lined with Whatman No. 1 filter paper (Whatman, UK) with no treatment (Un), 20 µl of hexane (H) or 20 µl of 1 mg/ml nepetalactone (N). The open containers were placed within 30 cm^3 fabric cages (Megaview Science, Taiwan) to avoid a build-up of the solvent damaging parasitoid development, the mummies would remain until emergence. Treatments were applied only once and each treatment group was stored separately to avoid cross contamination.

The bioassay arena was made of a square of metal placed between two glass sheets (Figure 4.1). A four-pointed star shape hole was cut centre of the metal square creating the arena in which the parasitoid could move. The odour sources were introduced via glass adaptors affixed to the end of each arm. Within these glass adaptors, a circle of 2.5cm diameter filter paper was placed containing 20 µl of nepetalactone in the treatment arm (either at a concentration of 1 mg/ml or 10 mg/ml) or 20 µl of hexane in the control arms. The treatment was only issued into one arm; other arms used as controls have only hexane applied to the filter paper, also in a 20 µl dose. Airflow was created by drawing air from the centre of the arena at 800 ml/min, buffered by a 1 L glass chamber. Air drawn from the centre of the arena ensured that odour contamination of the control arms did not occur. This was confirmed using visualisations with titanium tetrachloride. The filters supplying odour were changed after every complete set of runs (a set equalling 4 runs; 1 of each treatment set looked at, which took approximately 1 hour).

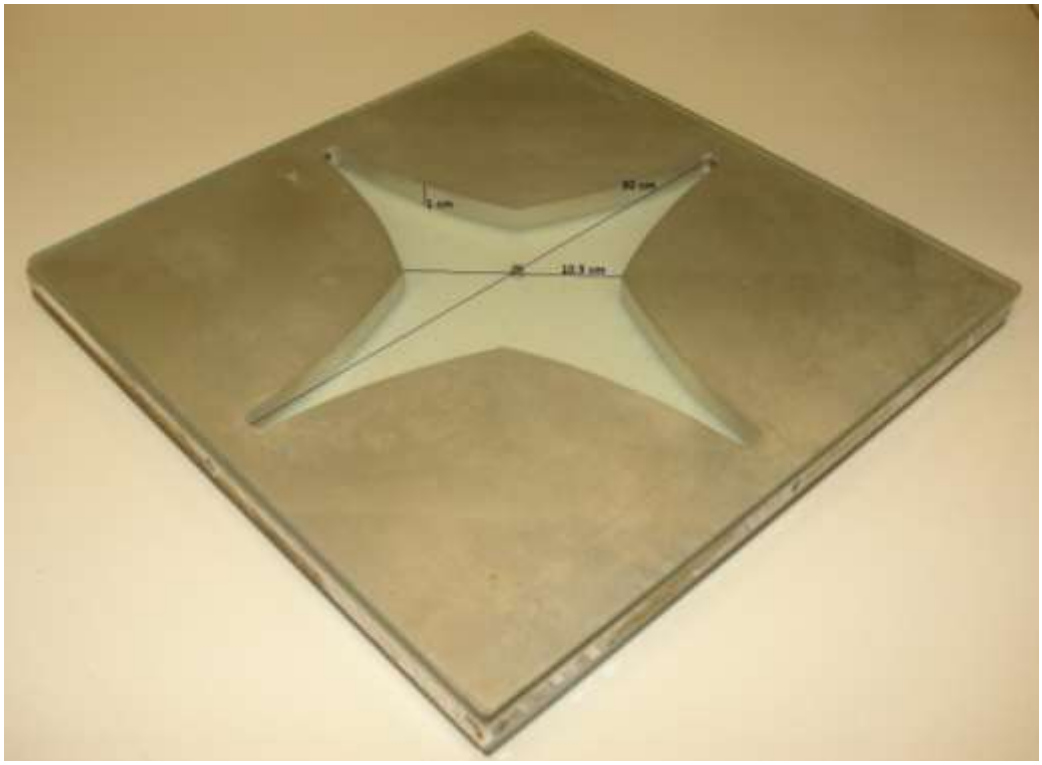


Figure 4.1 Four-arm olfactometer

The four-arm olfactometer was used in the retention time experiments. Holes at the tip of each arm allow placement of the odour source. Air was withdrawn from the opening in the centre.

To commence the bioassay, a vial containing an individual female was inverted and placed over the hole on the upper side of glass. This allowed the parasitoid to walk from the vial and into the four-arm arena. The arena was lit from below by fluorescent tubing with a diffusion filter, lighting from below was used to encouraged the movement of parasitoids onto the base of the arena. A sheet of paper was affixed to the outer side of the glass to diffuse lighting from underneath. A camera (Model: TK-C1360B, JVC) was held in place above the arena using a clamp stand and connected to a DVD recorder. All experimental runs were recorded onto DVDs to be re-analysed at a later date if necessary. Divisions were drawn to split the arena into four equal areas, each containing an arm and the quarter of the central area leading to it. The parasitoid was given 12 minutes in the arena with the recording starting once the wasp left the vial and entered the arena. As the parasitoid moved freely around the arena the time spent in the area of each arm was noted. If the parasitoid spent time directly below the centre, where the air was withdrawn, or on the lines separating any of the quadrants this was discounted from the total time. Methods for recording the olfactometer data were taken from Vet *et al.* (1983) and Saïd *et al.* (2006). Additional time was given (twelve minutes rather than ten) to adjust for the longer periods of time

the parasitoids were observed spending in the central area. A total of 16 replicates were completed per treatment.

A one-sample T-test was used to compare the percentage of time spent in a given arm and the percentage of visits to an arm to a predicted value of 0.25 which represents the expected value if movement around the arena was at random. A paired T-test was used to determine the difference between the mean time per visit to the treatment arm and the control arms. All statistical tests were carried out on Minitab 16 statistical software (© 2010 Minitab Inc).

4.2.4 Attack/success rate

The rate at which the parasitoid attacks, and the percentage of these attacks that result in successful larvae development, in part determine the success of parasitoid application in biological control. The attack rate bioassay was used to determine if differences were seen in the frequency at which *A. colemani* attacks the hosts when in a host rich environment, the frequency of its attacks that result in the successful development of larvae and how the presence of a highly reliable cue like nepetalactone may affect this.

Parasitoids were reared and pre-treated following the methods used for four-arm olfactometry bioassay (Ch. 4.2.3). It has been shown by Blande *et al.* (2004) that *Diaeretiella rapae* attack rate is highest 2-5 days after emergence, accordingly all parasitoids were used 2 days after emergence. Sixteen hours before trials were run, 35 aphids were placed on each leaf measuring 6.5 cm width and greater than 6.5 cm in length on a mature Chinese cabbage 5-7 weeks old. Aphids in the 3rd instar of development were chosen as these are shown to be preferred by many related parasitoid species such as *Aphidius rubifolii* (Gilbert & Gutierrez, 1973) and *Aphidius sonchi* (Shu-sheng & Hughes, 1984) though on different hosts. It is recognised that host size also has an effect on ovipostion preference in *A. colemani* (Lin & Ives, 2003) and for this reason hosts were selected that appeared to be equal in size.

Parasitoids were satiated with 20% honey solution and during rearing and preparation had no contact with aphids and so, in this sense, remained naïve. Throughout this study the term naïve refers to parasitoid having no ovipositional experience. The parasitoids were placed in the bioassay environment (20 ± 1°C, 50% humidity) for an hour. During this hour the leaf discs could be prepared. To prepare the leaf discs around 30ml of 1.5% agar solution was poured into a 7 cm Petri dish; this setup was similar to that used by Blande *et al.* (2004). A preliminary trial showed that the leaf in this media and condition was able to survive 10 days, at which point mummies would

be clearly formed. Scissors used to cut the leaves were sterilised in 90% ethanol to reduce the chance of infection. As the agar cooled the leaf was gently embedded into the surface ensuring that the stem was in contact with the agar and could receive sufficient moisture. Caution was taken not to place the plant in before the agar was sufficiently cooled as this instantly kills the aphids or causes them to scatter. Counts were made of the aphids to ensure that there were exactly 30 present on each leaf; any surplus aphids were removed with a size 0 paintbrush (SG250, Royal & Langnickel, IN, USA).

To start the bioassay a Petri dish containing the leaf disc was placed inside a modified cage. The modified cage was a wire-frame cage covered in transparent plastic sheeting made from incineration bags (Fisherbrand, UK). The plastic sheeting reduces the possibility of odour contamination in other samples and can be disposed of between treatments; the wire frame was washed with 70% ethanol between trials. Odour was introduced by a 10 µl glass microcapillary (Drummond Scientific Co., USA), allowing a release rate of 0.3 µl per minute by evaporation (Glinwood, 1998). The lid of the Petri dish was removed and the parasitoid was released into the centre of the leaf. The parasitoid was given 15 minutes in which time her behaviour was observed, some of the runs were recorded onto DVD using a TK-C1360B colour camera (JVC) to be viewed later allowing for simultaneous trials to be conducted. Note was taken of each attack made and repeat attacks. Other behaviours, such as the time spent grooming and the number of times the antennae made contact with the host without then making an oviposition attempt, were also recorded. Ovipostion was noted as distinct contact between the parasitoid ovipositor and an aphid host. Although repeat attacks were noted this can only be seen as approximations since no markings were made on the aphids and repetition was noted from memory. Accordingly, the runs that were recorded onto to DVD were watched through only once with no rewinding or pausing of the footage to ensure that any observational errors were consistent with real-time recordings. After 15 minutes the parasitoid and odour-containing microcapillary were removed. The lid was returned to the Petri dish and it was stored in a controlled environment room (21 ± 3°C, 16:8 light:dark) for 10 days.

Ten days after the bioassay was conducted the sample dish was taken out and the number of mummies that had formed was counted; the number of mummies as a proportion of attacks made was considered the success rate. To analyse the data in each instance a two-sample T-test was conducted between the untreated control group (no additional odour stimuli present in pre-treatment or during assay, represented as Un-Un) and the hexane control group (only hexane present in pre-treatment and during assay, represented as H-H). If no difference was seen between the control groups, Un-Un could be omitted from further analysis. A general linear model could then be run analysing the interaction between all remaining treatments

and pre-treatments. To achieve a normal distribution it was necessary to log transform the data for the time of first attack prior to analysis.

4.2.5 Square assay

To test the odour preference of *A. colemani* and the extent to which a response may be enhanced a square assay was employed following Ameixa and Kindlemann (2011). The equipment and procedure for this experiment were adapted from Ameixa and Kindlmann (2011). Treatments in the olfactometer and experimental procedure used were exactly as seen in the previous square assay (Ch. 3.2.3). Differences in the experimental procedure were seen in the learning experience of the parasitoids before entry into the olfactometer. Three different experience groups were tested: parasitoids with no oviposition experience (naïve), experience with 6-methyl-5-hepten-2-one and experience with 6-methyl-5-hepten-2-one and nepetalactone combined (for details of how experience was administered see Ch. 4.2.2). To commence the assay a parasitoid was placed in the centre of the arena. The parasitoid was given 10 minutes to move freely within the arena; the time spent within each square of the arena was recorded. As used in the square assay without experience (3.2.3), treatments present in the olfactometer were: 6-methyl-5-hepten-2-one, nepetalactone, 6-methyl-5-hepten-2-one with nepetalactone (a 1:1 ratio) and no treatment. After every 3 repeats, the surrounding equipment was rotated. The positioning of the treatments was changed after every 12 replicates.

4.2.6 Motivation assay

The motivation assay allowed the study of movement patterns observed in *Aphidius colemani* when exposed to a range of treatments. The various parameters measured can be indicative of foraging patterns employed by the parasitoid.

The motivation assay arena was constructed of a Petri dish 9 cm in diameter with a 2 cm x 2 cm square removed from the centre. The central opening was covered with gauze and a 3 cm diameter Petri dish was affixed to the underside. 1 cm to the side of the square a circle was cut of approximately 0.5 cm diameter which provided an entry point for the parasitoid. The larger upper dish formed the assay arena while the smaller dish on the underside was the odour source chamber. The parasitoids were placed in the environment 30 minutes before the start of the experiment to allow them to acclimate. During this time they were provided with a wick of 20% honey solution. The lid of a vial containing a parasitoid was placed in the 0.5 cm diameter hole in the base of the arena. A glass covering prevented parasitoids from flying out of the arena but still allowed for a recording of the assay to be made from above (TK-C1360B colour camera, JVC). The behaviour of each parasitoid was recorded for eight minutes.

It has previously been found with the hymenopteran parasitoid *Pseudeucoila bochei* that following a host encounter the female wasp will continue searching for 6-8 minutes (Luck *et al.*, 1979) the time was also similar to that used in tracking studies of the more closely related *Aphidius uzbekistanicus* (Micha & Wyss, 1996). Recordings were all analysed using Ethovision software (v 3.1 Noldus Information Technology). Four treatments involved naïve parasitoids in the assay with either: no stimulus, an infested leaf or 1 µl of nepetalactone at a high (10 mg/ml) or low (1 mg/ml) concentration. Two treatments containing experienced parasitoids; one with no stimulus present following experience and in the other the parasitoid had nepetalactone present during experience (as described in Ch. 4.2.2) and had the same pheromone application in the assay as was present during learning.

4.2.7 Leaf location assay

The leaf location assay provides an opportunity to test the foraging efficiency of the parasitoid wasps in a localised scale. By allowing the wasps to search in an arena containing a host patch it was possible to assess the difference that behavioural changes, induced through learning, can have on foraging efficiency. The same arena was used for the leaf location assay as the square assay though divided into 64 squares (each 3 cm x 3 cm). The outer most ring of squares was described as Zone 1 and the second most outer as Zone 2 (Figure 4.2). Using the true random number generator www.random.org (Mads, 2012) number values were provided before the experiment started. The leaf was then placed in the assigned square within the assigned zone. The innermost squares were not used as possible locations for the leaf, as it was a concern that the parasitoid may find the leaf and hosts too quickly. A leaf infested with around 15 aphids was cut to 2.5 cm x 2.5 cm. Once a position for the leaf was allocated that position would be used for four trials (one for each treatment). If an odour was provided alongside the leaf it was done so on a 1 cm x 2 cm piece of filter paper placed under the leaf. Treatment was provided in 2 µl doses of a 1 mg/ml solution. The four most central squares were considered a neutral zone in which the parasitoid could be placed to commence the assay. Movement through the arena was only counted once the parasitoid had left the centre. The parasitoid was given 10 minutes in the arena during which time its behaviours and the number of squares it visited were recorded. If the parasitoid located the leaf and made an ovipositional stab at one of the aphids, the assay was stopped. The time taken for the parasitoid to locate the leaf was compared using an ANCOVA, with distance from the starting point being the covariate. The time between arriving on the leaf and conducting the first ovipositional attack was analysed using a one-way ANOVA. All statistics were carried out on Minitab 16.

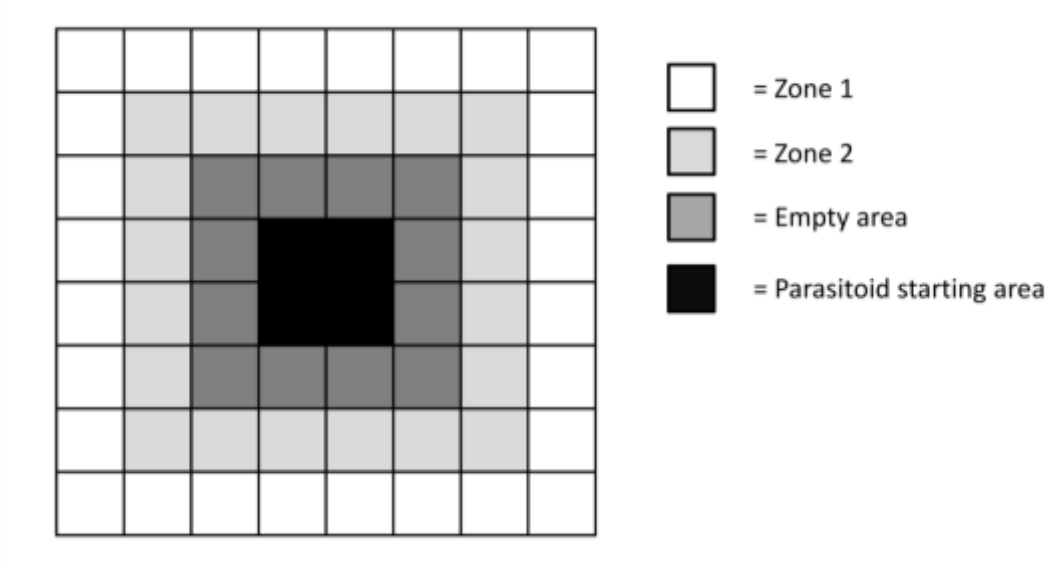


Figure 4.2 Layout of the leaf location assay.

The glass-based square olfactometer used for the leaf-location assay. The arena was divided into three zones with each square measuring 3 x 3 cm, giving a total size of 24 cm^2.

4.3 Results

4.3.1 Four-arm olfactometry

The mean percentage of time spent in the treatment arm was compared to the expected time using a one-sample t-test following the method of analysis used in Webster et al. (2008). For time and number of visits the expected value was 0.25, the null hypothesis was that the parasitoid will spend a quarter of the total time or total visits made in/to the treatment arm of the olfactometer. It was observed that the percentage of time spent in each arm does not vary between treatment sets (Table 4.1). There was no difference in the number of visits made to the treatment arm in any of the treatment sets used (Table 4.2).

Table 4.1 Retention time of Aphidius colemani in the four-arm olfactometer after exposure to various pre-treatments and treatments. The P value was acquired by comparing the proportion of time spent in treatment arm to the expected proportion (0.25) using a one-sample t-test. NS = Not Significant.

Pre treatment	Treatment	N	% of time spent in treatment arm	P
None	Nepetalactone 1 mg/ml	16	22.5	0.692 NS
Hexane	Nepetalactone 1 mg/ml	16	21.8	0.486 NS
Nepetalactone 1 mg/ml	Nepetalactone 1 mg/ml	16	19.2	0.451 NS
None	Nepetalactone 10 mg/ml	16	30.3	0.655 NS

Table 4.2 Percentage of visits to an arm by Aphidius colemani after exposure to a pre-treatment/treatment set. The P value was acquired by comparing the proportion of visits made to the treatment arm to the expected proportion (0.25) using a one-sample t-test. NS = Not Significant.

Pre treatment	Treatment	N	% of visits to treatment arm	P
None	Nepetalactone 1 mg/ml	16	17.7	0.266 NS
Hexane	Nepetalactone 1 mg/ml	16	18.3	0.073 NS
Nepetalactone 1 mg/ml	Nepetalactone 1 mg/ml	16	19.4	0.075 NS
None	Nepetalactone 10 mg/ml	16	27.4	0.366 NS

Assessing the mean time spent by the parasitoid in the odour patch per visit was a good measure of the retention effect on the individual because it eliminates the number of visits made as a factor, but may also give an idea of the preference the parasitoid had to this patch when it did visit.

A trend appeared to be emerging that parasitoids exposed to the hexane pre-treatment and subsequently a treatment of nepetalactone at a concentration of 1 mg/ml (T_{15} = -2.1, P = 0.053) or those exposed to nepetalactone in the pre-treatment and again in the assay (T_{15} = -2.06, P = 0.057) spent less time per visit in the treatment arms; no difference was seen for any other pre-treatment/treatment set (Figure 4.3). Treatments were compared using a paired t-test, unlike the previous parameters which were analysed using a t-test to an expected value of 0.25, since no expected value can be easily obtained for the mean time spent per visit.

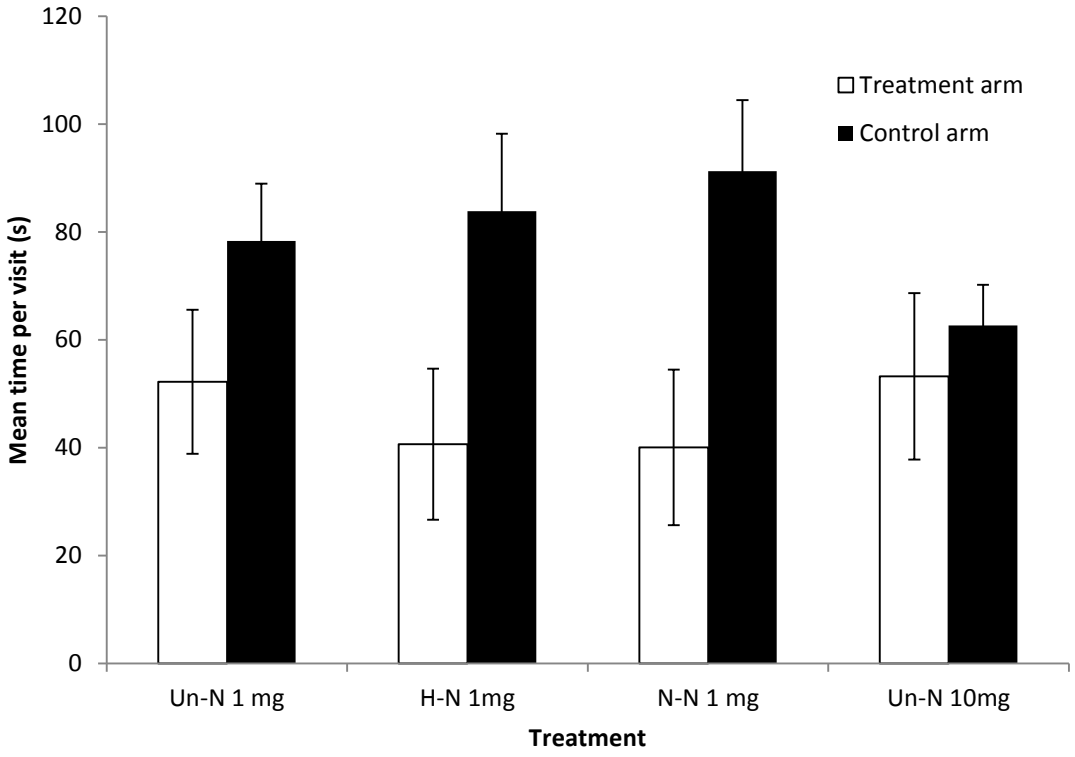


Figure 4.3 Mean time spent in a given arm per visit.

Each treatment arm was compared to the control arm using a paired t-test. The first letter set denotes the pretreatment and second letter represents the treatment during the assay where Un = no treatment, H = hexane and N = nepetalactone.

4.3.2 Attack/Success rate

No difference was seen between the untreated control and the hexane control in the number of attacks (T_{24} = 0.02, P = 0.984) or number of mummies formed (T_{18} = -0.11, P = 0.917). Number of attacks was not affected by pre-treatment ($F_{1,42}$ = 0.25, P = 0.619), test treatment ($F_{1,42}$ = 0.01, P = 0.908) or their interaction ($F_{1,42}$ < 0.01, P = 0.947). No difference in the number of mummies formed was seen as an effect of pre-treatment ($F_{1,42}$ = 0.7, P = 0.407), test treatment ($F_{1,42}$ = 0.77, P = 0.386) or their interaction ($F_{1,42}$ < 0.01, P = 0.970) (Figure 4.4).

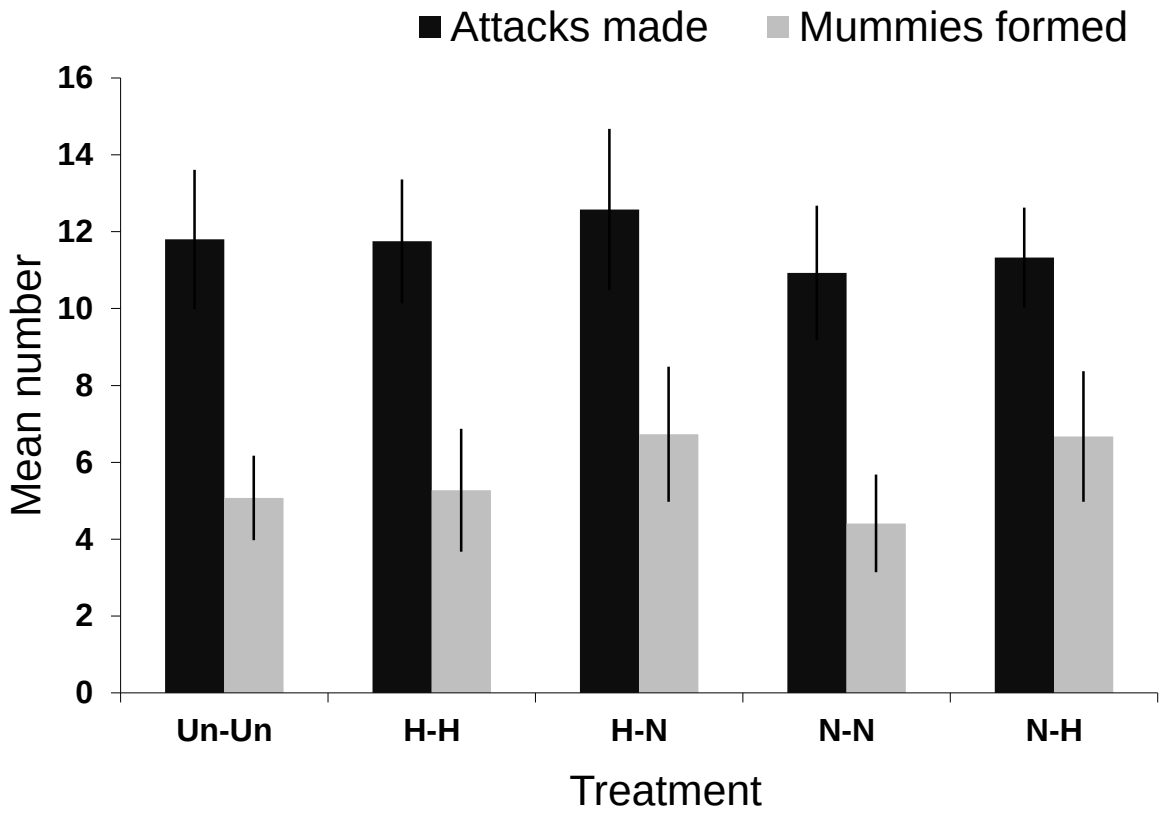


Figure 4.4 Attack rate and success rate in parasitoids

Mean attack rate in each treatment and the mean number of mummies after 10 days. Un = Untreated, H = Hexane and N = Nepetalactone with the first letter representing the pre-treatment and the second the treatment exposure during the bioassay. No difference was observed in the number of attacks made or mummies formed as a consequence of pre-treatment or test treatment (P > 0.05).

During the experiment parasitoids were observed to groom their ovipositor between ovipositional attacks. It was hypothesised that the additional olfactory information available in the environment may reduce the time that parasitoids spend grooming, as they seek to optimise the time spent ovipositing. Hexane did not alter the time spent

grooming between the control groups (T_{24} = 0.67, P = 0.509). Grooming time was not affected by pre-treatment ($F_{1,46}$ = 0.26, P = 0.613), test treatment ($F_{1,46}$ = 0.8, P = 0.375) or the interaction between them ($F_{1,46}$ = 0.5, P = 0.482) (Figure 4.5).

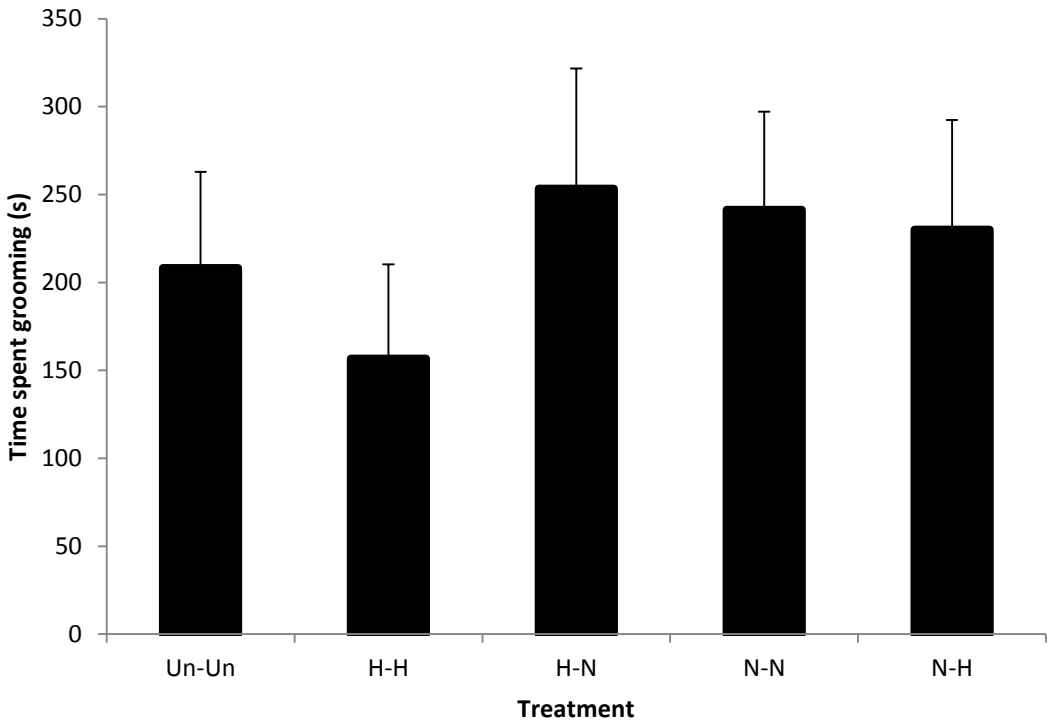


Figure 4.5 Time spent grooming within the assay for each treatment.

Un = Untreated, H = Hexane and N = Nepetalactone with the first letter representing the pre-treatment and the second the treatment exposure during the bioassay. No treatment was found to increase or decrease grooming time (P > 0.05).

The time that it takes the parasitoid to make its initial attack may be critical in reducing the aphid populations: the longer it takes to make an initial oviposition attempt whilst in the presence of the aphids the longer they have to detect the parasitoid and scatter, decreasing the ease with which it will be able to locate subsequent hosts. No difference was seen between the time to first attack in the untreated and hexane control groups (T_{24} = 1.06, P = 0.301). The time taken between the parasitoid first entering the assay and making its first oviposition attack did not vary as a result of pre-treatment ($F_{1,46}$ = 1.8, P = 0.186), test treatment ($F_{1,46}$ = 0.08, P = 0.772) or their interaction ($F_{1,46}$ = 1.86, P = 0.179) (Figure 4.6).

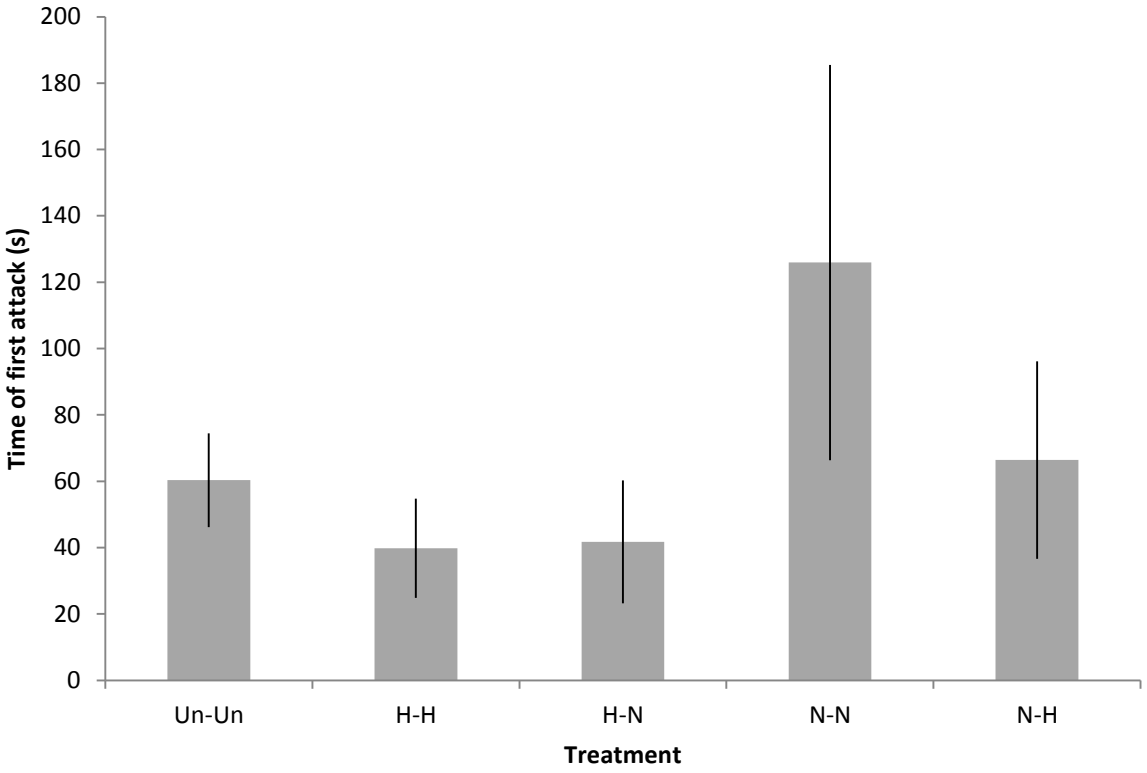


Figure 4.6 Time of first attack.

Effect of treatment on the time taken before the first attack from *A. colemani* in the attack rate bioassay. No difference was seen between any of the treatments ($P > 0.05$). Letters preceding the hyphen denote the pre-treatment and letters after the hyphen represent the stimuli present during the assay where Un = no treatment, H = hexane and N = nepetalactone.

Success rate was given by the proportion of total aphids attacked that subsequently formed into mummies. It was confirmed that no differences in success rate existed between untreated parasitoids (Un-Un) to those only exposed to hexane (H-H) ($T_{15} = -1.08$, $P = 0.298$) (Figure 4.7). The success rate was shown to have no significant difference due to pre-treatment ($F_{1,37} = 2.03$, $P = 0.162$), treatment ($F_{1,37} = 1.20$, $P = 0.281$) or the interactions between them ($F_{1,37} < 0.01$, $P = 0.995$) (Figure 4.7). It was shown that the mean success rate of ovipositional attacks in this environment when no additional stimuli are present was around 40% (40.35 SE ± 7.62).

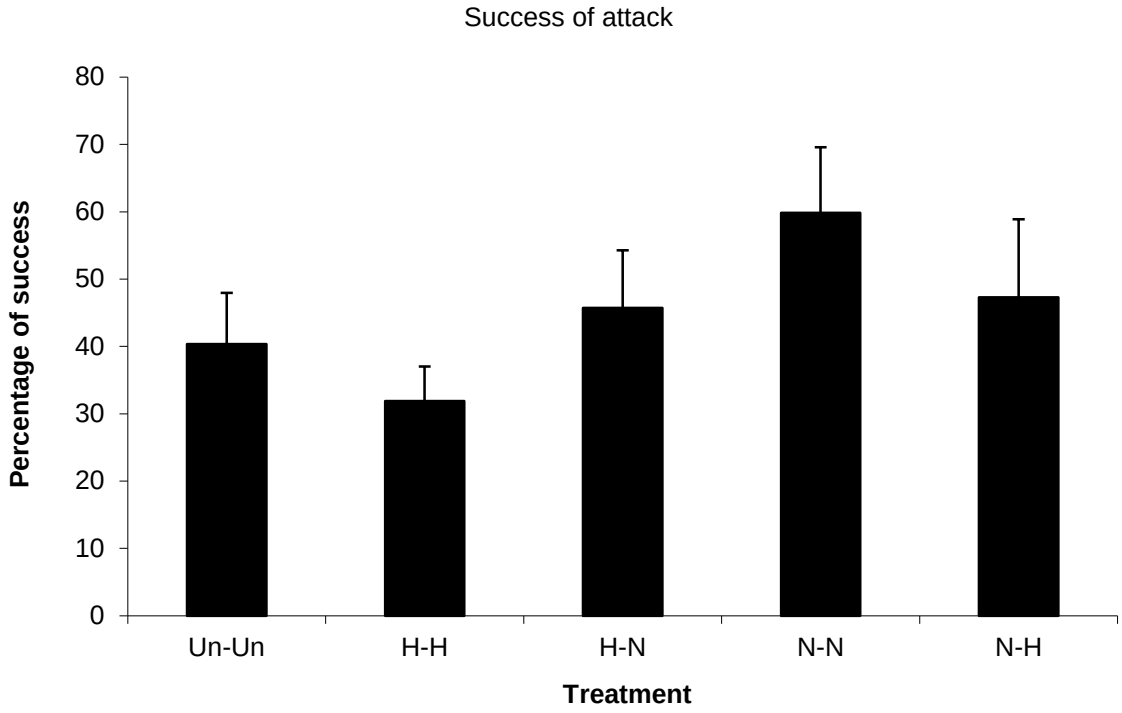


Figure 4.7 Success rate of ovipositional attacks.

Success rate is represented as the proportion of ovipostional attacks resulting in successful mummification of the aphid host. No differences are observed between any of the treatment groups ($P > 0.05$).

4.3.3 Square assay

None of the treatments were found to be more attractive regardless of the experience of the parasitoid (Figure 4.8). In treatment 1, in which parasitoids have no ovipositional experience, they spent significantly less time in the square containing 6-methyl-5-hepten-2-one ($T_{27} = -2.52$, $P = 0.018$). In treatment 3, where the parasitoid had pre-assay ovipositional experience with 6-methyl-5-hepten-2-one and nepetalactone present, it was found to spend less time in the nepetalactone treatment square ($T_{26} = -3.30$, $P = 0.003$). Low numbers seen for all treatments demonstrates a considerable amount of time the parasitoids spent in the central region of the assay or in transition squares.

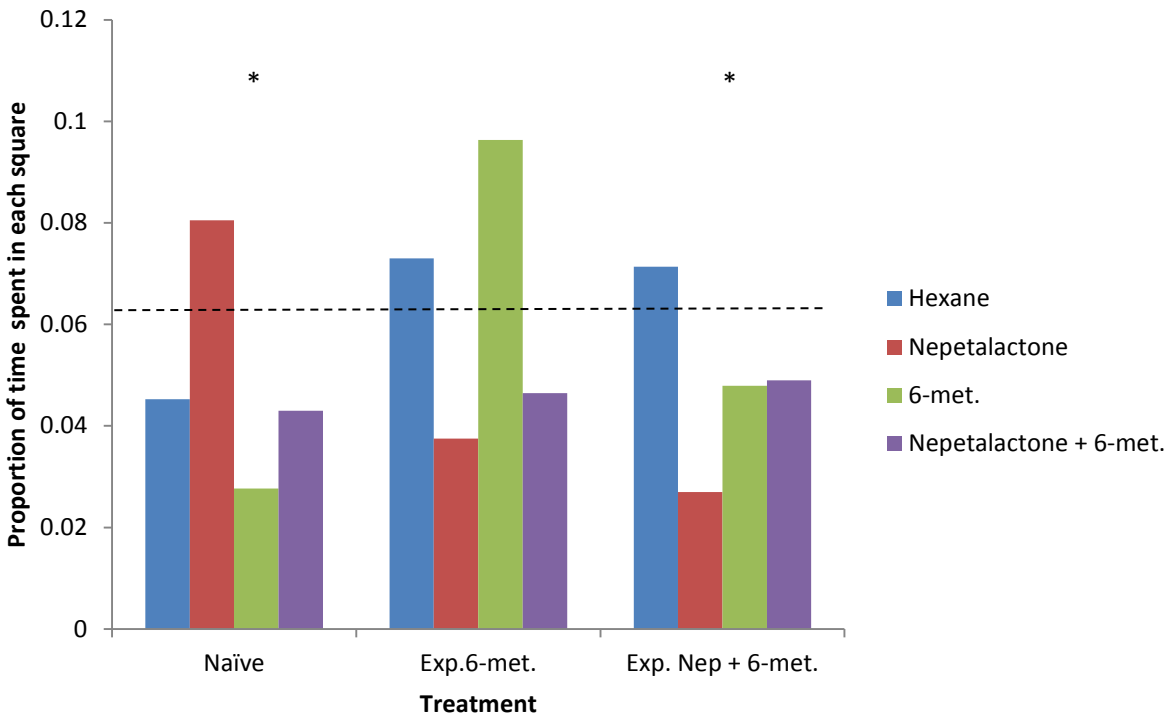


Figure 4.8 Time spent in square assay treatments.

Proportion of time spent in each of the treatment squares for each treatment set. * $P < 0.05$ for time spent in squares within the treatment set. The dashed line represents the expected time to be spent in each of the squares if movement was completely random.

The squares adjacent to the corner treatment square could be combined to be analysed as one quadrant. This analysis accounts for disproportionate time spent in the centre because it includes all areas of the olfactometer except the entry point. Given movement at random it would be expected that the wasp would spend 25% of its time in each quadrant. In treatments of wasps experienced with 6-methyl-5-hepten-2-one and those experienced with a combination of nepetalactone and 6-methyl-5-hepten-2-one the time spent in each quadrant did not vary significantly from the expected 25%. However, naïve parasitoids were found to spend significantly more time in the nepetalactone quadrant (39.91% of the total time, $T_{28} = 2.17$, $P = 0.039$) and a trend appeared to be emerging that wasps spent less time in 6-methyl-5-hepten-2-one (16% of the total time, $T_{28} = -2.02$, $P = 0.053$) (Figure 4.9).

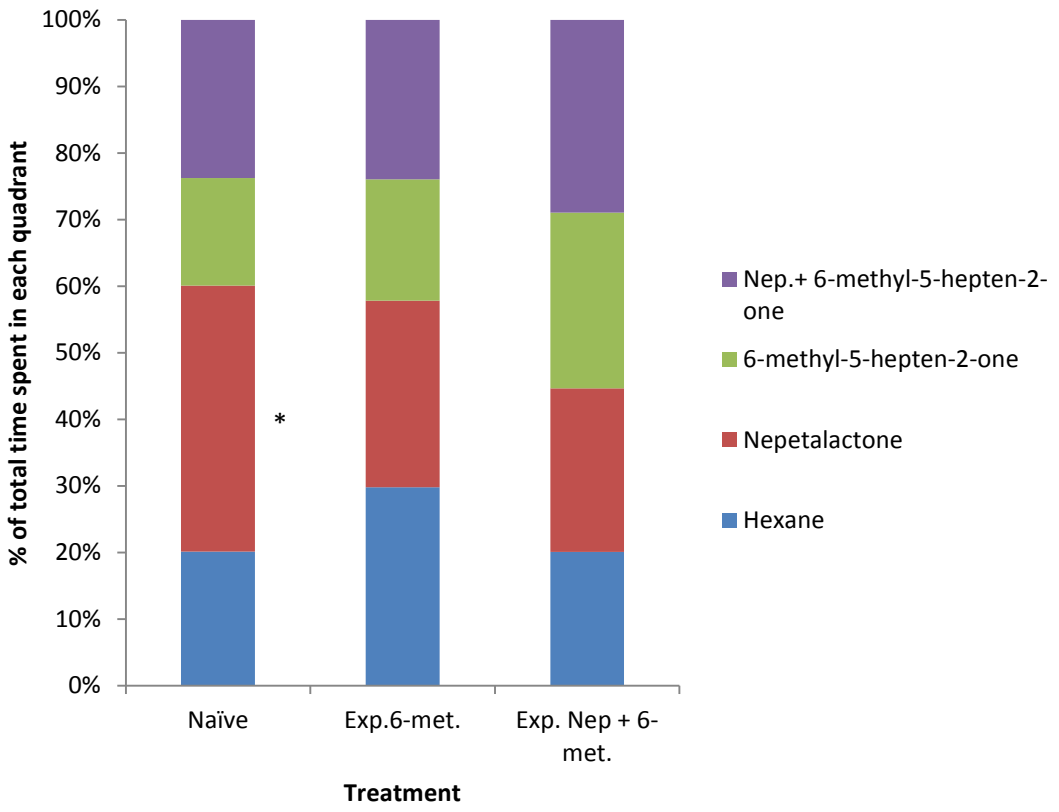


Figure 4.9 Time spent in square assay quadrants.

Time spent in each of the quadrants of the olfactometer for each treatment set. Exp. = Experience with, 6-met. = 6-methyl-5-hepten-2-one, Nep = nepetalactone. * $P < 0.05$ for the bar to which it is adjacent. N = 28

4.3.4 Motivation assay

Treatments were found to differ significantly in the affect that they had on the total distance moved ($F_{5,100} = 2.45$, $P = 0.039$), meander ($F_{5,90} = 2.87$, $P = 0.019$) and velocity ($F_{5,100} = 2.75$, $P = 0.023$) of *A. colemani*. An increase in the rate of turning, or klinokinesis, is typical amongst foraging individuals (Hassell & Southwood, 1978). Looking at this aspect of the behaviour pattern of the parasitoid helps to determine the triggers required to induce foraging behaviour, a desirable result in an area known to contain aphids. Tukey posthoc tests for each parameter showed that the difference in meander was between the control group (a naïve parasitoid with no stimulus) and the naïve parasitoid exposed to a high concentration of nepetalactone (10 mg/ml) (Figure 4.10).

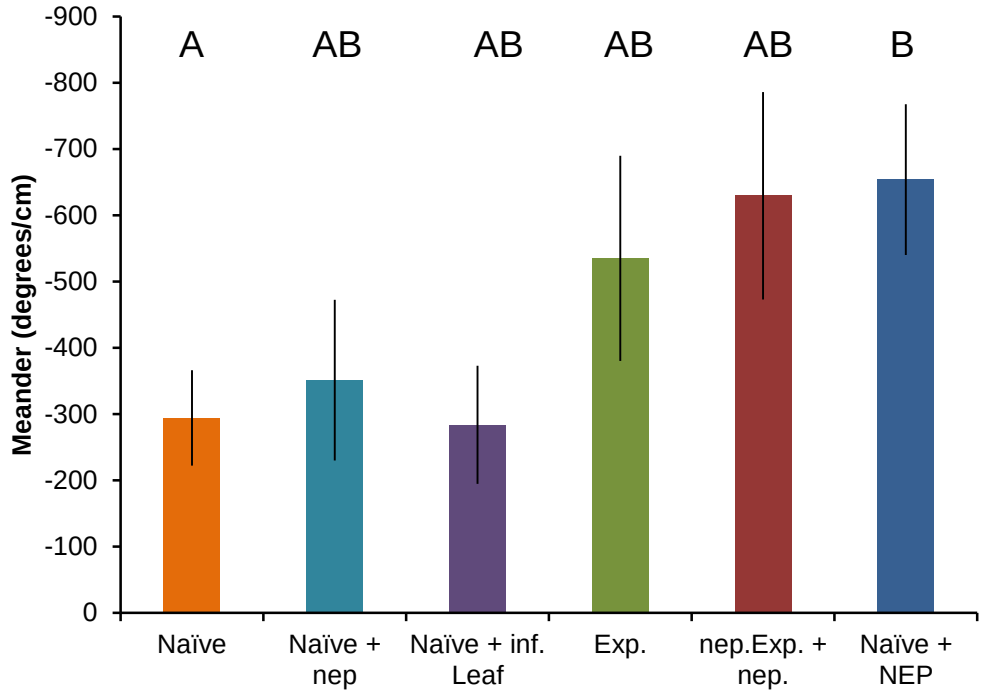


Figure 4.10 Effect of treatment on the turning of A. colemani.

Naïve = no previous ovipositional experience, Exp. = ovipositional experience, nep. = 1 mg/ml concentration of nepetalactone, NEP = 10 mg/ml concentration of nepetalactone and inf. Leaf = an infested leaf. Treatments described following the '+' sign demonstrate that they were present during the assay. Those not sharing a letter are significantly different, $P > 0.05$.

In addition to enhanced klinokinesis, insect foraging is also associated with negative orthokinesis (a reduction in speed) (Hassell & Southwood, 1978). Negative orthokinesis was observed in wasps that had a learning experience of nepetalactone and were re-introduced to it at a later stage (Figure 4.11).

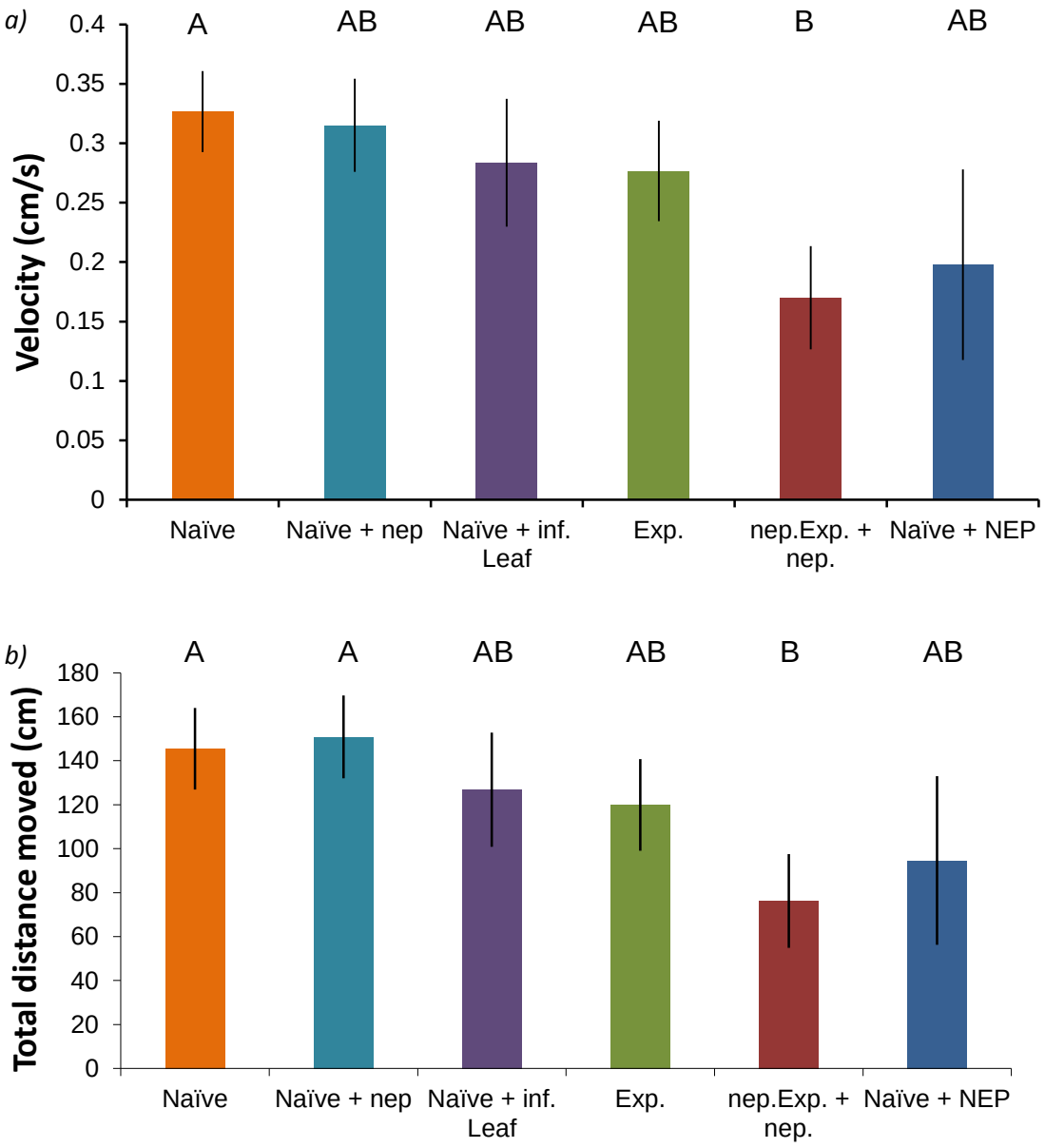


Figure 4.11 Parasitoid orthokinesis.

Results of the motivation assay showing negative orthokinesis as *a)* velocity and *b)* distance moved in response to the parasitoid having learnt a response and then having the odour reintroduced. Naïve = no previous ovipositional experience, Exp. = ovipositional experience, nep. = 1 mg/ml concentration of nepetalactone, NEP = 10 mg/ml concentration of nepetalactone and inf. Leaf = an infested leaf. Treatments described following the '+' sign demonstrate that they were present during the assay. Those not sharing a letter are significantly different, $P > 0.05$.

4.3.5 Leaf location assay

Differences in the foraging pattern of *A. colemani* in response nepetalactone may be representative of an altered foraging pattern, which may improve host location within a patch. The leaf location assay provided a restricted area to test the ability of the parasitoid to locate an infested leaf following learning experiences. Across all

treatments only 21.1% of parasitoids tested (27 of 128) were successful in locating the leaf. The number of individuals successfully locating the leaf material showed little variation across samples (± 0.48) with no significant effect of treatment (3df, χ = 0.406) (Figure 4.12). For those locating the leaf, a difference was seen in the time taken before the first ovipositional attack was made ($F_{3,23}$ = 3.14, P = 0.045). A Tukey post-hoc test revealed that the difference was seen in the experienced wasps with no additional stimuli which took longer to attempt oviposition than all other treatments.

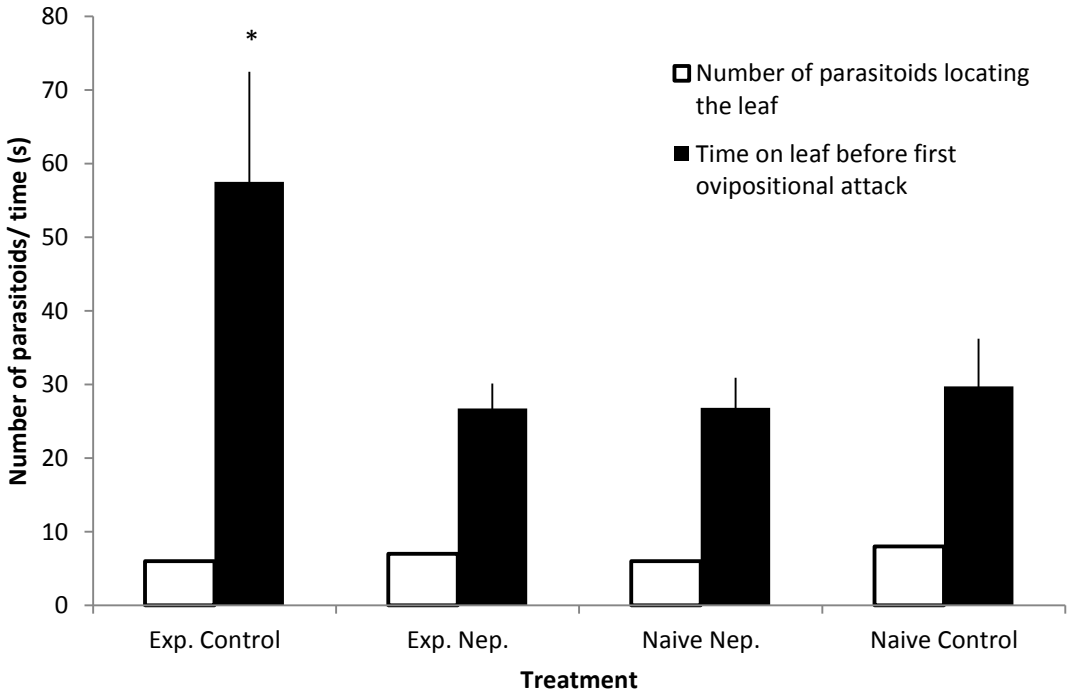


Figure 4.12 Parasitoid leaf and host location.

Number of parasitoids locating the leaf and the time on the leaf before an ovipositional attack was observed. No difference was seen between any of the treatments for numbers locating the leaf, though time to first attack for those finding the leaf was greater in experienced wasps with no additional odour present in the assay * P < 0.05

Looking exclusively at samples in which the leaf was located; no difference was seen between the time taken to locate the leaf as an effect of treatment ($F_{3,23}$ = 0.24, P = 0.870) (Figure 4.13).

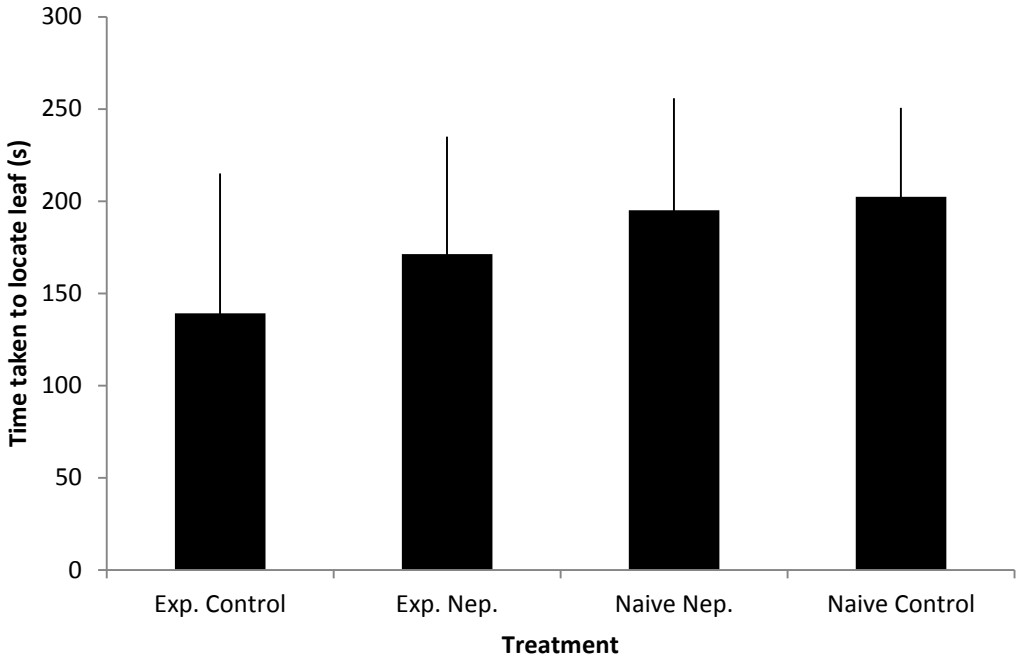


Figure 4.13 Time taken for leaf location.

Time taken for parasitoids to successfully locate the leaf for each treatment. This graphs excludes parasitoids which failed to find the leaf.

The arena remained open throughout the assay allowing the parasitoid to fly out if the odour was not sufficiently attractive. The number of parasitoids flying from the arena was no different between treatments (3df, χ = 0.895) nor was the time at which they escaped ($F_{3,52}$ = 0.42, P = 0.739) (Figure 4.14).

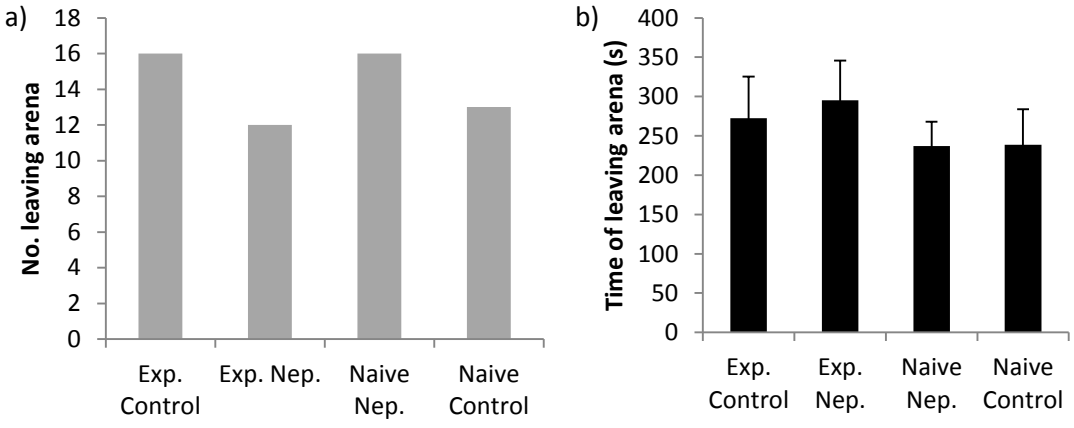


Figure 4.14 a) and b) Parasitoids leaving the arena during leaf location.

a) Numbers of individuals leaving the arena for each treatment and *b)* the times at which they flew out of the arena.

4.4 Discussion

The learning capacity of many hymenoptera species is well established (Giurfa *et al.*, 2001, Laska *et al.*, 1999). One of the objectives of this study was to elucidate whether these learning processes occur with a cue already known to be innate. Manipulation of parasitoid behaviour through learning may be a useful tool to enhance efficacy of wasps foraging in field or glasshouse environments and therefore a practical application to such work was also considered.

Emergence conditioning (EC) offers interesting insight into parasitoid learning but was also one of the most practical manners in which the learning response may be manipulated. In an applied context an advantage of EC is that no additional time is wasted on preparing the parasitoid with a learning experience but that it will learn as a natural part of its release, allowing for large numbers of wasps to be simultaneously taught an odour. It is known that sex pheromones are able to induce success-motivated foraging in insects (Bell, 1990) and it was hypothesised that such behaviour may be observed with *A. colemani*, leading it to stay searching within a patch for longer when given evidence of the host. Emergence conditioning to the sex pheromone odour was not effective in altering the rate of parasitoid attack or the retention time of the wasp. It has been demonstrated that parasitoids *Aphidius ervi* (Glinwood *et al.*, 1999a, Poppy *et al.*, 1997), *Aphidius eadyi* (Glinwood *et al.*, 1999a) and *Praon* species (Hardie *et al.*, 1994a) parasitoid species are attracted to nepetalactone without experience in other assays. Contrary to this, wasps that had EC with either hexane or nepetalactone showed a trend of decreased visitation to the treated nepetalactone arm ($P = 0.053$ and 0.057, respectively); a trend which was matched in the overall percentage of visits made to the nepetalactone arm ($P = 0.073$ and 0.075, respectively) that may have proved significant if sample error could have been reduced. Although the response was inconsistent with the results seen for other parasitoid species it is consistent with recent work on *A. colemani* (Ameixa & Kindlmann, 2011) and experiments in this study using unexperienced *A. colemani* (See Ch. 3).

As demonstrated in Vinson's five stages of host selection (1976), not every parasitoid 'attack' results in an egg being successful oviposition. Initial experiments looked at success in the earlier stages of foraging, host habitat location and host location, but here a look was taken at the success in the later stages of the host selection process: host acceptance and host suitability. It is recognised that in each stage of the host selection process chemical reception plays a role. In many parasitoids it is known that after insertion of the ovipositor into the host the parasitoid may still select not to deposit an egg (Rosenheim & Rosen, 1991). The host acceptance stage is an essential

element in determining the success of parasitoid biocontrol systems. It is hypothesised that the female is likely to deposit an egg in a greater proportion of her attacks if there are more reliable cues within the environment to indicate that the host is viable. It has been shown in *Diaeretiella rapae* that the attack rate of the parasitoid and the time before it makes its first attack can be affected by the volatiles present in the environment (Blande *et al.*, 2004), though this has only been studied with plant volatiles, a response which is highly plastic for parasitoids. The attack rate or success rate were not affected by EC using nepetalactone. The current understanding of EC shows it to be effective in enhancing responses which are highly plastic (Storeck *et al.*, 2000, van Emden *et al.*, 1996), such as the preference for certain host plant complexes (HPC). Without experience the parasitoid will respond to such volatiles (Villagra *et al.*, 2007), however, preference to another HPC will be seen if experience is given. A plastic response to HPC is expected because hosts may be abundant on different plants within different environments or seasons, in contrast the components of the aphid sex pheromone are not subject to temporal variation and similarly it is not expected that the parasitoid would hold such a plastic response to them. In this respect EC is considered a weaker form of learning; it often serves to confirm the environment of the wasp but can be overwritten by a 'stronger' experience. It was also recognised that optimisation of attack rate and speed of attack within a restricted arena may be limited, and in a larger environment it would first be necessary for the parasitoid to find its host.

The association of a reward, such as oviposition, may subsequently make an environment more attractive to the parasitoid. This phenomenon of associative learning is thought to be common to all hymenoptera (Vet *et al.*, 1990). It is hypothesised that in the case of innate odour preferences no alpha-conditioning (enhancement of a pre-existing response) can occur because the innate response is already too strong. Using the 'stronger' ovipositional experience in a square olfactometer there was no increased attraction or retention to the odours than would be expected by chance. Following an ovipositional learning experience of nepetalactone it was found that the nepetalactone treated area was visited less in the square olfactometer. This disproved the hypothesis that visitation to the area, or time spent there, would increase following an experience of nepetalactone with a positive reward, as provided through ovipositional experience. It was expected that the spatial scale dealt with in this experiment may, in part, explain why such results are not seen. It was also anticipated that the result may not be as simple as an attraction or retention but rather a varied and complex serious of behavioural changes represented by an alteration of the overall foraging pattern of the wasp. Although the treatments were placed in the corners of the olfactometer with an airflow coming out of the centre there was expected to be a distribution of the odour across the quadrant, which was

confirmed with visualisations using titanium tetrachloride smoke. As a consequence of the odour dispersal it may be expected that behavioural modifications are observed as parasitoids enter the peripheral area of the odour stream. If an odour was responsible for an immediate arrestant effect the parasitoid may be seen to enter a quadrant less often, however, if the response was elicited after reaching a higher threshold it would be expected that a reduction in the visits would occur closer to the source as the wasp moves up the concentration gradient. Evidence of an arrestant effect following learning was supported by results of the motivation assay, where a decrease in movement was recorded for individuals exposed to nepetalactone following a learning experience with the odour. The slowing of movement when in, or near, a host patch is beneficial to foraging as it increases the time spent within a patch, and therefore the likelihood of encountering a host while in that patch. Although it was observed that the movement was not entirely halted, and instead a slower rate of movement was observed. This may represent a more thorough exploration of the patch and, along with increased turning that was seen in high concentration of nepetalactone, is typical of foraging behaviours (Hassell & Southwood, 1978).

The switch in foraging behaviours triggered by the reintroduction of nepetalactone presumably enhances foraging success, otherwise selection pressures would have acted against these behaviours, especially when the time wasted would be in a potentially host-rich environment. To test the theory that short-distance foraging was enhanced by learning of nepetalactone, the leaf-location assay was run. An experience of nepetalactone did not cause an increase, or decrease, in the number of parasitoids: leaving the arena, locating the hosts or the time it took them to do either. It may be that no effect was seen in the overall number of parasitoids locating the leaf because the overall numbers were very low with only 27 individuals out of 128 finding the leaf in the course of the 10 minute assay. As a consequence, the number of aphid attacks also remained low. Interestingly experienced individuals encountering the leaf took longer to make their first oviposition attempt than any wasps from any other treatment, which was the opposite to what was found in the single-attack assay (Ch. 3.3.4); where experience led to earlier ovipositional attempts on the host. The difference that was observed may be due to differences in the experimental procedure. Parasitoids in the single-attack assay are taken directly from a host-rich environment and released into an assay where an aphid was instantly available, however, in the leaf-location assay the parasitoid experience a foraging stage in which it must search for the leaf in an environment which does not reflect the environment that it has come from, or would naturally encounter. It may be this time spent on the glass base of the olfactometer, without the plethora of visual or olfactory cues it previously experienced, that not only leads to a longer delay in oviposition once a leaf and host are encountered, but that also causes the low rate with which the leaf was located. In the

leaf-location assay the lag between being introduced to the arena and the locating the hosts means the parasitoid may be acting as though a new patch has been entered and require time to affirm that aphids present there are viable hosts.

The absence of a clear attraction in any of the assays led to the hypothesis that either no such behavioural response exists for *A. colemani* or that the response is only observed at a greater distance, presumably mediated by a concentration of the odour or other concomitant stimuli. To confirm the latter it will be necessary to run assays in a larger spatial scale. The former hypothesis does not exclude the possibility that nepetalactone, and the learning of the nepetalactone odour, does elicit alterations in the foraging behaviour of the parasitoid, for which evidence has been found in retention time assays, the square olfactometer and the motivation assay. It still remains unclear if the changes in foraging pattern will enhance the success of the parasitoid during host location, though again it is likely that the spatial scale will play a role in this. A change in the foraging pattern may decrease the likelihood that a wasp will pass over a host patch when foraging on a larger scale, possibly increasing the chance of landing near the host patch to commence probing and walking foraging behaviours. Only the later stage of this foraging process would have been observed in the olfactometers, and thus may have excluded the initial foraging flight which may be of primary importance.

Exploiting the learning capacity of *A. colemani* still offers potential to enhance foraging responses, but this may be best applied to overcome varied rearing backgrounds and not the enhancement of already innate responses. It was ascertained that foraging behaviour of *A. colemani* was altered by a learning experience with nepetalactone but requires a strong reinforcement found in the form of oviposition success. The response to nepetalactone following learning was also different to what has been noted for parasitoids responding to nepetalactone. It has been hypothesised by Glinwood (1998) that the response seen to nepetalactone may not be as simple as attraction, an observation later supported by Powell (2004) suggesting it is the overall spatial distribution of parasitoids which may be affected. This theory was supported by demonstrating alteration in the foraging strategies of the wasps in response to the nepetalactone odour.

EC may not be a sufficiently 'reliable' form of learning to enhance the nepetalactone response, as it provides information regarding the previous generation, and ovipositional experience, which provides information about the current environment, may be required for alpha conditioning to occur. In the leaf location assay an attempt was made to apply this concept and gauge the improvements that may be made to foraging but it is recognised that any understanding of the practical implications of

this work requires studies in a more complex spatial-temporal environment that includes all aspects of the parasitoid foraging.

5 The value of parasitism and nepetalactone in an applied context

Evaluating the effect of aphid feeding on plant fitness and testing parasitoid response to nepetalactone in a more complex spatial-temporal environment

5.1 Introduction

Studies have found nepetalactone responsible for the attraction and alteration of spatial distribution of various aphid parasitoid species (Glinwood *et al.*, 1999b, Powell *et al.*, 2004). It was demonstrated that asexual *M. persicae* are capable of detecting the sex pheromone components and, that in high concentrations, they may be deterred by it (Chapter 2). In chapters 3 and 4 it was shown that a synergy of the host-associated cues and the sex pheromone retained *A. colemani* in an area and that, through learning, it was possible to manipulate its pattern of foraging. These laboratory assays provide vital information on the use of nepetalactone to manipulate the behaviour of *A. colemani* and understand how it may affect *M. persicae* in the environment; but it does require a focus on specific aspects of the insects' behavioural responses. Furthermore, to understand how successful the release of parasitoids will be with nepetalactone, it is necessary to first understand how much damage is being done to the plants by aphid feeding. In this chapter, knowledge of parasitoid learning and response to nepetalactone gained in laboratory assays was applied in a more complex spatial-temporal situation with an aim to determine how behavioural or numerical manipulation of parasitoid release may benefit plant fitness. To do this it is necessary to understand the role of parasitoids in the glasshouse and the challenges facing successful biological control implementation.

5.1.1 Effect of aphid feeding on plant fitness

The purpose of increasing the rate of parasitism within the glasshouse would be with the ultimate goal of increasing the fitness of the plants involved and the yield of the crop, or in the case of ornamental horticulture, the healthy appearance of the foliage. Despite the primary importance of this goal, few studies evaluate this outcome (Chattopadhyay *et al.*, 2001) and most focus on specific aspects of the natural enemies' performance or behaviour. In this study, growth of the foliage was used as an indicator of the fitness of Chinese cabbage to compare the effect of aphid feeding in different population densities. Once this was established it was possible to effectively gauge the benefits that parasitoids will have in the environment through any reduction in the aphid population or its growth.

5.1.2 Parasitoid application in biological control

Parasitoids have been a consistent feature in glasshouse biological control programmes since the early 19th century (Orr & Suh, 1998). In modern glasshouses parasitoids are frequently utilised as part of an integrated pest management (IPM) strategy. IPM typically aims to restrict the growth of pest populations by incorporating various methods of both natural and chemical control. Using a myriad of control strategies creates problems for pests trying to adapt to a specific control method and reduces the overall pesticide application required (Ehler, 2006). Parasitoids are often a vital component of IPM, with hymenopteran parasitoids having been present in 66% of successful programmes (Thacker, 2002). Parasitoids used in such programmes are often sourced from biological control agencies where they are mass reared on a range of host/plant combinations prior to release in glasshouses. To provide the large numbers of parasitoids required in biological control programmes, mass rearing is normally carried out by a commercial company before the parasitoids are distributed to glasshouses. Once in the glasshouse, parasitoids are released in areas where a pest problem is suspected, such as in this case with aphids.

5.1.3 Challenges to successful parasitoid application

The success of parasitoids in IPM often relies on the release of large numbers of *A. colemani* (van Steenis, 1995). The inefficiency of parasitoid foraging is often reported for glasshouse studies and appears contrary to evidence obtained in laboratory assays. Previous laboratory studies demonstrate parasitoids to have an acute sense of olfaction, up to 94 times more sensitive than the most sophisticated human technologies (Rains *et al.*, 2004). Parasitoids combine this proficiency in odour detection with a propensity for complex learning of their environment (Meiners *et al.*, 2003), the adaptability of which is capable of making them highly efficient biosensors able to detect minutiae of odour variation within their environment (Fernández-Grandon *et al.*, 2011). This leaves the question: why are parasitoids not always able to locate their hosts and successfully establish when aphids are present?

The relative inefficiency of foraging in a glasshouse environment can be explained by several factors relating to the rearing and the release of commercially available parasitoids. Poor quality of the parasitoids available arises partly due to inbreeding of populations and transportation of the live insects (Fernandez & Nentwig, 1997). Parasitoids reared in large scale are often reared on whichever host is most readily available. It has been shown that, although parasitoids are frequently capable of oviposition in a variety of hosts, some hosts will provide fitter offspring (Antolin *et al.*, 2006). This can often be related to the size of the host available, with a larger host

resulting in larger, and consequently, more fecund progeny (Sequeira & Mackauer, 1992b). Although it is recognised that problems occur at this early stage, alterations to the rearing and transportation process would be difficult to regulate and would offer no insight into the ecology of organisms, a focus of this study. For this reason the problems faced following the release of the control agents into the glasshouse environment are addressed. As discussed previously, learning occurs before, and during the process of, parasitoid emergence from the host (Ch.4). This may be one of the most considerable challenges to the successful introduction of biological control agents into the field or glasshouse environment. The wasps often encounter an environment radically different to that in which they were reared and may be programmed to search for a host plant complex (HPC) that does not exist in the new environment. The plasticity of learning in generalist parasitoids is demonstrated in their success at locating and ovipositing in the hosts available in biological control systems, however it is the rapidity with which they do it which may be compromised.

5.1.4 Potential solutions to foraging inefficiency

Our work, and that of previous studies, provides two approaches to overcoming the challenges faced by different rearing backgrounds. The first would be a high reward experience following the emergence of the wasp. It has been shown that adult experience takes precedence over other learnt cues if the reward is sufficiently strong (Storeck *et al.*, 2000 for more details see Ch. 4.1.4). The reward which infers the greatest amount of information regarding a host is oviposition in a viable host. An early provision of the HPC available in the new environment may be sufficient to prepare the foraging wasp for the odours relating to successful oviposition. The second option to overcoming foraging inefficiency in a new environment is the provision of a strong and highly reliable cue to which the parasitoid demonstrates an innate response. Stimulating an innate response in the parasitoid may bypass issues related to learning of redundant cues if the parasitoid preferentially focusses on the innate response. Nepetalactone may be the solution; eliciting the innate response necessary to enhance parasitoid foraging within an area, regardless of their rearing background. Although two solutions to the problem are proposed, they are not mutually exclusive and it may be found that a combination of both methods will provide an optimal enhancement of parasitoid foraging.

This chapter aims to establish how the behavioural and numerical manipulation of *A. colemani* present in the environment may benefit the growth of plants. To achieve this it is necessary to ascertain the impact that aphid attack is having on the plant fitness, to determine how reduction in the aphid population will benefit the plants. Although fitness in plant models is often measured by reproductive fitness, flower numbers, fruit set, seed set, the number of seeds or the number of fruits (Leimu *et al.*, 2006), here

vegetative fitness of the plants was used which more accurately relates to the interests of glasshouse growers where the continued reproduction of the plants is not always essential but the appearance of leaves may be critical. Once the fitness cost of aphid attack was realised, a more complex spatial-temporal environment was employed to test the effect that nepetalactone may have on successful parasitism. Attempts are also made to manipulate the parasitoid behaviour by associative learning of nepetalactone with an oviposition reward and by altering the parasitoid numbers released to determine how parasitoid presence may indirectly affect aphid population growth.

5.2 Materials and Methods

5.2.1 Effect of aphid feeding on vegetative fitness

An assay was conducted to determine the impact that aphid feeding was having directly on plant fitness. Chinese cabbage (*Brassica pekinensis* cv. Wong Bok) were grown inside a controlled environment growth room for three weeks. At three weeks old, 16 of the plants showing the most similar leaf sizes were selected. Plants were randomly assigned to four groups (labels were made and then shuffled face-down before being stuck to each plant pot with the assigned groups now visible). The groups were: 0 aphids (control), 10 aphids, 20 aphids and 50 aphids. The leaves of each plant were measured by laying the leaf flat against a red laminated card with a scale bar along the side. Red was chosen as it provided the greatest contrast to the green leaves. An image was then taken using a digital camera (DSC-W55 Sony, Japan) to be analysed with ImageJ software (v. 1.46). A contrast threshold was acquired to separate the leaf from the background. The scale on the card in the background was used to provide reference; once the leaf was highlighted the 'calculate area' tool could be used to provide the area of the leaf. After all leaves were measured, aphids were applied to the four outmost leaves evenly (or as evenly as possible given that not all numbers were exactly divisible by four). Each treatment group had a separate tray (76 x 37.5 cm) filled to around 2 cm with water and surrounded by 10 x 25 cm yellow sticky traps (Fargro, UK). These were used to decrease the potential for cross contamination between treatments. Yellow sticky traps were also hung to provide monitoring data on the alatae movement within the growth room. The growth room was maintained at 25°C, 60% humidity with a 16:8 day:night light cycle. Every second day of the experiment two plants were selected from each treatment and the number of aphids were counted with the assistance of a mechanical hand tally click counter (ENM counting instruments, UK). The plants selected from the groups for counting alternated on each count day and were used to estimate the rate of aphid population growth. After 10 days, following a final aphid count for all treatments, each plant had

the outer four leaves' areas measured in the same manner as the start of the experiment. The number of leaves for each plant was also counted. For each trial, four plants were used in each treatment, these were considered only pseudo-replicates used to provide a more accurate mean for each trial. Four true biological replicates were completed for each treatment.

Despite the consistency of the environment for all treatments and elimination of any confounding variables, differences were observed in growth rate of the control group Chinese cabbages between trials. It is speculated that such variation relates to different seed quality between batches, something that it was not possible to control but was possible to account for. To account for the differences in growth rate, the trial groups were 'standardised' to that of the first trial in a similar to the procedure used in EAG studies (Kapitsky & Zhukovskaya, 1999) i.e. if the control group in trial 2 showed 7% more growth than the control group from trial 1 then all samples in trial 2 had 7% deducted from them, all trials were compared using this technique to the original control group. The data were analysed using a one-way ANOVA (Minitab 16). To determine where the differences lay, a Tukey posthoc test was applied.

5.2.2 Enhancing foraging through learning in a complex spatial-temporal environment

Laboratory bioassays provide essential information about the behavioural patterns of *A. colemani*, however, to truly recognise the impact that this may have in biological control, trials in a more complex spatial-temporal environment were required. The introduction of nepetalactone to different positions within the glasshouse allows us to determine if the compound was attractive to the parasitoids at this scale.

Two glasshouse compartments measuring 3 m x 4.82 m were used for this experiment. Within each glasshouse compartment there were allocated three positions for the plants, each position being 1.5 m from the parasitoid release point (Figure 5.1). Five plants were placed in each position with one in the centre and the other four forming a square around it, all plants used were Chinese cabbage in their third week of growth (for growing conditions see Ch.2.2.1). Outer plants were placed exactly 25 cm from the central plant. On each plant, 25 aphids were placed using a size 0 paintbrush. The aphids were all from 3rd instar to adult size taken from laboratory reared cultures (for details on the culturing see Ch. 2.2.1). Twenty-four hours after the aphids were placed on the plants, the treatments were added to each position. Treatments were administered through 1.5 ml amber autosampler vials (Sigma-Aldrich, UK). These vials had a 1 mm hole pierced in the centre of the lid and rubber seal. For nepetalactone a treatment of 50 µl of a 10 mg/ml solution was placed in the

vial and for the control groups 50 µl of hexane was used. The vials were affixed to a plant label which was then inserted into the soil with the vial remaining elevated 4 cm above the soil. Although nepetalactone is more likely to be available as an infused polymer, release rate from these strips was not found to be sufficiently reliable to quantify as introducing a known liquid quantity (Appendix B).

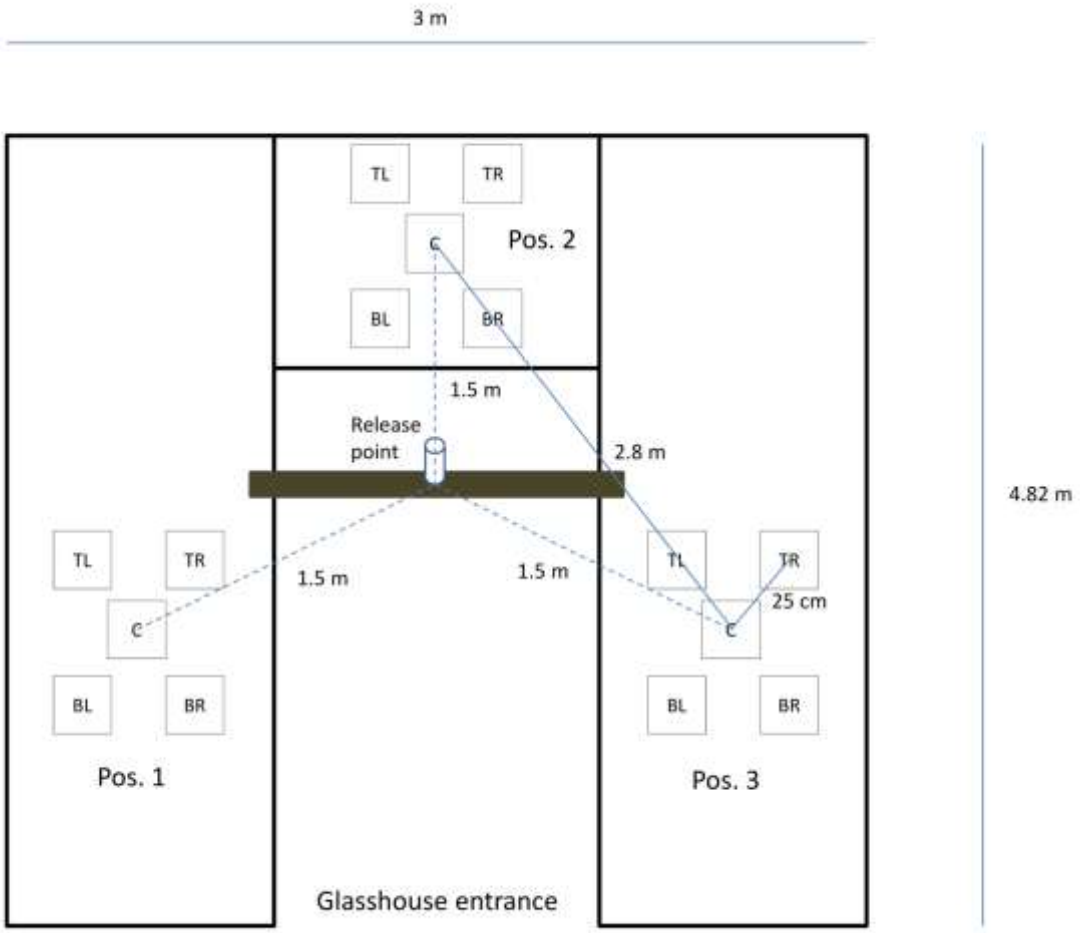


Figure 5.1 Layout of glasshouse compartment.

Layout of the glasshouse compartments used for glasshouse trials. Plant positions are given relative to the entrance. TL = Top left, TR = Top right, C = centre, BL = Bottom left and BR = Bottom right.

Five female parasitoids were pooted into an empty container produced by Koppert for the storage and transport of parasitoids (4 cm i.d., height 9 cm). All the parasitoids used were two days old, provided by Koppert (Koppert B.V., UK). Across the benches of the glasshouse a wooden beam was placed, the middle of which was the 'release point' where the release chamber was positioned. Release chambers were constructed by removing parts of a parasitoid storage container and affixing it to the opening of

another, allowing parasitoid exposure to a selected treatment as they exit the chamber (Figure 5.2).

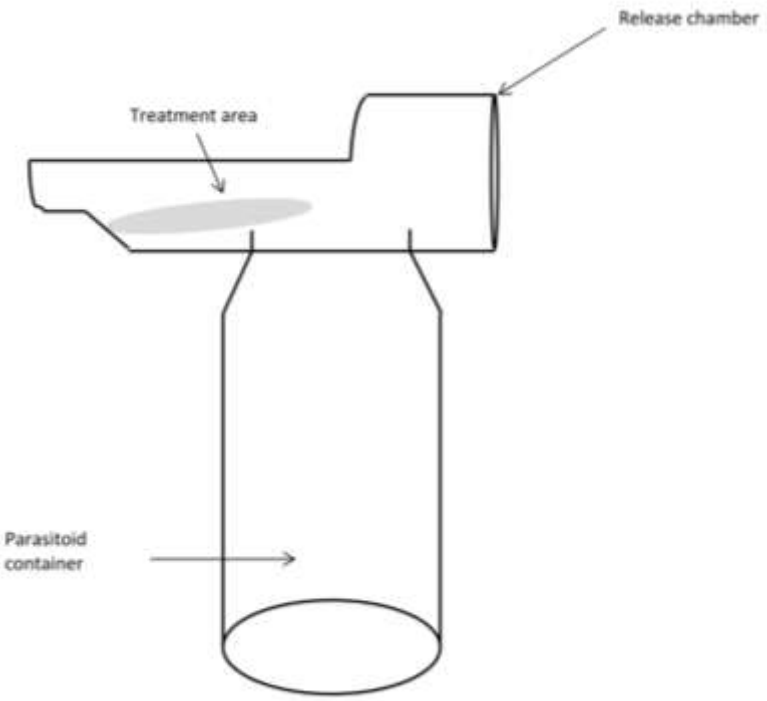


Figure 5.2 Parasitoid release chamber.

When used in trials five female parasitoids are pooted into the container before it was covered. Once in the glasshouse the lid was removed and the upper chamber with the treatment was affixed.

The parasitoids were given eight hours to exit the container, explore the environment and oviposit freely in aphid hosts. When the parasitoids were within the compartments no additional lighting was used and the flood benches in the glasshouse did not operate to reduce the likelihood that parasitoids would die by landing on a hot lamp or drowning. After eight hours had expired the release container and chamber were opened to count any wasps that had remained inside for the duration of the experiment. The number of parasitoids found on each plant was recorded and the wasps were destroyed. Each plant had a 30 cm x 25.5 cm bread bag (W.R. Wright & Sons Ltd., UK) placed around it and sealed against the pot using an elastic band. The plants were maintained in an environmental control room for 10 days at 21 ± 1°C, 16:8 day:night light cycle. Two days after each trial was conducted aphids present on each plant were counted. 10 days after the trial, total aphid numbers on central plants and the number of mummies present on each plant were counted. The treatment positions varied between each run of the experiment allowing all positions to be tested and the glasshouses in which the treatments were used was also varied allowing an equal amount of runs in each glasshouse with each treatment and position.

In treatments all other factors remained constant except treatments applied in the release chamber. The three treatments used were:

Control: Release chamber empty

HPC: Chinese cabbage leaf with 10 aphids in release chamber

HPC + Nep: Infested leaf (as previous) with 2 µl of 10 mg/ml nepetalactone on a 1 cm x 2 cm piece of filter paper

5.2.3 Manipulating parasitoid numbers in a complex spatial-temporal environment

An assay was conducted to determine the effect of parasitoid presence on aphid populations in a glasshouse environment when presented in varying numbers and with nepetalactone present. The assay was designed to evaluate the direct effect of parasitoid numbers on mummy formation in addition to the indirect effects on aphid population growth.

Trials to manipulate parasitoid numbers were completed using the same methods and glasshouse compartments as the trials assessing learning. The only differences to the set-ups were the addition of uninfested 'trap' plants, removal of the release chambers and altering the numbers of parasitoids released. A total of eight treatments were used which represented variation in the number of parasitoids released and the presence or absence of nepetalactone. Treatments were defined by the release of 0, 10, 25 or 50 female *A. colemani* into the glasshouse with nepetalactone located at one of the plant positions; all other plant positions contained a vial of hexane. Each treatment was mirrored by a control group in which no nepetalactone was applied and instead hexane was provided at each position.

Four trap plants placed at each position were intended to attract aphids that had attempted to disperse from the treatment plants, providing an indication of the rate at which aphids attempted to escape the plant. Trap plants were intact Chinese cabbage plants at the same stage of growth (week three) which were positioned between the infested plants (Figure 5.3).

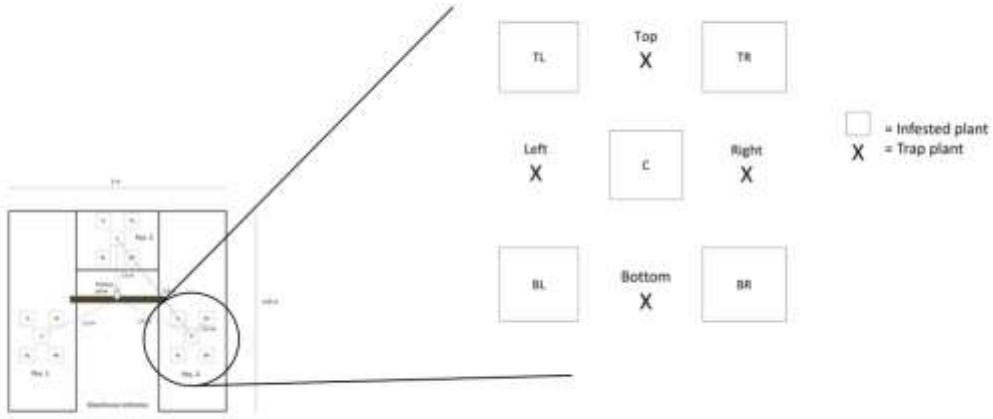


Figure 5.3 Placement of trap plants.

Infested and uninfested (trap) plants at each position within the glasshouse, the remaining layout remains the same as Figure 5.1. Directional assignments are given relative to the glasshouse entrance.

Counts of the aphids and mummies were performed as in the 1st set of glasshouse trials. In addition, the number of aphids found on trap plants at the end of each run was counted and these plants were maintained for 10 days when a mummy count could also be made. Attempts were made to balance the final aphid counts made for plants with mummies and those without. Therefore, if all three of the centre plants from a trial had mummies, additional counts would be made from a plant at each position which did not have any mummies providing a balanced final aphid count representing three plants with mummies and three plants without. This was not always possible because some trials led to mummy formation in every position, or an absence of mummies across most samples.

5.3 Results

5.3.1 Vegetative fitness

Aphid feeding caused a reduction in the final number of leaves found on each plant ($F_{3,73}$ = 6.45, P = 0.001) (Figure 5.4). A Tukey post-hoc test reveals that the differences lie between the control group and cabbages containing 20 or 50 aphids. Although variation in the leaf numbers between treatments remained low (10.82 ± 0.17, with a mode of 12) the difference was consistent enough to be significant across treatments.

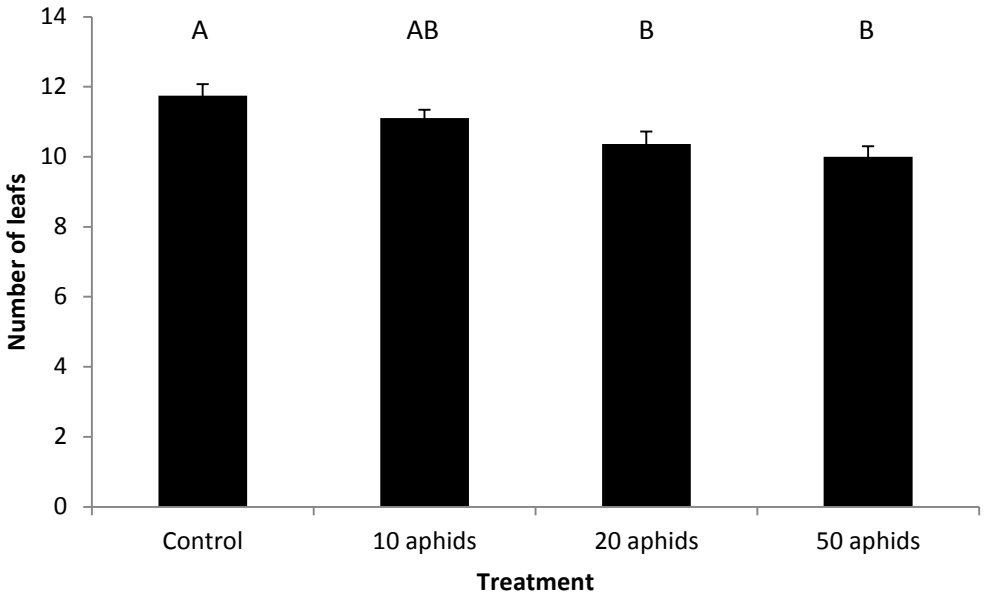


Figure 5.4 Leaf numbers as an effect of aphid attack.

The number of leaves counted after 10 days of the experiment. Treatments of 0, 10, 20 and 50 aphids. Those not sharing a letter are significantly different as determined by a one-way ANOVA and Tukey posthoc test ($F_{3,73} = 6.45$, $P = 0.001$).

The percentage of leaf growth was also observed to be different between treatments ($F_{3,20} = 5.12$, $P = 0.009$). A Tukey posthoc test revealed that growth was greater for plants in the control than for those with an initial population of 50 aphids (Figure 5.5).

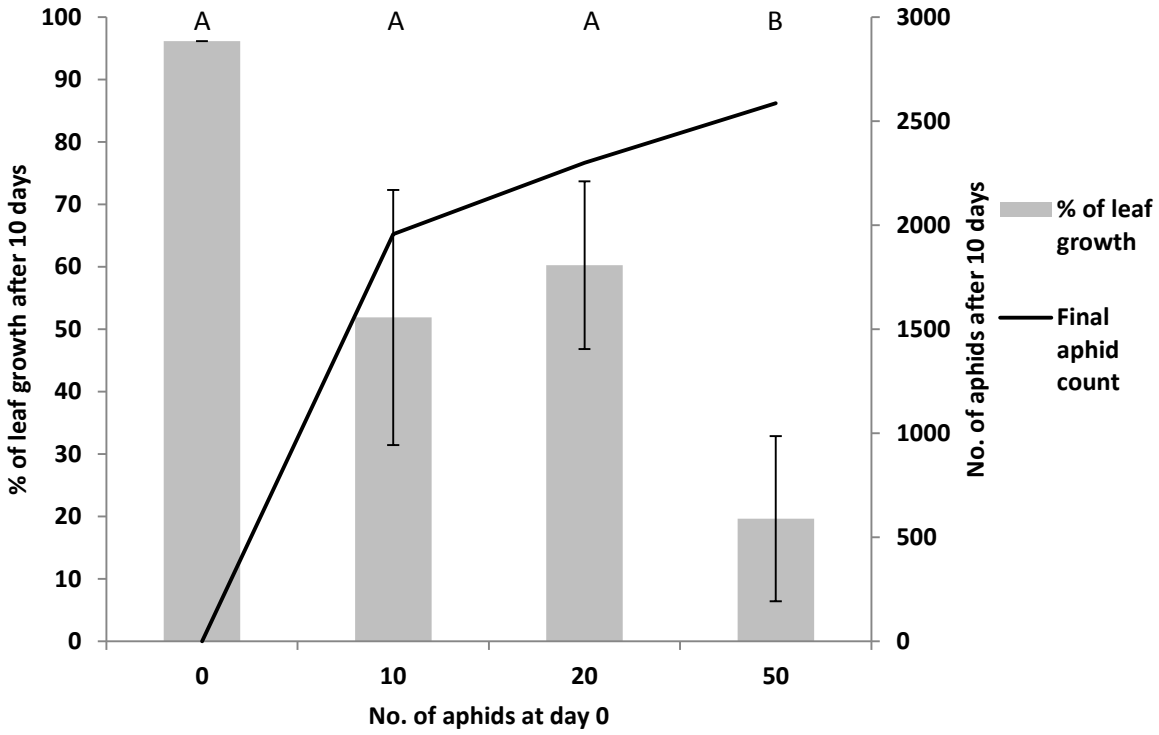


Figure 5.5 Percentage of leaf growth following aphid attack.

Leaf growth is seen for 3 week-old Chinese cabbage over a 10 day period and the impact of *M. persicae* feeding. The total number of aphids after the 10 days of reproduction can also be seen. Those not sharing a letter have significantly different levels of leaf growth ($F_{3,20} = 5.12$, $P = 0.009$).

Final aphid population size was found to differ between treatments ($F_{3,28} = 27.48$, $P < 0.001$), though it was found that this difference was only observed between treatments containing aphids and the control. With the control group removed, no difference was seen between treatments in the final count (after 10 days) of the aphid population ($F_{2,21} = 1$, $P = 0.384$). Differences in the aphid populations were only seen to emerge in the fourth day following infestation ($F_{2,21} = 158.05$, $P < 0.001$) and were evident to the eighth day ($F_{2,21} = 13.83$, $P < 0.001$) (Figure 5.6*a-e*). Tukey posthoc analysis revealed that differences existed between all treatments on the fourth and sixth day of the experiment, however, by the eighth day the aphid count for treatments of 10 and 20 aphids had begun to converge, and only the treatment of 50 aphids was significantly greater (Figure 5.6*a-e*).

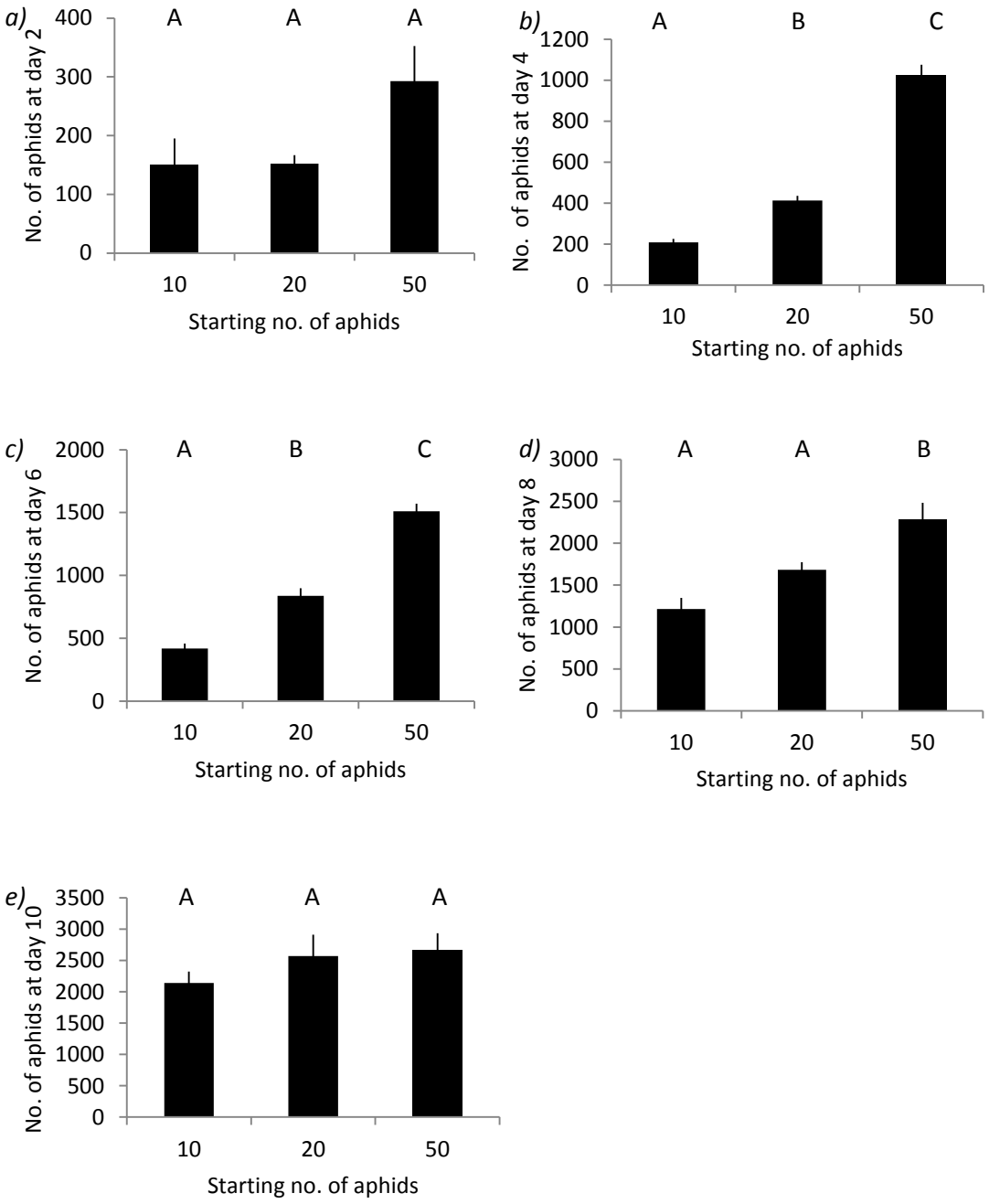


Figure 5.6a)-e) Aphid populations from days 2-10 of the foliar fitness experiments.

Within each chart those not sharing a letter are significantly different ($P < 0.01$). Control group is not shown, though was significantly different in each stage of growth ($P < 0.001$) with a mean of 0.2 aphids.

5.3.2 Enhancing foraging through learning in a complex spatial-temporal environment

Excluding the effect of any release treatments used, plants alongside nepetalactone vials showed no greater rate of parasitism than hexane (H_1 = 0.01, P = 0.942). Around 5.62 ± 0.97 mummies were found to be formed following parasitoid exposure in the control groups. The position of the plant within the glasshouse had no effect on the rate of parasitism observed (H_2 = 2.71, P = 0.258). None of the parasitoids were found in the release chamber at the end of any experimental day, for any of the treatments on any of the sampling days.

No difference in parasitism was observed between treatment groups ($F_{2,267}$ = 0.92, P = 0.398) implying that at this stage learning had no immediate effect on the proficiency of the parasitoid in locating hosts (Figure 5.7).

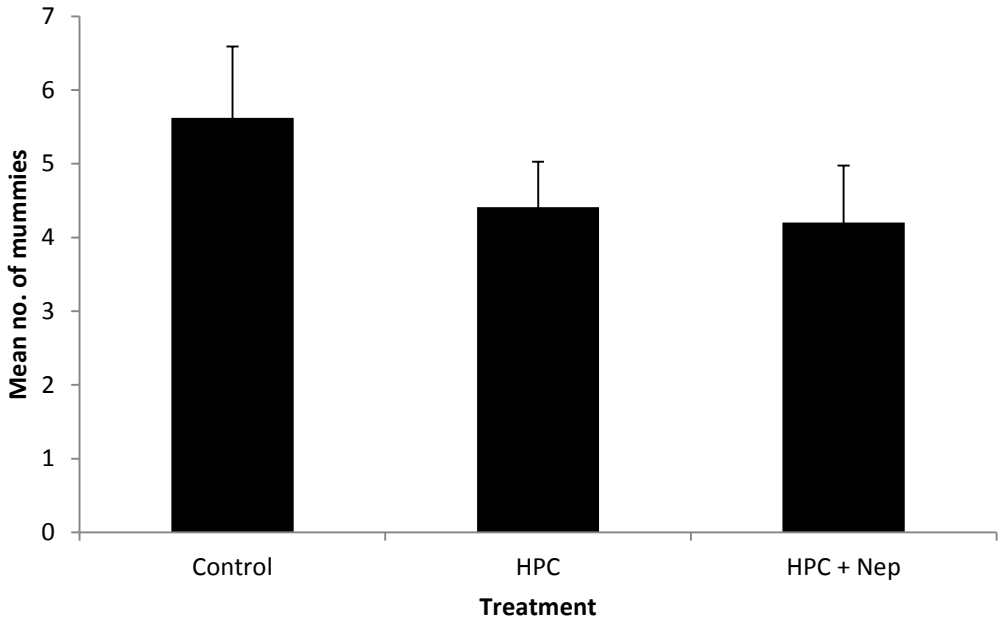


Figure 5.7 Number of mummies by treatment.

Comparison of the mean number of mummies formed for each treatment set. Each treatment includes the release of 5 naïve female *A. colemani* which alter only in the treatments contained in the release chamber. Release chamber treatments are: Control = no treatment, HPC = a leaf infested with 10 aphids and HPC + Nep = a leaf infested with 10 aphids and nepetalactone. No treatment was found to contain significantly more mummies ($F_{2,267}$ = 0.92, P = 0.398).

The number of aphids counted at two and 10 days after parasitoid release reveal no difference in size of the aphid populations between treatment groups at day two ($F_{2,51}$ = 1.29, P = 0.284) or at day 10 ($F_{2,51}$ = 0.32, P = 0.73). Despite no difference in the number of mummies between treatments, if they were divided by attacked and not attacked a difference was observed. To study the potential effect of parasitoid attack

on an aphid population, 'attacked' was defined as plants containing mummies; the only certain way of demonstrating parasitoid visitation. Plants containing mummies had significantly less aphids than those with no mummies ($F_{1,52}$= 39.16, P < 0.001) (Figure 5.8).

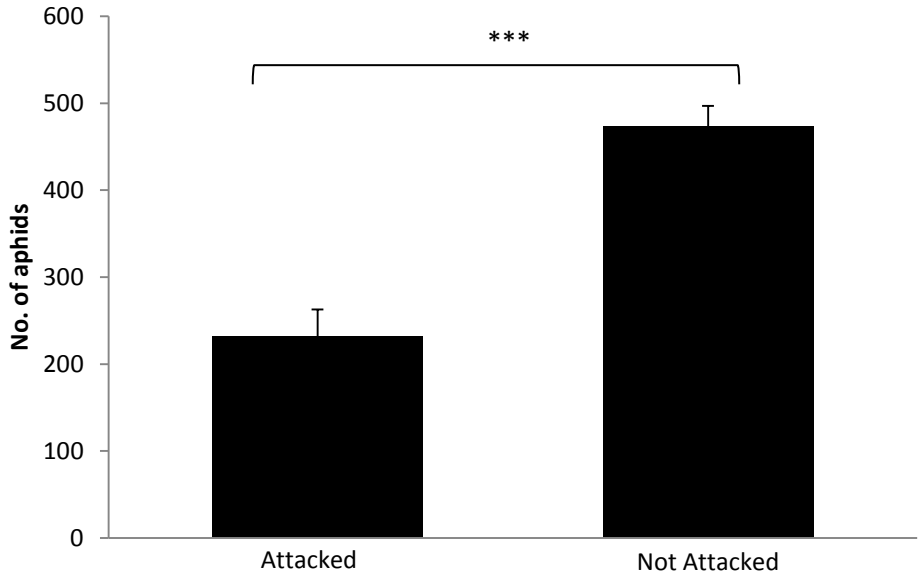


Figure 5.8 Effect of parasitoid attack on aphid population growth.

The number of aphids found on the plants 10 days after exposure to parasitoids. The aphid populations were all started with 25 *M. persicae* individuals. Where 'Attacked' are those showing the formation of any mummies after 10 days and 'Not Attacked' are populations which show no mummies having formed. *** P < 0.001. N = 54 (22 attacked and 32 not attacked).

Looking only at samples containing mummies, a regression analysis was carried-out to find any relationship between the number of mummies forming and the number of aphids found at the end of the experiment. No correlation was shown to exist between number of mummies on the plant and the final aphid population count ($F_{1,20}$= 0.68, P = 0.42) (Figure 5.9), implying the reduction in the aphid populations observed in the study was not directly related to the number of successful oviposition attempts but may be influenced by other factors related to the parasitoid visiting the plant.

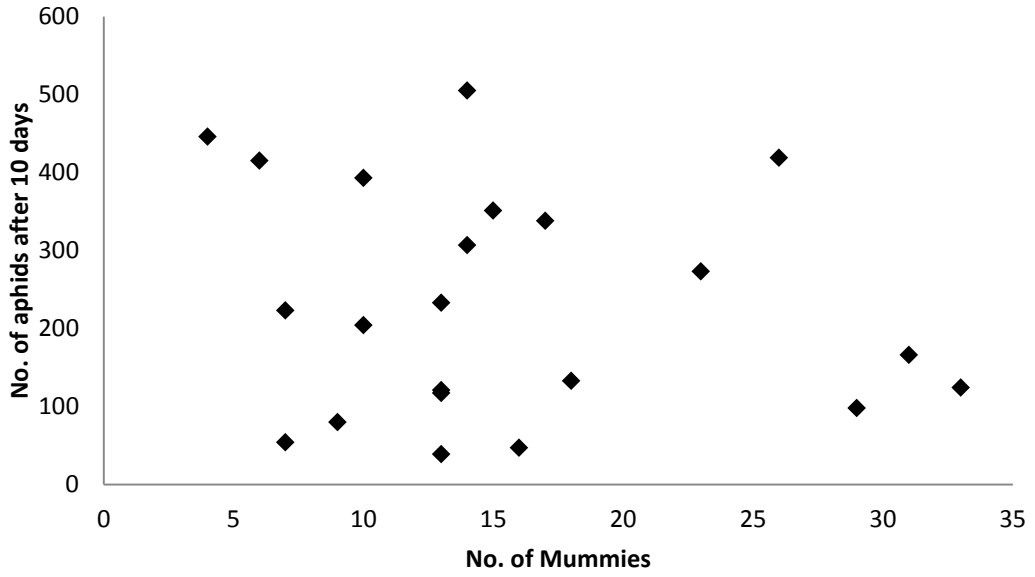


Figure 5.9 Correlation of mummies to aphid population growth.

Including only populations which contained *A. colemani* mummies, the number of mummies found after the 10 day period and the number of *M. persicae* were not correlated ($F_{1,20}$= 0.68, P = 0.42).

5.3.3 Manipulating parasitoid numbers in a complex spatial-temporal environment

Differences were observed between the numbers of mummies as a result of the parasitoid numbers ($F_{3,464}$ = 95.83, P < 0.001), though were not affected by odour treatment ($F_{1,464}$ = 1.84, P = 0.176) or the interaction of parasitoid numbers and treatment ($F_{3,464}$ = 2.4, P = 0.067) (Figure 5.10).

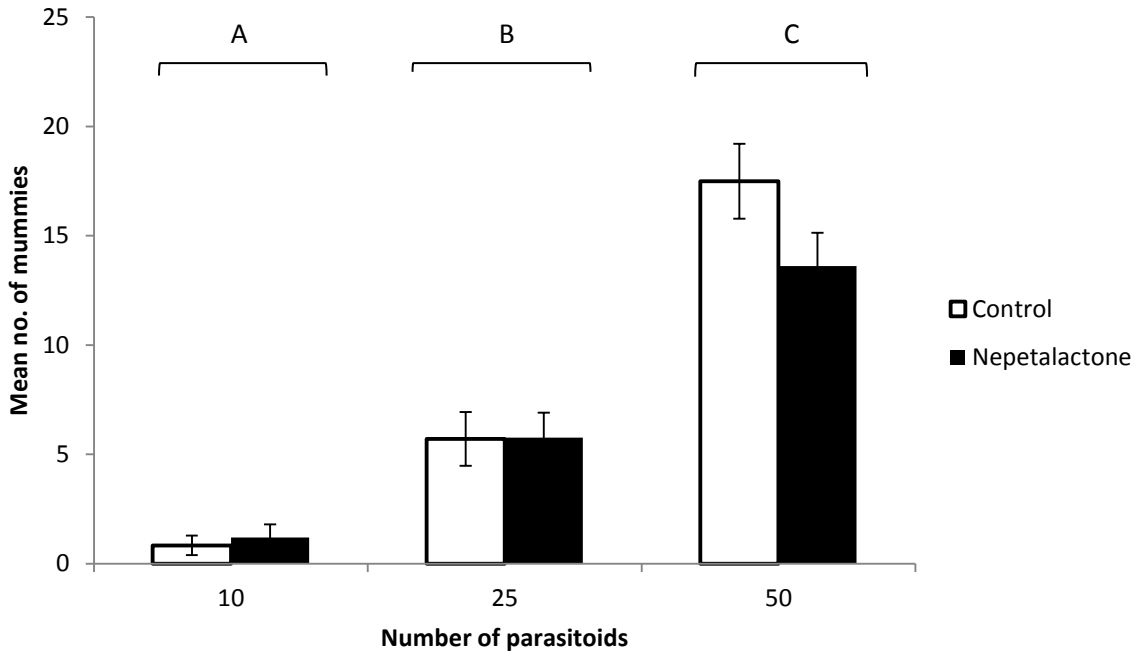


Figure 5.10 Number of mummies found for each treatment group.

Treatment groups are defined by the number of *A. colemani* females released and the presence or absence of nepetalactone in the glasshouse. Number of mummies was only affected by the number of parasitoids released into the glasshouse and not by the odour present ($F_{3,464} = 2.4$, $P = 0.067$). The control group of 0 parasitoids was included in the analysis but is not represented in the figure as no mummies formed. Those not sharing a letter are significantly different ($F_{3,464} = 95.83$, $P < 0.001$).

Similarly the aphid count after two days was affected by the number of parasitoids released into the environment ($F_{3,435} = 51.29$, $P < 0.001$) but not odour ($F_{1,435} = 0.96$, $P = 0.327$) or the interaction of the two ($F_{3,435} = 0.34$, $P = 0.799$). A Tukey posthoc test shows where the differences existed (Figure 5.11).

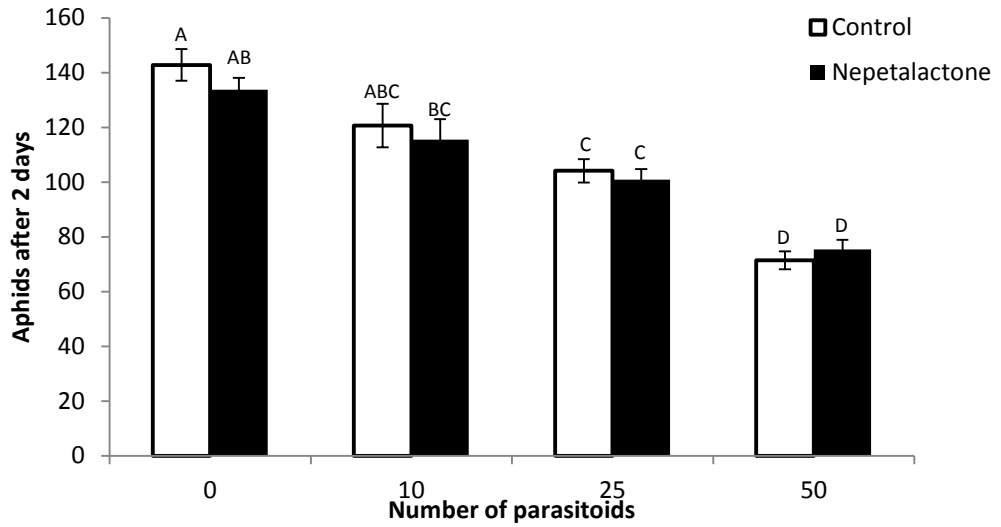


Figure 5.11 Effect of nepetalactone and parasitoid numbers on day 2 aphid populations.

Effect of the treatment (0, 10, 25 or 50 parasitoids released with or without nepetalactone) on the number of aphids present after two days. Those not sharing letters are significantly different ($F_{3,435} = 51.29$, $P < 0.001$).

The effect of parasitoid release that was observed for day two aphid counts was not seen in the final (day 10) aphid counts in any samples other than the control groups ($F_{3,118} = 37.67$ $P < 0.001$) (Figure 5.12). As seen at two days, neither the odour treatment ($F_{1,118} = 1.66$ $P = 0.2$) or the odour/parasitoid interaction ($F_{3,118} = 1.2$ $P = 0.312$) affected aphid population size.

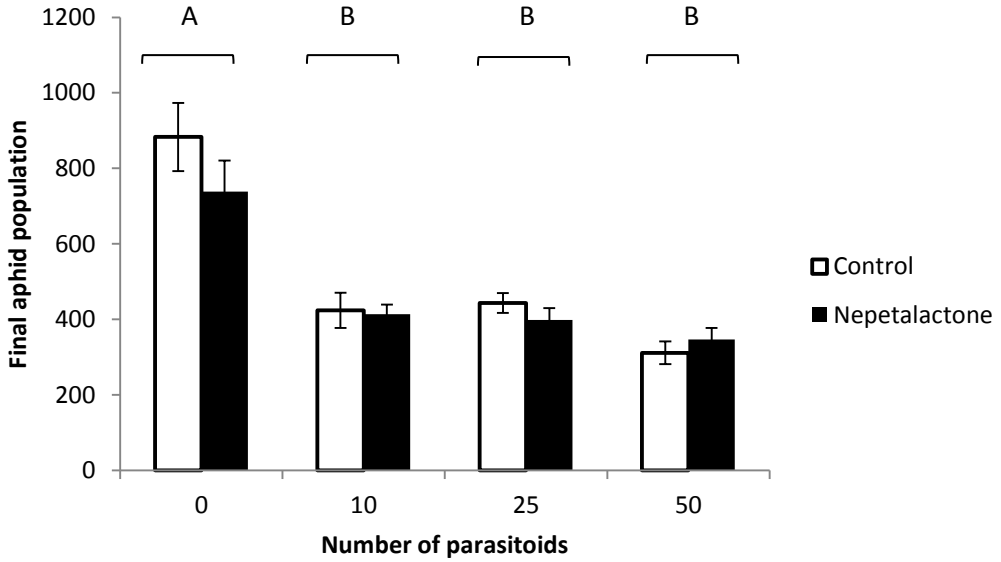


Figure 5.12 Effect of nepetalactone and parasitoid numbers on final aphid population.

Those not sharing a letter are significantly different ($F_{3,118} = 37.67$ $P < 0.001$).

Within treatments using nepetalactone, the pheromone was placed at one of three possible positions within the glasshouse. It was found that nepetalactone did not increase or decrease the number of mummies around the vials with the release of 10 ($F_{1,58}$ = 2.02, P = 0.16), 25 ($F_{1,57}$ = 0.14, P = 0.705) or 50 parasitoids ($F_{1,56}$ = 0.51, P = 0.477). Similarly nepetalactone did not cause any localised differences in the number of aphids after two days in treatments of 0, 10, 25 or 50 parasitoids ($F_{1,214}$ = 0.03, P = 0.873) (Figure 5.13).

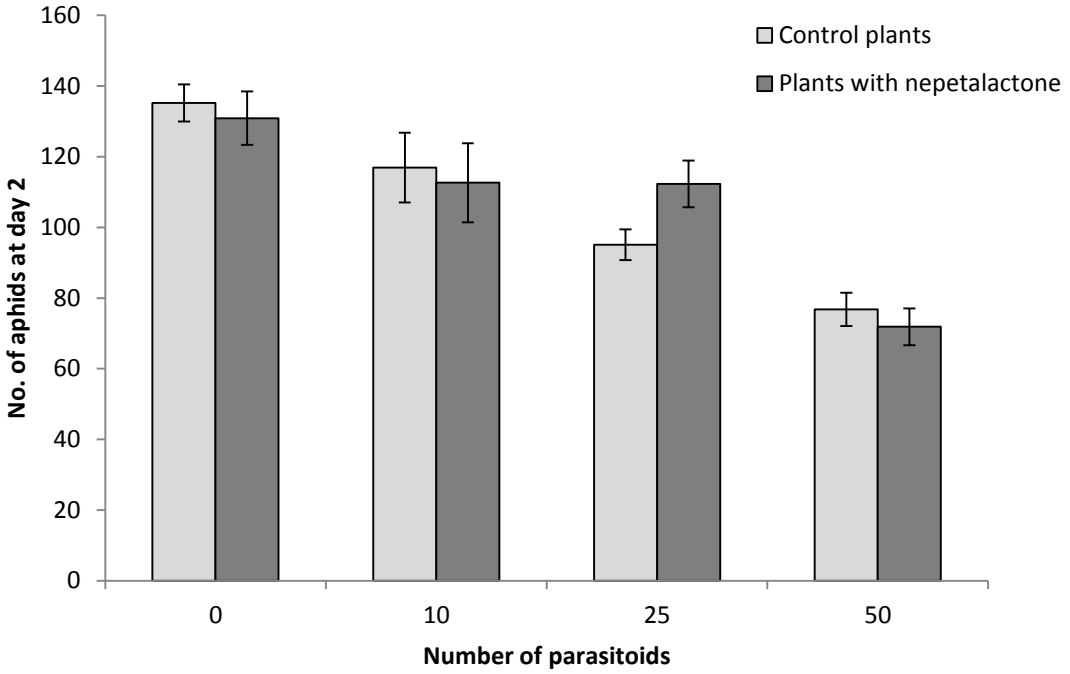


Figure 5.13 The effect of nepetalactone proximity on 2 day aphid counts.

Comparing the mean number of aphids two days after parasitoid attack in glasshouses containing nepetalactone. Values are given for plants alongside the nepetalactone and those at a greater distance from the source ('Control plants'). No significant differences were seen as result of proximity to nepetalactone ($F_{1,214}$ = 0.03, P = 0.873).

No difference was seen in the number of trap plants containing aphids for each treatment ($F_{7,24}$ = 1.47, P = 0.224) or the number of aphids found on the trap plants ($F_{7,24}$ = 0.88, P = 0.535). The proportion of aphids resulting in mummies on standard treatment plants was calculated for each parasitoid release and used as the expected value for trap plant mummification using a one-sample t-test. Populations of aphids established on trap plants were shown to have the same proportion of mummies as would be expected for regular experimental plants for 10 (T_3 = 0.11, P = 0.918), 25 (T_3 = 0.62, P = 0.577) or 50 parasitoid releases (T_4 = 0.62, P = 0.354) (Figure 5.14).

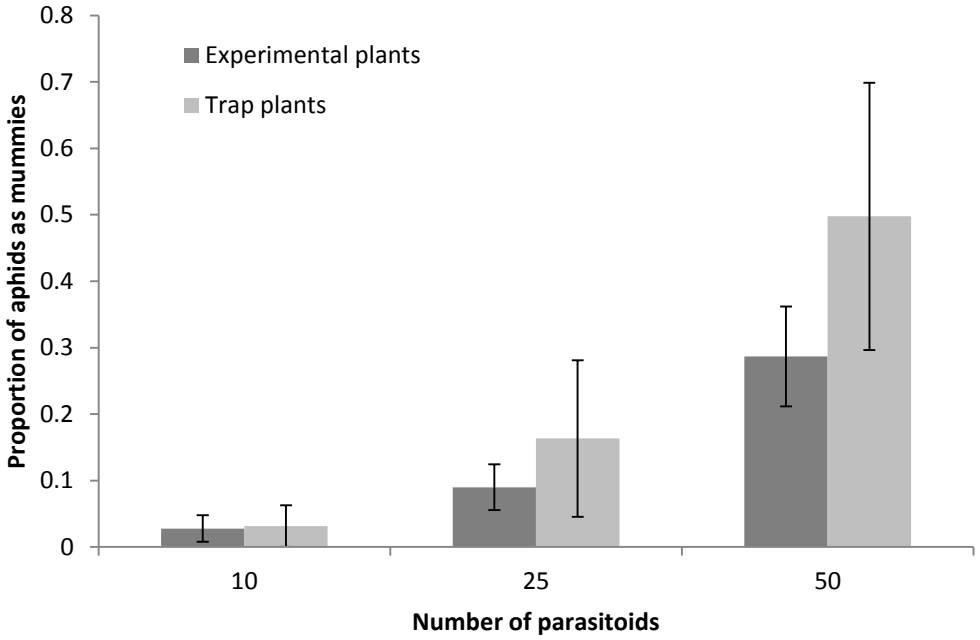


Figure 5.14 Proportion of mummies in trap and experimental plants.

The proportion of aphids found to be successfully mummified after 10 days in experimental and trap plants. Trap plants are found not to have significantly different proportions of parasitized aphids than is expected to be seen throughout the population, given the number of parasitoids released ($P > 0.05$).

Combining all treatments provided the opportunity to evaluate the differences between plants with mummies, those with no evidence of attack and the control group in which it is known no parasitoids were present. It was found that there was a difference in the day two ($F_{2,440} = 53.64$, $P < 0.001$) and final aphid count between these groups ($F_{2,123} = 61.51$, $P < 0.001$). A Tukey posthoc showed all treatments to differ, with the control group showing a greater aphid population than unattacked plants and attacked plants exhibiting the fewest aphids (Figure 5.15).

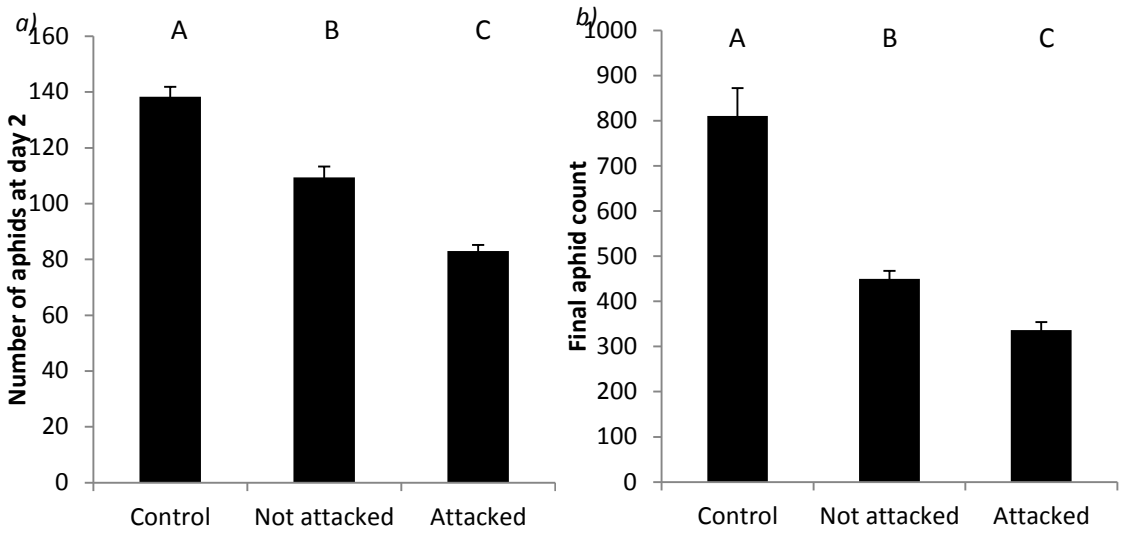


Figure 5.15 Effect of parasitoid visit on the growth of aphid populations.

Effect of parasitoid attack is seen at *a)* 2 days and *b)* 10 days. 'Attacked' are those plants containing any mummies, 'Not attacked' are plants in glasshouses with parasitoids though showed no mummies and 'Control' plants were never exposed to parasitoids. Those not sharing a letter are significantly different ($P < 0.001$).

Although a greater number of parasitoids did result in reduced aphid population size after two days and in the final count (Figure 5.15). No direct correlation appeared to exist linking successful oviposition, as demonstrated by the number of mummies appearing, to the reduction of aphids at day two ($F_{1,139} = 3.58$, $P = 0.061$) or in the final count ($F_{1,41} = 2.63$, $P = 0.112$) (Figure 5.16 *a* & *b*).

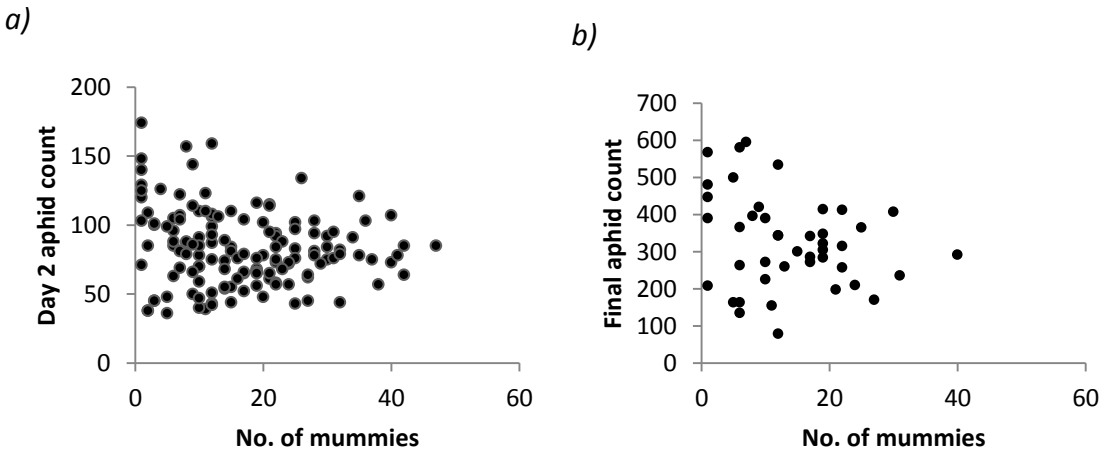


Figure 5.16 Correlation between mummies and aphid numbers.

Including only plants which contained *A. colemani* mummies no correlation was present between the number of mummies found *a)* two days after attack ($F_{1,139} = 3.58$, $P = 0.061$) or *b)* after 10 days when the final count was made ($F_{1,41} = 2.63$, $P = 0.112$).

Despite no correlation in the number of mummies found and the aphid population size, a greater number of parasitoids present in the environment did lead to a reduction in aphid population size even in plants showing no sign of attack. Differences were seen both at day two ($F_{3,298}$ = 19.72, P < 0.001) and in the final aphid count taken after 10 days ($F_{3,73}$ = 18.22, P < 0.001) (Figure 5.17*a & b*).

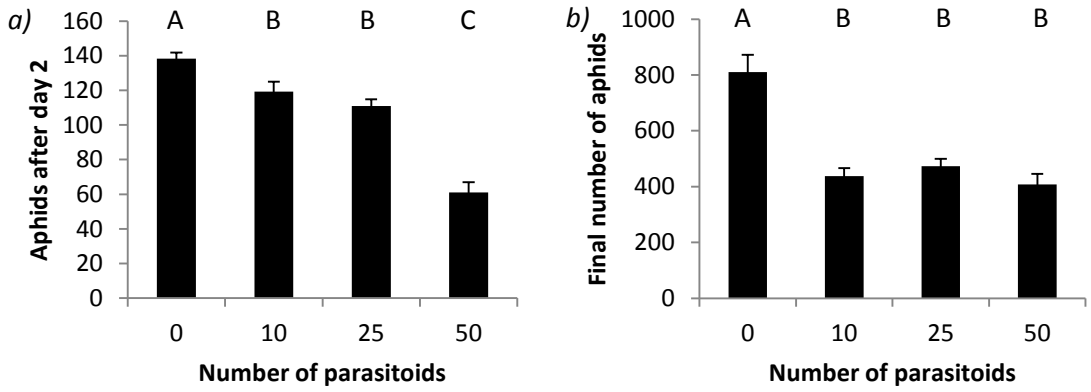


Figure 5.17 **Effect of parasitoid numbers on 'unattacked' aphids.**
Combined data from the control and nepetalactone glasshouses comparing the aphid population at *a)* two and *b)* 10 days in plants showing no sign of parasitoid attack. Those not sharing a letter are significantly different (P < 0.001).

5.4 Discussion

In this study it was possible to determine the impact of aphid feeding on the vegetative fitness of Chinese cabbage and the impact that parasitoids can have on aphid population dynamics when present in the environment. Although the work provides insights into how biological control can be enhanced it did not demonstrate an effective application of nepetalactone in this spatial-temporal scale.

5.4.1 Vegetative fitness

Biological control programmes are frequently defined by the objective of reducing pest populations (Fauvergue *et al.*, 2012), while this is an essential function of biological control programmes, it ignores the overall aim to increase the health of the plant and, in food crops, the yield. Few studies evaluate how the success of natural enemies actually benefits crop yields (Östman *et al.*, 2003). The current understanding of tritrophic systems lends to the theory that plants 'cry for help' by the production of volatiles (Dicke, 2009, Gershenzon, 2007, Kessler & Baldwin, 2001), however, there is little evidence to demonstrate the benefits of volatile output or parasitoid visitation to the plant. In this study leaf growth was looked at as an indicator of fitness. Vegetative fitness is a useful indicator of fitness because it does not rely on seed production from

the plant and can, therefore, be completed in a shorter time, but also because foliar growth accurately reflects damage to the plant which would affect ornamental growers or crops in which the leaf is for human consumption. It was shown that 10 days after establishing a population of 20 or 50 *M. persicae* the leaf growth of the plant was hindered, both in terms of the number of leaves forming and size of the leaves. After the 10 days for which the trial ran there was no difference in the size of the aphid populations of the infested plants, despite their different starting values. This is most likely a function of the plants reaching carrying capacity and demonstrates the importance of the early infestation on the plant fitness. The difference observed in vegetative fitness for plants starting with 20 aphids, though not with 10 aphids, illustrates that over this time period there was an initial infestation threshold after which leaf growth was likely to be inhibited, it is predicted that this lies between 10 and 20 *M. persicae* adults. These data provided evidence that aphid feeding does negatively affect vegetative growth, from this it was possible to infer that a reduction in the aphid population, or inhibition of aphid population growth, will benefit the plant. The obvious next step to increase plant fitness would be to reduce aphid population growth, mitigating against the damage done by feeding. It was hypothesised that this may be achieved through the introduction of nepetalactone and learning by the wasps as they enter the new environment.

5.4.2 Learning in a complex spatial-temporal environment

In the previous chapter (Ch. 4) it was found that learning of the nepetalactone altered the foraging pattern of female *A. colemani*, but did not increase the efficiency of host location when tested in the laboratory. It was speculated that for the foraging efficiency to be increased a larger spatial-temporal scale was required. In Ch. 3 it was shown that a synergy of aphid-related volatiles and nepetalactone would cause a greater retention of parasitoids; this was considered along with trials conducted by Glinwood (1999a) showing that the nepetalactone may function as an arrestant for specialist parasitoids at distances over a metre, supporting the idea that a larger spatial-temporal environment was necessary. It is proposed here that the response elicited in A. *colemani* may also be an arrestant effect. If the odour was responsible for an arrestant effect on the wasps at a distance it was anticipated that a greater number of mummies would be seen in control positions within the glasshouse (placed approximately 2.8 m from the nepetalactone vial), or if the odour was attractive to them, or arrested them only at closer proximity, there would be a greater number of mummies alongside the nepetalactone vial. The number of mummies was found to be equal for all positions within the glasshouse showing that at this level of spatial-temporal complexity the nepetalactone is not sufficient to alter foraging within

patches. However, as previously discussed the odour may be affecting parasitoid behaviour at distance from the release source.

If the rate of parasitism had been greater across the whole environment due to the presence of nepetalactone it would not have been recognised in these trials where no control was present without the odour. Learning also failed to increase the success of foraging within the glasshouse. It is possible that the benefits to learning recognised in this study i.e. increased speed of attack and an altered pattern of foraging, do not confer benefits on this scale. The increased speed and success seen in attacks by *A. colemani* following ovipositional experience may have played a role in the initial moments following release of the parasitoids, however, given the temporal scale of the assay (eight hours to attack freely) such a difference may have been overlooked as those that did encounter host gained experience and were able to expend their eggload. It was speculated that differences in the foraging pattern following an encounter of the pheromone-HPC blend and retention by such an odour may be more relevant to a field environment, where the parasitoid is often in flight. The encounter of the HPC-pheromone combination may elicit foraging within the patch, though the spatial scale of the glasshouse may be too confined to observe this effect.

Although none of the treatments had an effect on the success of the foraging parasitoids it was found that those that showed evidence of attack, as observed through mummy formation, had reduced aphid populations. It was expected that attack from parasitoids, which results in the death of the host, inhibits the growth of aphid populations, however, due to the long development period of the parasitoid larvae (10-14 days) this was not guaranteed. *A. colemani* is a koinobiont parasitoid, meaning it allows the aphid to continue feeding and growing during development of the wasp larva. Aphids continue to grow and remain mobile for around nine days following parasitism (Le Lann *et al.*, 2011). It has been noted that aphids are able to continue reproduction following oviposition (Starý, 1970) and parasitoids are, therefore, not capable of the rapid population control which can be achieved with predatory biological control agents. However, it is noted that the reproductive potential of an aphid following oviposition is decreased (Ch. 3.3.4). Reduction in the aphid populations does not appear to be entirely accounted for by parasitoid attack. If the mummification of aphids was entirely responsible for the inhibition of population growth it would be anticipated that the number of mummies formed would correlate inversely to the population growth. No such correlation was seen between mummy formation and aphid population growth in samples showing evidence of parasitoid attack; suggesting other factors may be involved in the suppression of aphid reproductive potential. From this it was hypothesised that the visit of *A. colemani* to an infested leaf will cause a disturbance to the aphid population that temporarily

reduces the reproductive rate of the individuals. The most likely cause of such a reduction in fitness was dispersion caused by the production of the alarm pheromone, (*E*)-β-farnesene, which has been shown to reduce the pea aphid populations on plants (Bruce *et al.*, 2005). The scattering effect caused by the (*E*)-β-farnesene may not lead to reduced reproductive potential but an increase in the dispersal, which was not measured.

5.4.3 Manipulating release numbers

This issue of dispersal was addressed in the following series of trials by the use of 'trap plants' surrounding the experimental groups. Number of aphids on trap plants was not affected by treatment. This indicates that dispersal was not greater in any treatment set and, therefore, was less likely to be import in explaining differences observed in aphid population growth. It is realised that the trap plants may not fully account for the dispersal from the aphid populations, as not all of those escaping will encounter one of the plants. It may give an indication of how successful the aphids are following dispersal, which may be an equally relevant factor in determining the success of biological control strategies. It is known that the aphid alarm pheromone can increase the rate at which aphids will drop from the plant (Verheggen *et al.*, 2009, Wohlers, 1981) and it is known that mortality of those dropping from plants is greatly increased (Chau & Mackauer, 1997), though many may find their way back onto a suitable host plant. It was expected that the dispersal to trap plants would have been higher for plants subjected to greater parasitoid numbers as individuals attempted to avoid parasitism, with no differences between trap plants in treatments this hypothesis was not supported. It may still be the case that the aphids drop from the plant but fail to find their way onto another suitable host, or continue to avoid the area until the threat has left, which may not happen in the temporal scale of this experiment.

Initially it was assumed that more attacked aphids would also leave the plant than non-attacked individuals to increase their inclusive fitness (Hamilton, 1964a, Hamilton, 1964b). The clonal nature of the aphid population means an individual's genes are often best preserved by protecting conspecifics in the area. Following parasitism the aphid is a danger to the remaining population as it carries a natural enemy within its body. It was hypothesised that an infected aphid would make efforts to leave the remaining population; this may manifest itself either as reduction in aphid populations with no mummies seen as the aphid attempts to move out of range of its colony or a greater presence of aphids (and specifically parasitized aphids) on the surrounding trap plants. Aphids on trap plants were found to develop into mummies at the same proportion to those in the rest of the glasshouse: around 3% with 10, 9% with 25 and 29% with 50 parasitoids were released. This demonstrates that aphids were not moving to trap plants more frequently as a consequence of parasitism. Previous

studies have shown that while inclusive fitness does play a role in the aphid behavioural response to parasitoid attack it is not manifested by a greater avoidance of the population but a continued exposure to the threat of predation by other natural enemies (McAllister & Roitberg, 1987).

Using a larger sample range it was possible to confirm that parasitoid attack inhibited the growth of the aphid population but that there was no correlation between the number of aphids and the number of mummies on a plant. In these trials it was possible to compare control groups which had not been subject to parasitoid attack and those which had been exposed to different numbers of parasitoids, regardless of whether or not they were attacked. Using this analysis it was possible to determine that the presence of parasitoids was sufficient in decreasing aphid populations, with differences seen in the aphid populations at two days and after 10 days for those exposed to parasitoids and those not exposed. It was recognised throughout the work that the day two count may be a more reliable indicator of how the aphid population was affected, as numbers tend to converge around 10 days, likely to be a consequence of plants reaching, or nearing, their carrying capacity. As the plants were young and well maintained in the trials, it appeared that carrying capacity was limited more by the space available on the young Chinese cabbage leaves than any other resource. *M. persicae* population growth inhibition could be seen two days after exposure to parasitoids. Of the parasitoid-exposed groups, those with 50 females released showed the greatest inhibition of population growth. This confirms that the extent of the disturbance to the aphid population can be related to population growth inhibition. The other possible explanation for the reduction in aphid population is that the exposure to greater levels of attacks led to the direct death of the hosts. It may be that in many incidences *M. persicae* are located by several parasitoids, and exposed to repeated ovipositional attempts. The immune system would be rapidly overwhelmed in these individuals and may cause an immediate death of the host as several wasp larvae tried to develop inside. Introductions of parasitoids in biological control programmes do not normally have the set aim of eliminating the pest population but rather keeping it under a sustainable threshold. By allowing the population to persist at a low level the natural enemies are also able to persist, decreasing the necessity for constant reapplication of control agents. However, it is accepted that if populations of the parasitoid can persist, as was seen in trials, the instant death of some individuals may be an advantage to suppressing aphid population growth. Regardless of the mode by which the population growth was reduced, it was demonstrated that there was a benefit to parasitoid release other than the direct rate of parasitism.

The recognition that factors other than successful parasitism are important to pest population control raises several questions regarding previous and future studies on

parasitoid efficacy. Studies into parasitoid foraging frequently rely on the mummy count, or the success of parasitism, in a given area as an indicator of the success of a trial (Glinwood *et al.*, 1998, Hawkins *et al.*, 1993, Heimpel & Jervis, 2005, Lane *et al.*, 1999, Roschewitz *et al.*, 2005, Wäckers & Bruin, 2005). This research demonstrates that success of aphid control is not entirely determined by successful oviposition by the parasitoid and that the wasp may be having a positive impact even when visitation to an area is not detected.

6 General Discussion

The overall aim of this research was to establish how the aphid sex pheromone affects a host, its parasitoid, and the host-parasitoid interaction in a tritrophic system. The first step to achieving this was to determine the effect nepetalactone would have on the behaviour and performance of *M. persicae*. Following this, electrophysiological and behavioural assays were utilised to focus on the foraging behaviour of the parasitoid *A. colemani*, a critical stage in parasitoid success and one where olfactory cues are expected to play the greatest role. Little is known about learning in *A. colemani*, whilst previous studies with other parasitoid species have demonstrated a high proficiency for olfactory learning, the absence of knowledge regarding this widely used generalist make it an ideal model to test theories of learning and compare with existing knowledge of other species. To increase spatial-temporal complexity and provide a context more accurately reflecting a natural system, theories of parasitoid manipulation through nepetalactone in the tritrophic system were applied in a series of glasshouse trials. Work in the final chapter addressed the impact of aphid feeding on plant fitness, how learning affects parasitoid foraging, how parasitoid density affects aphid population growth and the role nepetalactone plays in these situations.

6.1 Key findings from each chapter

At the beginning of this thesis a series of hypotheses were formed regarding nepetalactone in a tritrophic system (Ch. 1.8.1). The following hypotheses were addressed with regard to new information acquired through the course of the study.

Chapter 2

1. *M. persicae* virginoparae are capable of detection of the aphid sex pheromone.

Using EAG it was possible to confirm a greater response for the pheromone components than was seen for solvent controls.

2. Virginoparae avoid nepetalactone as a means to avoiding conspecifics.

Despite virginoparae being capable of detecting nepetalactone, there was no evidence that it was used as a means to detecting, or avoiding, conspecifics. In a Y-tube olfactometer virginoparae did avoid high concentrations of nepetalactone but showed no preference for infested or uninfested plants. It is still possible that such a behavioural trait exists but only for the avoidance of populations of sexually

reproductive females and not asexual conspecifics; however the conditions of the experiment mean the theory is not supported or disproven.

3. An avoidance of nepetalactone will negatively affect aphid performance at an individual and population level.

The avoidance of nepetalactone did not decrease aphid performance at an individual or population level.

Chapter 3

1. *A. colemani* are capable of detecting aphid sex pheromone components.

It was proven, using EAG, that *A. colemani* is able to detect the sex pheromone components, responding most strongly to the component nepetalactone.

2. A synergy exists between nepetalactone and host-associated volatiles.

There was an increased retention of the parasitoid within an area containing HIPVs and nepetalactone.

3. *A. colemani* will show greater ovipositional success with nepetalactone present.

Although attack rate was not increased by nepetalactone, the *Nepeta cataria* oil did decrease the time before parasitoids made their first attack and the success rate of those attacks. This finding supports the idea that parasitoids utilise ambient cues in the host acceptance stage of the host selection process.

Chapter 4

1. Emergence conditioning with nepetalactone increases parasitoid foraging efficiency during a subsequent encounter.

Theories of learning were explored to test if nepetalactone is subject to alpha conditioning (Ch.1.6.4). Emergence conditioning was not sufficient to alter parasitoid foraging through retention time or attraction to the compound.

2. Ovipositional experience with nepetalactone will lead to increased foraging efficiency when the odour is encountered.

Ovipositional experience did change the foraging pattern of the parasitoid which is believed to be a mechanism of enhancing foraging efficiency. Foraging efficiency was tested in a laboratory-based olfactometer but no difference was seen between wasps

having learnt the nepetalactone odour and those remaining naïve. Given this evidence it is believed that the alterations observed in foraging patterns are only of importance when foraging occurs in a greater spatial environment where flight is involved. This study into learning demonstrated that a 'strong' stimulus is required to alter behaviour of *A. colemani* to nepetalactone.

Chapter 5

1. Parasitism will benefit plant growth.

This hypothesis was accepted following a two-part experiment. Initially it was established that aphid feeding was detrimental to leaf growth in Chinese cabbage, following that it was shown that parasitoid attack led to a reduction in aphid populations. Using this deductive logic it was possible to conclude that the introduction of parasitoids at an early stage of aphid infestation would benefit the fitness of the host plant.

2. Nepetalactone will increase the rate of parasitism in the glasshouse.

Nepetalactone did not have an effect on the rate of parasitism either at a local scale (adjacent plants), or across the glasshouse as a whole.

3. Parasitoids trained to the host-plant complex will be more efficient in foraging.

Learning through ovipositional experience did not appear to have any effect on the foraging success of parasitoids at this scale. Importantly it was recognised that parasitism alone was not adequate in explaining the reduction in aphid populations, instead this may be explained by the disturbance of aphid populations through parasitoid visitation. The number of parasitoids introduced into the glasshouse was the only factor affecting the rate of parasitism and neither learning nor the presence of the nepetalactone odour altered the foraging success of *A. colemani* or led to changes in the growth of *M. persicae* populations.

6.2 Understanding the role of nepetalactone in a tritrophic context: a new synthesis

The work in this thesis applying aphid sex pheromone both in the laboratory and glasshouse environments failed to demonstrate the attraction of *A. colemani* parasitoids to nepetalactone as observed in previous studies on related aphid parasitoids. Positive responses have been demonstrated for various *Praon* species of parasitoids (Hardie *et al.*, 1994a, Hardie *et al.*, 1991, Lilley *et al.*, 1994b, Powell *et al.*, 1993), *Aphidius rhopalosiphi* (Glinwood *et al.*, 1998), *Diaeretiella rapae* (Gabryś *et al.*,

1997), *Aphidius ervi* and *Aphidius eadyi* (Glinwood *et al.*, 1999a). *A. colemani* displayed no clear attraction to the aphid sex pheromone and the only behavioural response observed when the odour was presented in isolation required a very high concentration, which elicited a change in the parasitoid's foraging pattern. It may simply be that *A. colemani*, which has never previously been shown to elicit a response to nepetalactone, does not utilise the odour in the same way that the other listed parasitoids do. Although this is a parsimonious explanation based on the evidence, it does remain unlikely that it would have evolved a unique response considering it is exposed to the same selection pressures as other parasitoids. This work does not stand alone in failing to find a distinct response of *A. colemani* to nepetalactone. The only other published work regarding *A. colemani* response to nepetalactone found that it had no effect on attraction or retention of the parasitoid when presented in isolation (Ameixa & Kindlmann, 2011). With the information from previous studies and that gained in this study it is possible to synthesise a theory on how the parasitoid utilises such an odour.

One of the primary explanatory variables in foraging differences between parasitoid species is whether or not they are host generalists or specialists. It was noted by Glinwood *et al.* (1998) that different search strategies may be employed by generalist and specialist parasitoids in response to nepetalactone, with the latter showing much greater sensitivity to the odour and/or being arrested at a greater distance to the source. They proposed three different explanations for this; it could be an attractant for the generalist and an arrestant for the specialist, it may be that the specialist responds more strongly to the HIPVs explaining the response at a greater distance or it may be that the response threshold to nepetalactone is simply higher in the generalist than the specialist. It is hypothesised here that nepetalactone primarily functions as an arrestant for generalists and specialist, though working at different thresholds. The difference between generalist and specialist foraging could be defined as a selection of the spatial scale in which to commence host location, switching from host habitat location (Figure 6.1). Specialists commence host location at an earlier stage than generalists. For generalists, plant volatiles may provide the initial foraging cues which stimulate an oriented flight for the wasp, as it has already been observed that parasitoids of various species fly upwind to plant volatiles, specifically those releasing volatiles relating to herbivore attack (Connor *et al.*, 2007, Guerrieri *et al.*, 2002, Potting *et al.*, 1999). Although parasitoids are capable of achieving some host specificity through the volatiles (De Moraes *et al.*, 1998), they are also observed responding to compounds common to all herbivore attack (Du *et al.*, 1998). To increase the specificity in host habitat location the parasitoid may, in addition to plant volatiles, resort to host-related cues present in the environment, such as nepetalactone, before selecting a habitat. All the semiochemicals in the environment

contribute to the parasitoids response, which is ultimately defined by the abundance and reliability of odours. The reliability of these odours is weighted differentially between specialist and generalist parasitoids, defining the foraging distance and approach of that species.

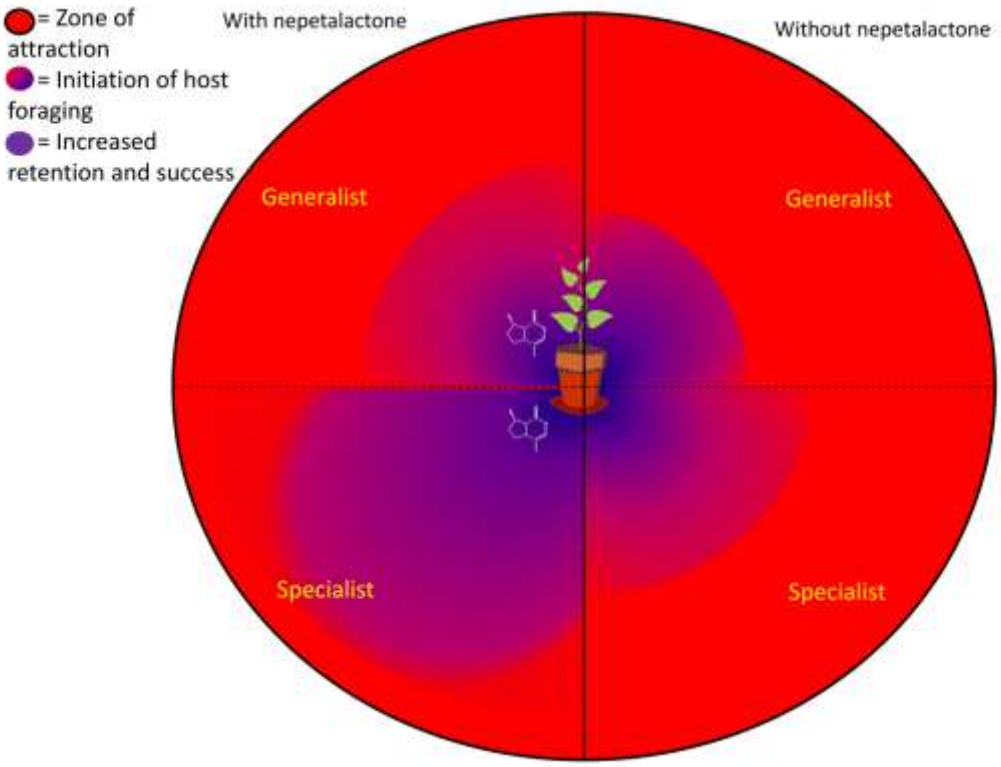


Figure 6.1 Visual representation of the effect of nepetalactone on parasitoid foraging.

This model is synthesised from results of this work and previous studies. It is suggested that nepetalactone does not attract parasitoids to the area but alters foraging, induces retention and increases the success of attacks when present alongside HIPVs. The effective range of the combination is believed to vary between specialist and generalist parasitoids.

This may explain why the retention of parasitoids on a leaf was increased by the presence of nepetalactone alongside an aphid-damaged plant. Simply put, it is speculated that the nepetalactone odour stimulates a retention response to the parasitoid rather than an attraction of the parasitoid to the odour source, once it has selected the host habitat it begins a more detailed, but confined, search which is apparent through increased turning and decreased locomotion. Once the host habitat is located, parasitoids may rely more strongly on contact kairomones, detected through probing of the leaf surface with the antennae. Although it is speculated that the primary role of nepetalactone for parasitoid generalists is in the location of the host habitat, it is recognised that the odour may affect choices in the later stages of

host selection. The single-attack assay demonstrated that eggs were laid more frequently, or showed greater success, when *Nepeta cataria* oil was present. It has already been suggested that chemical stimuli are important during oviposition (Vinson, 1976), however, only through contact and not those existing in the ambient environment. If this hypothesis on generalist parasitoid behaviour is accurate, the reported success of nepetalactone in previous studies, and this one, may be misleading. Wasps may be showing greater efficacy in foraging though not necessarily demonstrated by a clear attraction to the odour source and not through increased rates of oviposition but an increased rate of success. The study of parasitoid response to nepetalactone using the largest temporal-spatial scale failed to demonstrate attraction to areas containing the pheromone, however they did note an altered spatial distribution and reduction in the aphid populations (Powell *et al.*, 2004). These findings support the theory that nepetalactone is only likely to be effective at a larger spatial scale due to the role it plays in host habitat location. Once parasitoids receive a certain level of positive stimuli, an arrestant behaviour is initiated; ceasing flight and initiating a host foraging pattern. This threshold level is contributed to mostly by highly reliable odours that are available within the environment such as nepetalactone and HIPVs. Generalist may exercise a decreased dependency on HIPVs and rely more strongly on more reliable components common to all its host species, such as nepetalactone, but require a higher concentration. This model of parasitoid response threshold explains behavioural results observed in this work and previous studies, but remains speculative until further investigation can be completed to support or disprove its accuracy.

6.3 Potential benefits to biological control

The overall aim of this project was to establish how the sex pheromone affects *M. persicae*, *A. colemani* and their interaction in a tritrophic system. The work was completed with an academic focus, however, it was hoped that an understanding of the role of nepetalactone in the tritrophic system would provide opportunities for parasitoid manipulation that may enhance biological control. Although it has been felt that important insights were obtained into the ecology of a tritrophic system, no clear route or recommendation can be made regarding the application of this work to biological control programmes. The work on nepetalactone allowed the identification of various aspects of the tritrophic system in which nepetalactone may provide a benefit or a challenge to pest management (Table 6.1). This recognition of potential benefits/challenges to biological control is not intended to imply a balance as these factors are not weighted to account for their potential impact on a tritrophic system. The table is only provided as a simple representation of newly acquired knowledge in the field and how it may be important to biological control applications in the future.

Table 6.1 Findings from this thesis which may offer potential benefits or challenges to the application of aphid sex pheromones in biological control.

Relevant thesis chapter	Potential benefit	Potential challenge
Chapter 2	Aphid alatae formation, or overall dispersal, did not appear to be affected by nepetalactone	Avoidance of nepetalactone by aphid populations may result in decreased density around nepetalactone sources
	Aphid population growth may be reduced by presence of nepetalactone (only seen as trend)	
Chapter 3	*A. colemani*, a commonly used biological control agent, is capable of detecting aphid sex pheromone	Nepetalactone does not appear to attract *A. colemani*
	Nepetalactone requires aphid/plant volatiles to elicit retention response, reducing risk of parasitoid foraging in redundant patches	Observed retention effect means concentration of nepetalactone released will need to be carefully selected to ensure parasitoids approach source
	Success of parasitoid attack is increased in presence of *Nepeta cataria*	
Chapter 4	The parasitoid was able to learn nepetalactone when associated with oviposition resulting in an altered foraging pattern	Learning of nepetalactone did not increase the chance, or speed of host encounter during non-flight foraging
Chapter 5	Less aphids in a population ensures a higher vegetative fitness for the plant	Rate of parasitism in the glasshouse is not increased by nepetalactone
	Parasitoid release reduces aphid population size at a rate greater than indicated by attack alone	

6.4 Future work

As is the purpose of scientific research, this study provided new information and answered many of the original questions posed, though, as is common to scientific pursuit, this provided many more unanswered questions. Here an outline is given of some of the future work that would enhance the understanding of tritrophic interactions and those that may assist in biological control. Some of these suggestions for future work were not completed because of time constraints and others remained outside the remit of the project.

6.4.1 Nepetalactone to increase consistency of response

One of the main advantages that could be achieved with the introduction of nepetalactone could be in increasing the consistency of parasitoid response rather than optimising that of already capable individuals. It is recognised that one of the challenges facing successful parasitism in the glasshouse is the condition of the biological control agents upon arrival (Fernandez & Nentwig, 1997). During transportation parasitoids will often be exposed to periods of low temperatures; while this is beneficial to their longevity it can also affect their memory (van Emden *et al.*, 2008). This 'blank slate' provided by the cold storage experience may hinder parasitoid success but may also offer opportunities to tailor their response to the new environment. Subsequent learning experiences may be easier to imprint on such individuals. Without the memory of learning experience these individuals could see greater benefit in the presence of an innate cue such as nepetalactone to assist in identifying suitable foraging patches. The study of memory formation and learning manipulation would provide potentially beneficial insights on a practical level in addition to the academic interest in hymenopteran memory formation which may translate to other fields of study.

6.4.2 Understanding foraging strategies

It has been speculated here, and in previous work (Glinwood *et al.*, 1998) that foraging strategy of the parasitoid is largely dependent on the generalist or specialist nature of the wasp. Work by Glinwood *et al.* (1998) has addressed this issue but a more comprehensive analysis could be gained by future work involving the use of various generalist and specialist parasitoids foraging in the presence of a range of semiochemical cues (although only present as one variable at a time), hosts or monitoring traps alongside volatile collection equipment would be available at a series of distances from the source to determine effective range of each cue and test if this was consistent across generalist and specialist foragers. Ascertaining any threshold value that may exist that elicits behavioural changes would be beneficial to both an understanding of insect ecology and the application of semiochemicals in the field.

6.4.3 Indirect effects of nepetalactone

Taking a holistic approach, it would be of interest to note how parasitoid population dynamics may be altered by the pheromone. *A. colemani*, and other aphid parasitoids, display a haplodiploidy form of reproduction, permitting a level of control over the sex of the offspring suiting the environmental conditions. It is possible that the presence of the sex pheromone within the environment may falsely convey the abundance of hosts present. Females depositing eggs may be misled as to the availability of

resources and alter the sex balance of the offspring they produce. An imbalance in the sex ratio of following generations may prove unsustainable. Fisher (1930) demonstrated convincingly that a balanced sex ratio will arise through natural means, however, it has been shown in parasitoid populations that environmental cues can play a role in sex determination, leading to gender imbalances (Charnov *et al.*, 1981). Sex ratio imbalances are less likely to be an issue in the field environment where natural migration occurs but could be highly detrimental to glasshouse introductions which attempt to maintain continuous cultures.

An unexpected finding of this research was the ability of *M. persicae* virginoparae to respond to the aphid sex pheromone. It is speculated that detection of the pheromone is used by the aphid to avoid competition of some types, or that it persists as an evolutionary relic. Regardless of the function of nepetalactone to the ecology of asexual aphid populations it does demonstrate the need to consider all aspects of the multitrophic system; the existence of an odour in the environment inherently carries information regarding the source to any organism able to detect it. Within the model tritrophic system it was confirmed that two of the three levels are capable of detecting the aphid sex pheromone. Perception of the sex pheromone is described at the two levels where a response is most expected; aphids whose conspecifics produce the odour and parasitoids which utilise aphid volatiles in foraging, however, it is not unfeasible that the plant may also detect and utilise such an odour. It is known that signals sent out from a plant are capable of initiating a defence response in another (Guerrieri *et al.*, 2002). From this it is recognised that plants can 'predict' attack from environmental cues rather than relying on induction through direct herbivore damage, it is therefore possible that plants have also evolved the direct detection of the their predator: the herbivore. If this is the case it would be anticipated that reliable and related volatiles such as the honeydew, host frass, alarm and sex pheromones may induce a defence response in a plant, or produce an additive effect with herbivore induced volatiles. There was no opportunity during this study to test such a hypothesis though it is recognised that it may be of importance for future work. If an alteration is seen in the volatile output of plants following induction by the sex pheromone, or other host-related odours, it may provide an understanding of results obtained in previous studies and help to structure future applications of semiochemical technology.

One of the big questions in parasitoid ecology is the extent to which wasps may use a host marker pheromone (HMP). Throughout the literature the pheromones are often alluded to with little direct evidence of their existence in aphid parasitoids, or any chemical components described (Godfray, 1994). If HMPs exist it is necessary to understand the role they play within the ecosystem. Looking specifically at the

introduction of nepetalactone it would be vital to understand any interactions that occur. It should be noted also that it is not implied that the HMP is a cue 'voluntary' left by the parasitoid but may simply refer to the ability of the parasitoid, or another, to detect that visitation has occurred, this could be through odours bound to the cuticle which then adhere to the mummy surface or surrounding leaf area. A HMP may actually play a role in assisting foraging of the wasp; if detected from a distance the odour could be used to determine that hosts are present in an area, even if that area has already been visited by another individual. In this scenario nepetalactone could work with any HMP enhancing the reliability of the odour to attract or retain passing wasps. It is unlikely that a HMP would be detectable over such a distance as a strong selection pressure exists to maintain hidden from hyperparasitoids. It is more likely that a HMP would be a kairomone functioning to deter a wasp from ovipositing in an aphid which has already been attacked. The trade-off in hyperparasitoid detection mentioned above stresses that if a marker is to exist it must be of considerable value to avoid such selection pressures. Nepetalactone, or any other aphid-produced semiochemical, may mask the HMP rendering it ineffective. The 'single-attack rate' assay in chapter 3 attempted to address this and was initially intended to have another parasitoid introduced to the host with or without nepetalactone present. Unfortunately time was a constraint here but it is recommended that a similar study could be conducted to try and answer these questions.

6.4.4 Other avenues of semiochemical research

Although the focus of this work has been nepetalactone, the study has also led to the recognition of other avenues for future work in chemical ecology and how this knowledge can be applied to benefit biological control.

In the same way that control measures are often best as part of an integrated system it may be best to look at the application of nepetalactone as one facet of a programme using semiochemicals. Currently much focus has been placed on the potential use of the aphid alarm pheromone, (E)-β-farnasene, in controlling pest populations (Yu *et al.*, 2012). This is a recommendable approach because benefits of the pheromone are seen in the attraction of wasps and immediate detriment to the host. The odour is also relevant to aphids in the glasshouse which are normally asexual on crop plants and use the alarm pheromone but not nepetalactone. Parasitoids respond to nepetalactone (Glinwood *et al.*, 1999a, Glinwood *et al.*, 1998, Hardie *et al.*, 1994a, Hardie *et al.*, 1991, Lilley *et al.*, 1994b) but the odour may not be producing its optimal response when used with crop plants, as parasitoids may also rely on the association of this cue with other naturally occurring stimuli which are not available. (E)-β-farnasene is present in these populations, its composition does not vary by species and habituation to the odour will be detrimental to the aphids. For all the reasons listed, it suggested

that research into alarm pheromone should remain a primary focus. In addition to the current work being completed on parasitoid wasp response, it would be interesting to determine the impact that a regulated, though discontinuous, release of the odour would have on pest populations. If the compound is released with frequency to disrupt aphid populations it is possible that the wasps will habituate to the odour, it would be at this point that other semiochemicals, such as nepetalactone, may prove beneficial by exploiting the reduced dispersal potential of the aphids.

It was identified that aphid population growth is hindered by parasitoid attack (Ch. 5) and that the reduction is not directly related to the rate of mummy formation. The explanation given was that the disturbance caused by wasp visitation to the plants led to an increase in the general agitation of individuals and, therefore decrease in the energy spent on reproduction. It is also possible that the odours remaining following parasitism may have influenced the population growth. Initially this appears to be only a minor amendment to the theory but a reduction in aphid population as a result of odours left on the leaves, or associated with a parasitized individual could provide opportunities for the applications of such odours as a natural control measure in IPM.

Our study focussed on laboratory-based assays and trials run at a larger spatial-temporal environment which were conducted in glasshouse compartments. This scale suited the aim of the project but provided little information regarding the potential application in biological control at a field or large glasshouse scale. Trials have been conducted to investigate the effect nepetalactone in the field (Glinwood *et al.*, 1999b, Hardie *et al.*, 1994a) though they have focussed on the number of aphids parasitized and not the fitness of the plants, which is of greater importance. Future work into nepetalactone as a tool in biological control would benefit from more comprehensive large scale trials monitoring the plant fitness, aphid population growth, parasitoid diversity in the system and sustainability over time. Aphid counts following the glasshouse release of *A. colemani* demonstrate that by recording only mummy counts, studies may be overlooking many of the benefits achieved by the release of natural enemies. The need for a more holistic approach to understanding chemical ecology has been recognised by other researchers (Rand *et al.*, 2011, Sanders & van Veen, 2012, Van Huynh *et al.*, 2008) and is supported here. Attempts were made to assess the impact of nepetalactone on the system as a whole but the process of eliminating confounding variables may also have caused some important aspects of the ecology of the multitrophic system to be overlooked. Identification of virginoparae response to nepetalactone emphasised the importance of studying non-target organisms, though it was not possible in this study to evaluate how the sex pheromone may affect plants, predators or parasitoids at a higher trophic level. An example can be seen in hyperparasitoids which can be critical to the success of an integrated pest

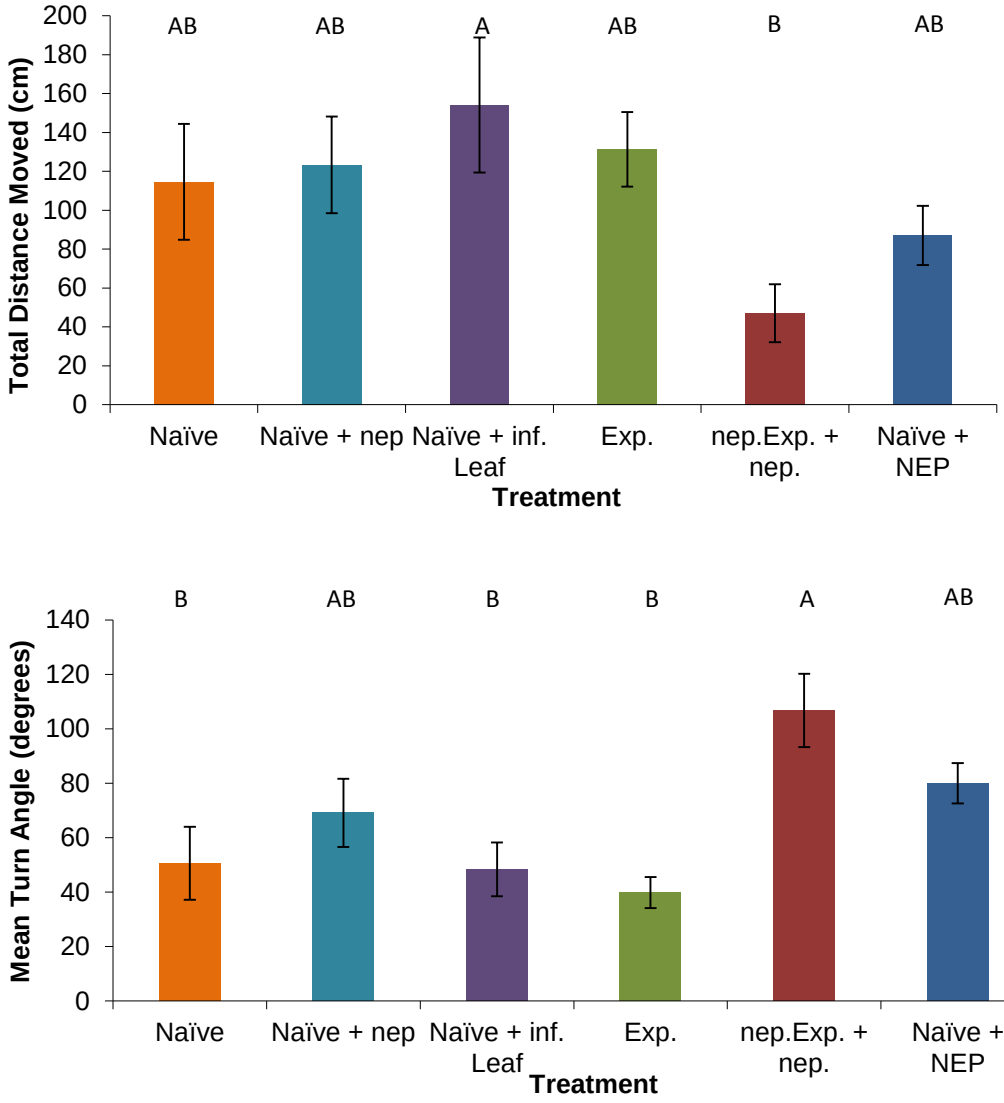
management system but have so far remained unstudied with regards to the aphid sex pheromone.

It is recommended research move away from studies focussing on confirming a specific recognised response to pheromones and instead understand a myriad of factors contribute to the response of individuals at all trophic levels. Once this is realised research can move away from categorising responses and will be able to concentrate on a comprehensive understanding of semiochemicals in a multitrophic system.

Chapters 2-4 of this work reflect attempts to increase knowledge of nepetalactone in the tritrophic system, though an interest in the potential for application was never lost. In Chapter 5 inroads were made to understanding how this knowledge may be applied in biological control, but time limited the opportunity to take this further. It is identified that in research a trade-off exists in what can be achieved in science and in application, though it need not be the case. Currently work on food security is challenged by the dichotomy that exists between 'pure' science and applied trials. It is likely that a great deal of this arises from the nature of funding across science, which is often divided by the necessity to produce a high standard of academic output but relies on grand claims regarding the benefit of such research. It is essential to realise that both methods of scientific research are vital to solve real-world problems and advance technologies. Efforts should be made to take knowledge gained in the laboratory to the field and feed those results back into the investigative process. This is a difficult goal to achieve because the pressure that currently exists on scientists to achieve a high output of peer-reviewed work, regardless of its utility to the real world. It is not suggested that work should be of a purely applied nature but that an integrated approach may best solve the problem. Ideally scientists will start out on the road to a new solution but not be afraid to create new paths to get there.

Appendix A

Effect of nepetalactone and nepetalactone learning on the foraging patterns of *Aphidius matricariae*. Design, analysis and treatments for these data are exactly as described for *A. colemani* (Ch. 4.2.6).



Appendix Figure 1 a) & b). A. matricariae foraging patterns.

Effect of treatment on a) the distance moved and b) the turning of *A. matricariae*. Naïve = no previous ovipositional experience, Exp. = ovipositional experience, nep. = 1 mg/ml concentration of nepetalactone, NEP = 10 mg/ml concentration of nepetalactone and inf. Leaf = an infested leaf. Treatments described following the '+' sign demonstrate that they were present during the assay. Those not sharing a letter are significantly different, $P > 0.05$.

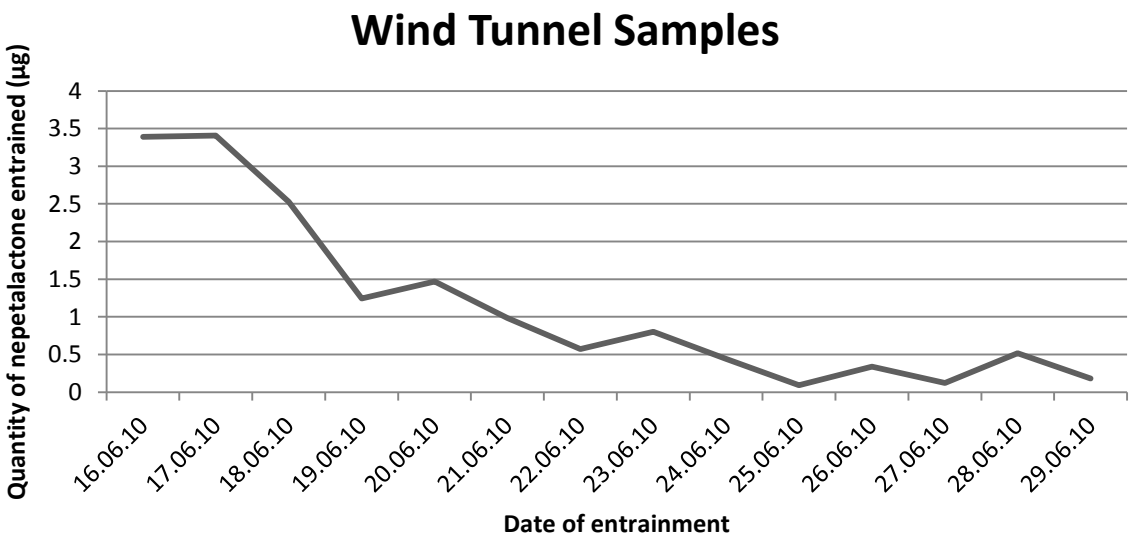
Appendix B

Nepetalactone infused PVC lures were produced by Agrisense (Suttera LLC, USA). These extrusions were initially used as an attractant in cat toys, since the nepetalactone stimulates cat play and can function as a feline attractant (Bland, 1979). Infused strips allow for the prolonged release of the nepetalactone and have the advantage of being inexpensive to produce (Birkett & Pickett, 2003). The exact release rate of these strips and for how long they will continue release detectable levels of nepetalactone is unknown, though a study was conducted by Graves (2003) to assess the release rate of a similar nepetalactone infusion on a different polymer. This study charted the weight loss of the nepetalactone from the strip over a period of around 60 days under different temperatures and wind speeds. It did not, however, give any indication of the remaining content of nepetalactone on the strip or how much of the pheromone continued to be emitted. For this study strips of the extrusion were cut to weigh 1 ± 0.01 g. 106 of these strips were cut in total with 6 being sampled on the first day. Entrainment samples of the strips were carried out in glass quikfit vessels (3.5 cm diameter) for 2 hours at an airflow of 800 ml/min. The volatiles were adsorbed onto 50 mg of porapak Q (50-80 mesh; Waters Associates, USA) held between two plugs of salinized wool. After the entrainment was complete the samples are eluted from the filters with 500 µl of redistilled diethyl ether. These are sealed in glass ampoules in the freezer (-20°C) until the samples are ready to be analysed. To clean the filters they were rinsed through 4 times with diethyl ether and left in a heated block at around 220°C for a minimum of 3 hours while a constant nitrogen flow of 200 ml/min was fed through.

Of the remaining 100 strips, 50 were placed in the glasshouse, suspended by paper clips and the other 50 were placed in a wind tunnel, also suspended by paper clips. Samples in the wind tunnel were left in the dark with airflow was set to 0.2 m/s. Humidity and temperature were recorded inside the wind tunnel and glasshouse using remote 'EasyLog' data loggers (Lascar Electronics, UK). In the glasshouse a light meter (Skye, UK) was also used daily to record light levels at solar noon. For a 14 day period daily entrainments were made of three strips from the glasshouse and three strips taken from the wind tunnel. Three strips were used from each environment to provide a more accurate mean for each sample day.

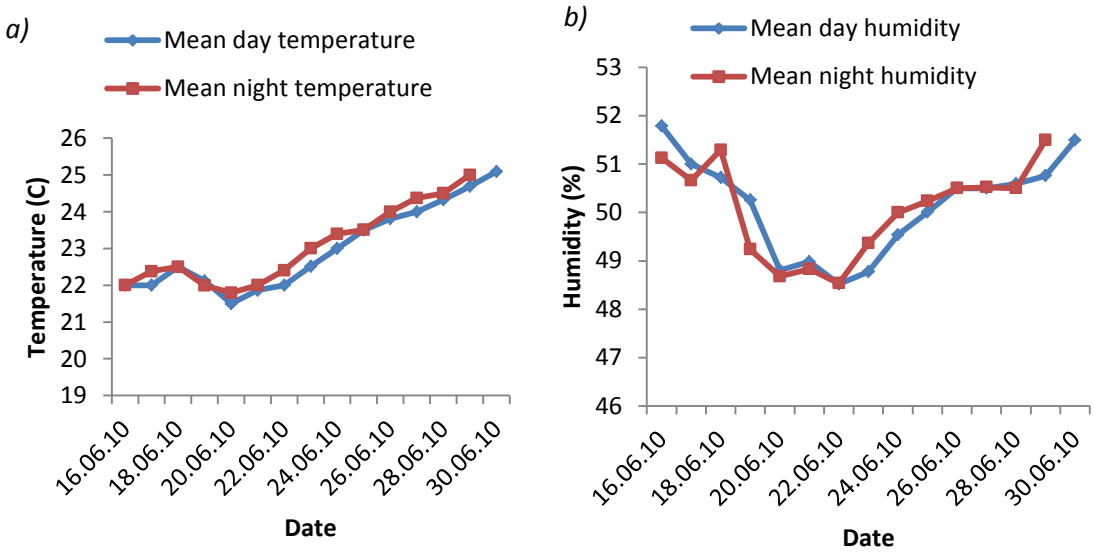
A preliminary long term study of the PVC infused strips showed that the nepetalactone was no longer detectable after a two week period. This led to the more detailed study of the release of the PVC strips for two weeks following their introduction into the glasshouse environment. Release rate of the strip was measured by daily entrainments made from the strip in both the glasshouse environment and a wind tunnel set to 2

m/s windspeed. Understanding the rate at which nepetalactone is released from the strips will be essential in regulating the use of the pheromone in the glasshouse environment.



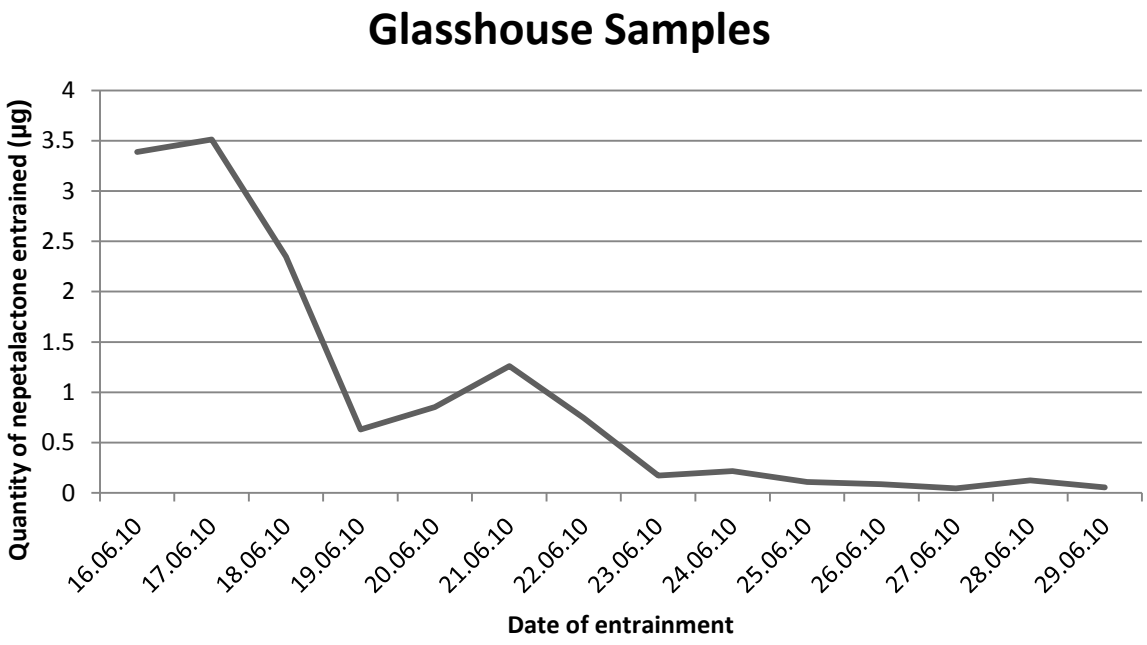
Appendix Figure 2 Quantity of nepetalactone entrained in the wind tunnel.

Mean daily entrainments from 1 g of PVC strip produced by Agrisense in the wind tunnel environment maintained at 2 m/s windspeed in an unlit room.



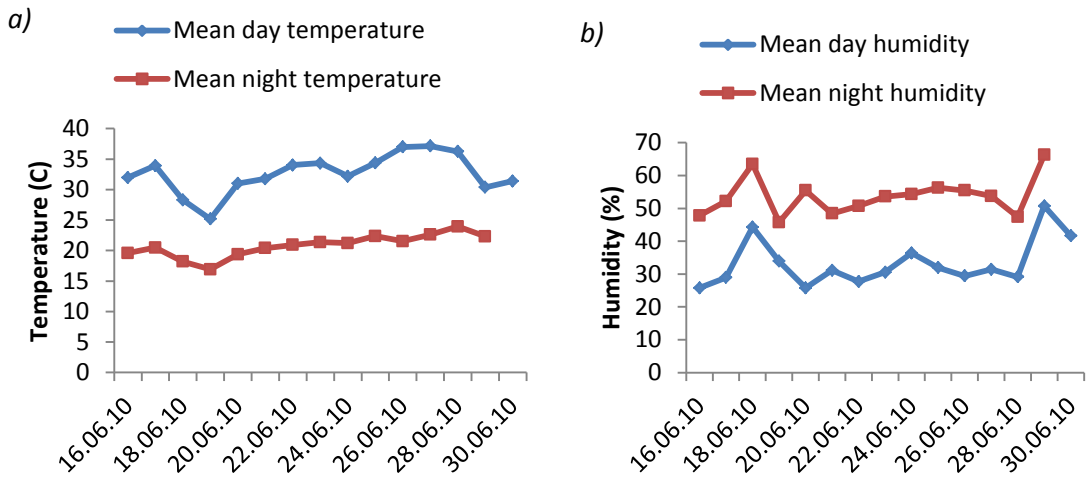
Appendix Figure 3 a) & b) Ambient wind tunnel conditions.

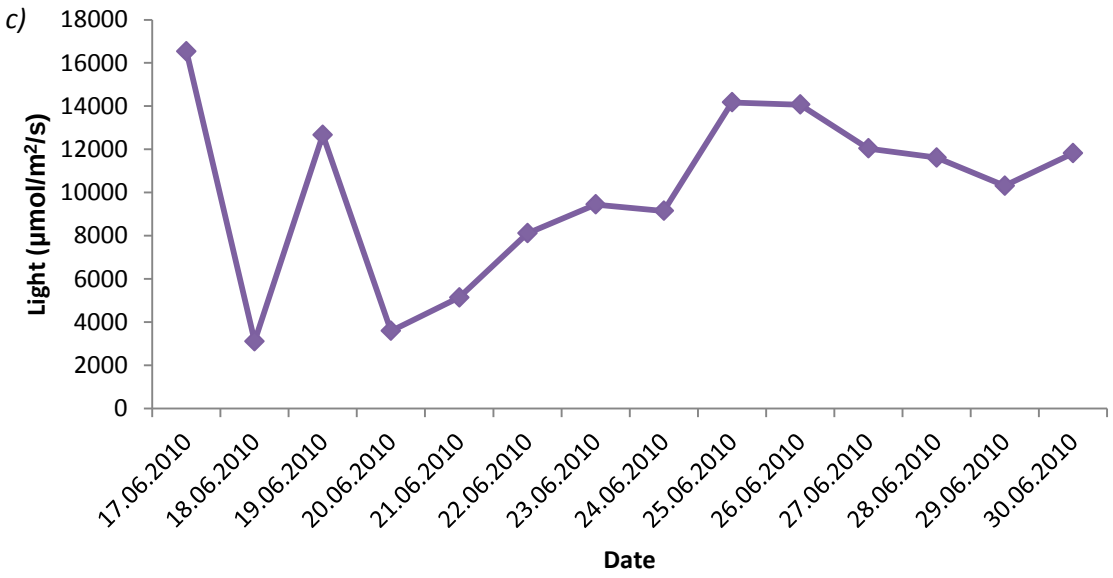
Using Easylog dataloggers information is provided from within the environment on the mean daily and nightly *a)* temperature and *b)* humidity over the two week period.



Appendix Figure 4 Quantity of nepetalactone entrained in the glasshouse.

Mean daily entrainments from 1 g of PVC strip produced by Agrisense in the glasshouse environment for a two week period during summer.





Appendix Figure 5 a)- c) Ambient glasshouse conditions.

Using Easylog dataloggers information is provided from within the environment on the mean daily and nightly *a)* temperature and *b)* humidity over the two week period. *c)* A lightmeter was also used to provide a light reading for each day at solar noon.

Bibliography

Ameixa OMCC & KindImann P (2011) Effect of synthetic and plant-extracted aphid pheromones on the behaviour of *Aphidius colemani*. *Journal of Applied Entomology,* 136, 292-301.

Antolin MF, Bjorksten TA & Vaughn TT (2006) Host-related fitness trade-offs in a presumed generalist parasitoid, *Diaeretiella rapae* (Hymenoptera: Aphidiidae). *Ecological Entomology,* 31, 242-254.

Atkinson D, Burnett F, Foster GN, Litterick A, Mullay M & Watson CA (2003) The Minimization of Pesticide Residues in Food: a Review of the Published Literature. *Food Standards Agency, London*.

Baker TC, Hansson BS, Löfstedt C & Löfqvist J (1988) Adaptation of antennal neurons in moths is associated with cessation of pheromone-mediated upwind flight. *Proceedings of the National Academy of Sciences,* 85, 9826.

Barker G, Rose C & Kistson H (2003) Protecting England and Wales From Plant Pests and Diseases. IN Office NA (Ed.).

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

Battaglia D, Poppy G, Powell W, Romano A, Tranfaglia A & Pennacchio F (2000) Physical and chemical cues influencing the oviposition behaviour of *Aphidius ervi*. *Entomologia experimentalis et applicata,* 94, 219-227.

Beale MH, Birkett MA, Bruce TJA, Chamberlain K, Field LM, Huttly AK, Martin JL, Parker R, Phillips AL & Pickett JA (2006) Aphid alarm pheromone produced by transgenic plants affects aphid and parasitoid behavior. *Proceedings of the National Academy of Sciences,* 103, 10509-10513.

Bell WJ (1990) Searching behavior patterns in insects. *Annual Review of Entomology,* 35, 447-467.

Bernstein C & Driessen G (1996) Patch-marking and optimal search patterns in the parasitoid *Venturia canescens*. *Journal of Animal Ecology*, 211-219.

Birkett MA & Pickett JA (2003) Aphid sex pheromones: from discovery to commercial production. *Phytochemistry,* 62, 651-656.

Blackman RL (1971) Variation in the photoperiodic response within natural populations of *Myzus persicae* (Sulz.). *Bulletin of Entomological Research,* 60, 533-546.

Blackman RL & Eastop VF (1984) *Aphids on the world's crops. An identification and information guide*, John Wiley.

Bland KP (1979) Tom-cat odour and other pheromones in feline reproduction. *Veterinary Research Communications,* 3, 125-136.

Blande JD, Pickett JA & Poppy GM (2004) Attack rate and success of the parasitoid *Diaeretiella rapae* on specialist and generalist feeding aphids. *Journal of chemical ecology,* 30, 1781-1795.

Boo KS, Choi MY, Chung IB, Eastop VF, Pickett JA, Wadhams LJ & Woodcock CM (2000) Sex pheromone of the peach aphid, *Tuberocephalus momonis*, and optimal blends for trapping males and females in the field. *Journal of chemical ecology,* 26, 601-609.

Boo KS, Chung IB, Han KS, Pickett JA & Wadhams LJ (1998) Response of the lacewing *Chrysopa cognata* to pheromones of its aphid prey. *Journal of chemical ecology,* 24, 631-643.

Bouchard Y & Cloutier C (1984) Honeydew as a source of host-searching kairomones for the aphid parasitoid *Aphidius nigripes* (Hymenoptera: Aphidiidae). *Canadian Journal of Zoology,* 62, 1513-1520.

Bruce TJA, Birkett MA, Blande J, Hooper AM, Martin JL, Khambay B, Prosser I, Smart LE & Wadhams LJ (2005) Response of economically important aphids to components of *Hemizygia petiolata* essential oil. *Pest management science,* 61, 1115-1121.

Bruce TJA, Matthes MC, Chamberlain K, Woodcock CM, Mohib A, Webster B, Smart LE, Birkett MA, Pickett JA & Napier JA (2008) cis-Jasmone induces *Arabidopsis* genes that affect the chemical ecology of multitrophic interactions with aphids and their parasitoids. *Proceedings of the National Academy of Sciences,* 105, 4553.

Budenberg WJ (1990) Honeydew as a contact kairomone for aphid parasitoids. *Entomologia experimentalis et applicata,* 55, 139-148.

Butenandt A, Beckmann R, Stamm D & Hevker E (1959) On the sex pheromone of the silkworm moth *Bombyx mori*. Isolation and structure. *Zeitschrift für Naturforschung B,* 14, 283-284.

Campbell AM, Cook FJ, Pickett JA, Pope TW, Wadhams LJ & Woodcock CM (2003) Responses of the aphids *Phorodon humuli* and *Rhopalosiphum padi* to sex pheromone stereochemistry in the field. *Journal of chemical ecology,* 29, 2225-2234.

Campbell AM & Mackauer M (1975) The effect of parasitism by *Aphidius smithi* (Hymenoptera: Aphidiidae) on reproduction and population growth of the pea aphid (Homoptera: Aphididae). *The Canadian Entomologist,* 107, 919-926.

Campbell CaM, Dawson GW, Griffiths DC, Pettersson J, Pickett JA, Wadhams LJ & Woodcock CM (1990) Sex attractant pheromone of damson-hop aphid *Phorodon humuli* (Homoptera, Aphididae). *Journal of chemical ecology,* 16, 3455-3465.

Carde RT & Minks AK (1995) Control of moth pests by mating disruption: successes and constraints. *Annual Review of Entomology,* 40, 559-585.

Charnov EL (1982) *The theory of sex allocation*, Princeton University Press.

Charnov EL, Los-Den Hartogh RL, Jones WT & Van Den Assem J (1981) Sex ratio evolution in a variable environment. *Nature,* 289, 27-33.

Chattopadhyay J, Sarkar R, Elena Fritzsche-Hoballah M, Turlings TCJ & Bersier LF (2001) Parasitoids may determine plant fitness-a mathematical model based on experimental data. *Journal of theoretical Biology,* 212, 295-302.

Chau A & Mackauer M (1997) Dropping of pea aphids from feeding site: a consequence of parasitism by the wasp, *Monoctonus paulensis*. *Entomologia experimentalis et applicata,* 83, 247-252.

Chen P, Zhang ZQ, Xu W, Wang K, Zhu G, Lu L & Liu H (1991) Effects of chemical control of the cotton aphid during the early season on cotton plants on natural enemies and yield. *Journal of Applied Entomology,* 111, 211-215.

Connor EC, Rott AS, Samietz J & Dorn S (2007) The role of the plant in attracting parasitoids: response to progressive mechanical wounding. *Entomologia experimentalis et applicata,* 125, 145-155.

Costamagna AC, Van Der Werf W, Bianchi F & Landis DA (2007) An exponential growth model with decreasing r captures bottom-up effects on the population growth of *Aphis glycines* Matsumura (Hemiptera: Aphididae). *Agricultural and Forest Entomology,* 9, 297-305.

Croze H (1970) *Searching image in Carrion Crows: Hunting strategy in a predator and some anti-predator devices in camouflaged prey*, Parey.

Dawson GW, Griffiths DC, Janes NF, Mudd A, Pickett JA, Wadhams LJ & Woodcock CM (1987) Identification of an aphid sex pheromone. *Nature,* 325, 614-616.

Dawson GW, Griffiths DC, Merritt LA, Mudd A, Pickett JA, Wadhams LJ & Woodcock CM (1988) The sex pheromone of the greenbug, Schizaphis graminum. *Entomologia experimentalis et applicata,* 48, 91-93.

Dawson GW, Griffiths DC, Merritt LA, Mudd A, Pickett JA, Wadhams LJ & Woodcock CM (1990) Aphid semiochemicals—a review, and recent advances on the sex pheromone. *Journal of chemical ecology,* 16, 3019-3030.

Dawson GW, Pickett JA & Smiley DWM (1996) The aphid sex pheromone cyclopentanoids: Synthesis in the elucidation of structure and biosynthetic pathways. *Bioorganic & Medicinal Chemistry,* 4, 351-361.

De Moraes CM, Lewis WJ, Pare PW, Alborn HT & Tumlinson JH (1998) Herbivore-infested plants selectively attract parasitoids. *Nature,* 393, 570-571.

Debach P & Rosen D (1991) *Biological control by natural enemies*, Cambridge University Press.

Dewhirst SY, Birkett MA, Fitzgerald JD, Stewart-Jones A, Wadhams LJ, Woodcock CM, Hardie J & Pickett JA (2008) Dolichodial: A New Aphid Sex Pheromone Component? *Journal of chemical ecology,* 34, 1575-1583.

Dicke M (2009) Behavioural and community ecology of plants that cry for help. *Plant, cell & environment,* 32, 654-665.

Dicke M, Gols R, Ludeking D & Posthumus MA (1999) Jasmonic acid and herbivory differentially induce carnivore-attracting plant volatiles in lima bean plants. *Journal of chemical ecology,* 25, 1907-1922.

Dixon AFG, Horth S & Kindlmann P (1993) Migration in insects: cost and strategies. *Journal of Animal Ecology,* 62, 182-190.

Douloumpaka S & Van Emden HF (2003) A maternal influence on the conditioning to plant cues of *Aphidius colemani* Viereck, parasitizing the aphid *Myzus persicae* Sulzer. *Physiological Entomology,* 28, 108-113.

Du YJ, Poppy GM, Powell W, Pickett JA, Wadhams LJ & Woodcock CM (1998) Identification of semiochemicals released during aphid feeding that attract parasitoid *Aphidius ervi*. *Journal of chemical ecology,* 24, 1355-1368.

Du YJ, Poppy GM, Powell W & Wadhams LJ (1997) Chemically mediated associative learning in the host foraging behavior of the aphid parasitoid *Aphidius ervi* (Hymenoptera: Braconidae). *Journal of Insect Behavior,* 10, 509-522.

Dukas R (2008) Evolutionary biology of insect learning. *Annu. Rev. Entomol.,* 53, 145-160.

Ehler LE (2006) Integrated pest management (IPM): definition, historical development and implementation, and the other IPM. *Pest management science,* 62, 787-789.

Eisenbach J & Mittler TE (1980) An aphid circadian rhythm: factors affecting the release of sex pheromone by oviparae of the greenbug, *Schizaphis graminum*. *Journal of Insect Physiology,* 26, 511-515.

Elzen GW, Williams HJ & Vinson SB (1983) Response by the parasitoid *Campoletis sonorensis* (Hymenoptera: Ichneumonidae) to chemicals (synomones) in plants: implications for host habitat location. *Environmental Entomology,* 12, 1873-1877.

Elzen GW, Williams HJ & Vinson SB (1986) Wind tunnel flight responses by hymenopterous parasitoid *Campoletis sonorensis* to cotton cultivars and lines. *Entomologia experimentalis et applicata,* 42, 285-289.

Europa (2008) New rules on pesticide residues to strengthen food safety in the European Union. IN Eu (Ed.).

Fabré J & Miall B (1911) *Social life in the insect world*, Unwin.

Fauvergue X, Vercken E, Malausa T & Hufbauer RA (2012) The biology of small, introduced populations, with special reference to biological control. *Evolutionary Applications,* 5, 424-443.

Fenton B, Birch ANE, Clyne C, Kasprovicz L, Malloch G, Mitchell C & Gordon SC (2004) No sex please - we're British aphids. The Scottish Crop Research Institute annual report.

Fernández-Grandon GM, Girling RD & Poppy GM (2011) Utilizing insect behavior in chemical detection by a behavioral biosensor. *Journal of Plant Interactions,* 6, 109-112.

Fernandez C & Nentwig W (1997) Quality control of the parasitoid *Aphidius colemani* (Hym., Aphidiidae) used for biological control in greenhouses. *Journal of Applied Entomology,* 121, 447-456.

Fischer S, Samietz J, Wäckers F & Dorn S (2001) Interaction of vibrational and visual cues in parasitoid host location. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology,* 187, 785-791.

Fisher RA (1930) *The genetical theory of natural selection*, Clarendon Press.

Foster SP, Denholm I, Harling ZK, Moores GD & Devonshire AL (1998) Intensification of insecticide resistance in UK field populations of the peach-potato aphid, *Myzus persicae* (Hemiptera: Aphididae) in 1996. *Bulletin of Entomological Research,* 88, 127-130.

Foster SP, Harrington R, Dewar AM, Denholm I & Devonshire AL (2002) Temporal and spatial dynamics of insecticide resistance in *Myzus persicae* (Hemiptera: Aphididae). *Pest management science,* 58, 895-907.

Gabryś BJ, Gadomski HJ, Klukowski Z, Pickett JA, Sobota GT, Wadhams LJ & Woodcock CM (1997) Sex pheromone of cabbage aphid *Brevicoryne brassicae*: identification and field trapping of male aphids and parasitoids. *Journal of chemical ecology,* 23, 1881-1890.

Gaston KJ (1991) The magnitude of global insect species richness. *Conservation Biology,* 5, 283-296.

Geng W, Xiangyu J, Li X, Zhang Z, Han D & Li Y (1997) Male peach aphid attraction in the field by sex pheromones. *Insect Science,* 4, 364-368.

Gershenzon J (2007) Plant volatiles carry both public and private messages. *Science's STKE,* 104, 5257.

Gilbert N & Gutierrez AP (1973) A plant-aphid-parasite relationship. *The Journal of Animal Ecology*, 323-340.

Girling RD, Hassall M & Turner JG (2007) Do turning biases by the 7-spot ladybird, *Coccinella septempunctata*, increase their foraging efficiency? *Behaviour,* 144, 143-163.

Girling RD, Hassall M, Turner JG & Poppy GM (2006) Behavioural responses of the aphid parasitoid *Diaeretiella rapae* to volatiles from *Arabidopsis thaliana* induced by *Myzus persicae*. *Entomologia experimentalis et applicata,* 120, 1-9.

Girling RD, Stewart-Jones A, Dherbecourt J, Staley JT, Wright DJ & Poppy GM (2011) Parasitoids select plants more heavily infested with their caterpillar hosts: a new approach to aid interpretation of plant headspace volatiles. *Proceedings of the Royal Society B: Biological Sciences,* 278, 2646-2653.

Giurfa M (2003) Cognitive neuroethology: dissecting non-elemental learning in a honeybee brain. *Current opinion in neurobiology,* 13, 726-735.

Giurfa M, Zhang S, Jenett A, Menzel R & Srinivasan MV (2001) The concepts of 'sameness' and 'difference' in an insect. *Nature,* 410, 930-932.

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

Glinwood R, Du YJ & Powell W (1999a) Responses to aphid sex pheromones by the pea aphid parasitoids *Aphidius ervi* and *Aphidius eadyi*. *Entomologia experimentalis et applicata,* 92, 227-232.

Glinwood R, Du YJ, Smiley D & Powell W (1999b) Comparative responses of parasitoids to synthetic and plant-extracted nepetalactone component of aphid sex pheromones. *Journal of chemical ecology,* 25, 1481-1488.

Glinwood RT, Powell W & Tripathi CPM (1998) Increased parasitization of aphids on trap plants alongside vials releasing synthetic aphid sex pheromone and effective range of the pheromone. *Biocontrol Science and Technology,* 8, 607-614.

Godfray HCJ (1994) *Parasitoids: behavioral and evolutionary ecology*, Princeton University Press.

Goldansaz SH & Mcneil JN (2003) Calling behaviour of the potato aphid *Macrosiphum euphorbiae* oviparae under laboratory and field conditions. *Ecological Entomology,* 28, 291-298.

Grant M (2003) *GM Nation?: The Findings of the Public Debate*, GM Public Debate Steering Board.

Grasswitz TR & Resse BD (1998) Biology and host selection behaviour of the aphid hyperparasitoid *Alloxysta victrix* in association with the primary parasitoid Aphidius colemani and the host aphid *Myzus persicae*. *Biocontrol,* 43, 261-271.

Graves SR (2003) The role of component ratio integrity in host-plant selection: a chemical and biological approach. University College London.

Guerra PA (2011) Evaluating the life-history trade-off between dispersal capability and reproduction in wing dimorphic insects: a meta-analysis. *Biological Reviews,* 86, 813-835.

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

Guerrieri E, Pennacchio F & Tremblay E (1997) Effect of Adult Experience on in-Flight Orientation to Plant and Plant-Host Complex Volatiles in *Aphidius ervi* Haliday (Hymenoptera, Braconidae). *Biological Control,* 10, 159-165.

Guerrieri E, Poppy GM, Powell W, Rao R & Pennacchio F (2002) Plant-to-plant communication mediating in-flight orientation of *Aphidius ervi*. *Journal of chemical ecology,* 28, 1703-1715.

Guerrieri E, Poppy GM, Powell W, Tremblay E & Pennacchio F (1999) Induction and systemic release of herbivore-induced plant volatiles mediating in-flight orientation of *Aphidius ervi*. *Journal of chemical ecology,* 25, 1247-1261.

Guldemond JA, Dixon AFG, Pickett JA, Wadhams LJ & Woodcock CM (1993) Specificity of sex pheromones, the role of host plant odour in the olfactory attraction of males, and mate recognition in the aphid *Cryptomyzus*. *Physiological Entomology,* 18, 137-143.

Hamilton WD (1964a) The genetical evolution of social behavior. I. *Journal of Theoretical Biology,* 7, 1-16.

Hamilton WD (1964b) The genetical evolution of social behaviour. II. *Journal of theoretical Biology,* 7, 17-52.

Hamilton WD (1971) Geometry for the selfish herd. *Journal of theoretical Biology,* 31, 295-311.

Hardie J (1990) The photoperiodic counter, quantitative day-length effects and scotophase timing in the vetch aphid *Megoura viciae*. *Journal of Insect Physiology,* 36, 939-949.

Hardie J, Hick AJ, Höller C, Mann J, Merritt L, Nottingham SF, Powell W, Wadhams LJ, Witthinrich J & Wright AF (1994a) The responses of *Praon* spp. parasitoids to aphid sex pheromone components in the field. *Entomologia experimentalis et applicata,* 71, 95-99.

Hardie J, Holyoak M, Nicholas J, Nottingham SF, Pickett JA, Wadhams LJ & Woodcock CM (1990) Aphid sex pheromone components: Age-dependent release by females and species-specific male response. *Chemoecology,* 1, 63-68.

Hardie J, Isaacs R, Pickett JA, Wadhams LJ & Woodcock CM (1994b) Methyl salicylate and (−)-(1R, 5S)-myrtenal are plant-derived repellents for black bean aphid, *Aphis fabae* Scop.(Homoptera: Aphididae). *Journal of chemical ecology,* 20, 2847-2855.

Hardie J, Nottingham SF, Dawson GW, Harrington R, Pickett JA & Wadhams LJ (1992) Attraction of field-flying aphid males to synthetic sex pheromone. *Chemoecology,* 3, 113-117.

Hardie J, Nottingham SF, Powell W & Wadhams LJ (1991) Synthetic aphid sex pheromone lures female parasitoids. *Entomologia experimentalis et applicata,* 61, 97-99.

Hardie J, Peace L, Pickett JA, Smiley DWM, Storer JR & Wadhams LJ (1997) Sex pheromone stereochemistry and purity affect field catches of male aphids. *Journal of chemical ecology,* 23, 2547-2554.

Hardie J, Storer J, Nottingham S, Peace L, Harrington R, Merritt L, Wadhams L & Wood D (1994c) The interaction of sex pheromone and plant volatiles for field attraction of male bird-cherry aphid, Rhopalosiphum padi.

Hardie J, Visser JH & Piron PGM (1994d) Perception of volatiles associated with sex and food by different adult forms of the black bean aphid, *Aphis fabae*. *Physiological Entomology,* 19, 278-284.

Hassell MP & Southwood TRE (1978) Foraging strategies of insects. *Annual Review of Ecology and Systematics,* 9, 75-98.

Hawkins BA, Thomas MB & Hochberg ME (1993) Refuge theory and biological control. *Science,* 262, 1429-1432.

He XZ & Wang Q (2008) Reproductive strategies of *Aphidius ervi* Haliday (Hymenoptera: Aphidiidae). *Biological Control,* 45, 281-287.

Heimpel GE & Jervis MA (2005) Does floral nectar improve biological control by parasitoids. *Plant-Provided Food and Plant–Carnivore Mutualism*, 267-304.

Hérard F, Keller MA, Lewis WJ & Tumlinson JH (1988) Beneficial arthropod behavior mediated by airborne semiochemicals. *Journal of chemical ecology,* 14, 1583-1596.
Hickman CP, Roberts LS, Larson A & L'anson H (2004) *Integrated principles of zoology*, McGraw-Hill.
Hoffmann MP & Frodsham A (1993) Natural enemies of vegetable insect pests. IN Extension CC (Ed.), Cornell University.
Horsfall JL (1924) *Life history studies of Myzus persicae Sulzer*, Pennsylvania State College Agricultural Experiment Station.
Hou Z & Yan F (1995) Electroantennogram responses of *Lysiphlebia japonica* Ashmead (Hymenoptera: Aphidiidae) to some cotton plant volatiles and cotton aphid pheromones. *Insect Science,* 2, 253-264.
Jeon H, Han KS & Boo KS (2003) Sex Pheromone of< i> Aphis spiraecola< /i>(Homoptera: Aphididae): Composition and Circadian Rhythm in Release. *Journal of Asia-Pacific Entomology,* 6, 159-165.
Kapitsky SV & Zhukovskaya MI (1999) New analogs of sex pheromones of American cockroach (*Periplaneta americana* L.): Behavioral and electroantennographic study. *Pheromones,* 6, 3-14.
Karlson P & Butenandt A (1959) Pheromones (ectohormones) in insects. *Annual Review of Entomology,* 4, 39-58.
Kennedy JS, Day MF & Eastop VF (1962) *A conspectus of aphids as vectors of plant viruses*, The Eastern Press Ltd.
Kessler A & Baldwin IT (2001) Defensive function of herbivore-induced plant volatile emissions in nature. *Science,* 291, 2141.
Kight SL, Steelman L, Coffey G, Lucente J & Castillo M (2008) Evidence of population-level lateralized behaviour in giant water bugs, *Belostoma flumineum* Say (Heteroptera: Belostomatidae): T-maze turning is left biased. *Behavioural processes,* 79, 66-69.
Kim JJ, Lee MH, Yoon CS, Kim H, Yoo JK & Kim KC (2001) Control of cotton aphid and greenhouse whitefly with a fungal pathogen. *Biological control of greenhouse pests*, 8-15.
Laing J (1937) Host-finding by insect parasites. 1. Observations on the finding of hosts by *Alysia manducator*, *Mormoniella vitripennis* and *Trichogramma evanescens*. *The Journal of Animal Ecology*, 298-317.
Lane SD, Mills NJ & Getz WM (1999) The effects of parasitoid fecundity and host taxon on the biological control of insect pests: the relationship between theory and data. *Ecological Entomology,* 24, 181-190.
Laska M, Galizia CG, Giurfa M & Menzel R (1999) Olfactory discrimination ability and odor structure–activity relationships in honeybees. *Chemical senses,* 24, 429-438.
Law JH & Regnier FE (1971) Pheromones. *Annual review of biochemistry,* 40, 533-548.
Le Lann C, Visser B, Van Baaren J, Van Alphen JJM & Ellers J (2011) Comparing resource exploitation and allocation of two closely related aphid parasitoids sharing the same host. *Evolutionary Ecology*, 1-16.
Leather SR & Dixon AFG (1984) Aphid growth and reproductive rates. *Entomologia experimentalis et applicata,* 35, 137-140.
Leimu R, Mutikainen P, Koricheva J & Fischer M (2006) How general are positive relationships between plant population size, fitness and genetic variation? *Journal of Ecology,* 94, 942-952.
Leroy PD, Schillings T, Farmakidis J, Heuskin S, Lognay G, Verheggen FJ, Brostaux Y, Haubruge E & Francis F (2011) Testing semiochemicals from aphid, plant and conspecific: attraction of *Harmonia axyridis*. *Insect Science*.
Lewis WJ & Tumlinson JH (1988) Host detection by chemically mediated associative learning in a parasitic wasp. *Nature,* 331, 257-259.
Lewis WJ, Vet LEM, Tumlinson JH, Van Lenteren JC & Papaj DR (1990) Variations in parasitoid foraging behavior: essential element of a sound biological control theory. *Environmental Entomology,* 19, 1183-1193.

Light DM, Flath RA, Buttery RG, Zalom FG, Rice RE, Dickens JC & Jang EB (1993) Host-plant green-leaf volatiles synergize the synthetic sex pheromones of the corn earworm and codling moth (Lepidoptera). *Chemoecology,* 4, 145-152.
Lilley R, Hardie J, Merritt L, Pickett JA, Wadhams LJ & Woodcock CM (1994a) The sex pheromone of the grain aphid, Sitobion avenae (Fab.)(Homoptera, Aphididae). *Chemoecology,* 5, 43-46.
Lilley R, Hardie J, Powell W & Wadhams LJ (1994b) The aphid sex pheromone: a novel host location cue for the parasitoid *Praon volucre*. *Brighton Crop Protection Conference - Pests and Diseases.* Thornton Health, UK.
Lin LA & Ives AR (2003) The effect of parasitoid host size preference on host population growth rates: an example of *Aphidius colemani* and *Aphis glycines*. *Ecological Entomology,* 28, 542-550.
Liu Y, Wang WL, Guo GX & Ji XL (2009) Volatile emission in wheat and parasitism by *Aphidius avenae* after exogenous application of salivary enzymes of *Sitobion avenae*. *Entomologia experimentalis et applicata,* 130, 215-221.
Loughrin JH, Manukian A, Heath RR, Turlings T & Tumlinson JH (1994) Diurnal cycle of emission of induced volatile terpenoids by herbivore-injured cotton plant. *Proceedings of the National Academy of Sciences,* 91, 11836.
Luck RF, Van Lenteren JC, Twine PH, Juenen L & Unruh T (1979) Prey or host searching behavior that leads to a sigmoid functional response in invertebrate predators and parasitoids. *Researches on Population Ecology,* 20, 257-264.
Lushai G & Loxdale HD (2004) Tracking movement in small insect pests, with special reference to aphid populations. *International journal of pest management,* 50, 307-315.
Macgillivray ME & Anderson GB (1958) Development of four species of aphids (Homoptera) on potato. *The Canadian Entomologist,* 90, 148-155.
Maddrell SHP (1969) Secretion by the Malpighian tubules of Rhodnius. The movements of ions and water. *Journal of Experimental Biology,* 51, 71-97.
Mads H (2012) www.random.org.
Malyshev SI (1968) Genesis of the Hymenoptera and the phases of their evolution. *Methuen and Co., London*.
Mandelbrot BB (1983) *The fractal geometry of nature*, Wh Freeman.
Marsh D (1975) Responses of male aphids to the female sex pheromone in *Megoura viciae* Buckton. *Journal of Entomology Series A, General Entomology,* 50, 43-64.
Martinez-Torres D, Foster SP, Field LM, Devonshire AL & Williamson MS (1999) A sodium channel point mutation is associated with resistance to DDT and pyrethroid insecticides in the peach-potato aphid, *Myzus persicae* (Sulzer)(Hemiptera: Aphididae). *Insect Molecular Biology,* 8, 339-346.
Mcallister MK & Roitberg BD (1987) Adaptive suicidal behaviour in pea aphids. *Nature,* 328, 797-799.
Mccall P & Kelly DW (2002) Learning and memory in disease vectors. *Trends in parasitology,* 18, 429-433.
Mcvean RIK & Dixon AFG (2001) The effect of plant drought-stress on populations of the pea aphid *Acyrthosiphon pisum*. *Ecological Entomology,* 26, 440-443.
Meiners T, Wäckers F & Lewis WJ (2003) Associative learning of complex odours in parasitoid host location. *Chemical senses,* 28, 231-236.
Messing RH & Rabasse JM (1995) Oviposition behaviour of the polyphagous aphid parasitoid *Aphidius colemani* Viereck (Hymenoptera: Aphidiidae). *Agriculture, ecosystems & environment,* 52, 13-17.
Micha SG & Wyss U (1996) Aphid alarm pheromone (E)-β-farnesene: A host finding kairomone for the aphid primary parasitoid *Aphidius uzbekistanicus* (Hymenoptera: Aphidiinae). *Chemoecology,* 7, 132-139.
Michaud JP & Mackauer M (1995) Oviposition behavior of *Monoctonus paulensis* (Hymenoptera: Aphidiidae): factors influencing reproductive allocation to hosts and host patches. *Annals of the Entomological Society of America,* 88, 220-226.
Moran NA (1992) The evolution of aphid life cycles. *Annual Review of Entomology,* 37, 321-348.

Nansen C, Macdonald KM, Rogers CD, Thomas M, Poppy GM & Baxter IH (2007) Effects of sex pheromone in electrostatic powder on mating behaviour by *Lobesia botrana* males. *Journal of Applied Entomology,* 131, 303-310.

Nasreddine L & Parent-Massin D (2002) Food contamination by metals and pesticides in the European Union. Should we worry? *Toxicology letters,* 127, 29-41.

Nottingham SF, Hardie J, Dawson GW, Hick AJ, Pickett JA, Wadhams LJ & Woodcock CM (1991) Behavioral and electrophysiological responses of aphids to host and nonhost plant volatiles. *Journal of chemical ecology,* 17, 1231-1242.

Olson DM, Rains GC, Meiners T, Takasu K, Tertuliano M, Tumlinson JH, Wäckers FL & Lewis WJ (2003) Parasitic wasps learn and report diverse chemicals with unique conditionable behaviors. *Chemical senses,* 28, 545-549.

Orr DB & Suh CPC (1998) Parasitoids and predators. *Biological and Biotechnological Control of Insect Pests. Ed. by Rechcigl, JE*, 3-34.

Östman Ö, Ekbom B & Bengtsson J (2003) Yield increase attributable to aphid predation by ground-living polyphagous natural enemies in spring barley in Sweden. *Ecological Economics,* 45, 149-158.

Paré PW & Tumlinson JH (1999) Plant volatiles as a defense against insect herbivores. *Plant Physiology,* 121, 325-332.

Park KC, Zhu J, Harris J, Ochieng SA & Baker TC (2001) Electroantennogram responses of a parasitic wasp, *Microplitis croceipes*, to host-related volatile and anthropogenic compounds. *Physiological Entomology,* 26, 69-77.

Peters TM & Barbosa P (1977) Influence of population density on size, fecundity, and developmental rate of insects in culture. *Annual Review of Entomology,* 22, 431-450.

Pettersson J (1970) An aphid sex attractant. *Insect Systematics 38; Evolution,* 1, 63-73.

Picard F (1922) Contribution a l'etude des parasites de *Pieris brassicae* L. *Bulletin biologique de la France et de la Belgique,* 56, 130.

Pickett JA, Aradottir GI, Birkett MA, Bruce TJA, Chamberlain K, Khan ZR, Midega CA, Smart LE & Woodcock CM (2012) Aspects of insect chemical ecology: exploitation of reception and detection as tools for deception of pests and beneficial insects. *Physiological Entomology,* 37, 2-9.

Pickett JA, Wadhams LJ, Woodcock CM & Hardie J (1992) The chemical ecology of aphids. *Annual Review of Entomology,* 37, 67-90.

Pope TW, Campbell CaM, Hardie J, Pickett JA & Wadhams LJ (2007) Interactions between host-plant volatiles and the sex pheromones of the bird cherry-oat aphid, *Rhopalosiphum padi* and the damson-hop aphid, *Phorodon humuli*. *Journal of chemical ecology,* 33, 157-165.

Pope TW, Girling RD, Staley JT, Trigodet B, Wright DJ, Leather SR, Van Emden HF & Poppy GM (2011) Effects of organic and conventional fertilizer treatments on host selection by the aphid parasitoid *Diaeretiella rapae*. *Journal of Applied Entomology,* 136, 445-455.

Pope TW, Kissen R, Grant M, Pickett JA, Rossiter JT & Powell G (2008) Comparative innate responses of the aphid parasitoid *Diaeretiella rapae* to alkenyl glucosinolate derived isothiocyanates, nitriles, and epithionitriles. *Journal of chemical ecology,* 34, 1302-1310.

Poppy GM & Powell W (2004) Genetic Manipulation of Natural Enemies: Can We Improve Biological Control by. *Genetics, evolution, and biological control*, 219.

Poppy GM, Powell W & Pennacchio F (1997) Aphid parasitoid responses to semiochemicals—Genetic, conditioned or learnt? *Biocontrol,* 42, 193-199.

Potting RPJ, Poppy GM & Schuler TH (1999) The role of volatiles from cruciferous plants and pre-flight experience in the foraging behaviour of the specialist parasitoid *Cotesia plutellae*. *Entomologia experimentalis et applicata,* 93, 87-95.

Powell W, A Hara S, Harling R, Holland J, Northing P, Thomas CFG & Walters KFA (2004) Managing biodiversity in field margins to enhance integrated pest control in arable crops (3-D farming project). *HGCA Project Report,* 356.

Powell W, Hardie J, Hick AJ, Höller C, Mann J, Merritt L, Nottingham SF, Wadhams LJ, Witthinrich J & Wright AF (1993) Responses of the parasitoid *Praon volucre* (Hymenoptera: Braconidae) to aphid sex pheromone lures in cereal fields in autumn: Implications for parasitoid manipulation. *European Journal of Entomology,* 90, 435-438.

Powell W, Pennacchio F, Poppy GM & Tremblay E (1998) Strategies Involved in the Location of Hosts by the Parasitoid *Aphidius ervi* Haliday (Hymenoptera: Braconidae: Aphidiinae). *Biological Control,* 11, 104-112.

Powell W & Pickett JA (2003) Manipulation of parasitoids for aphid pest management: progress and prospects. *Pest management science,* 59, 149-155.

Radcliffe EB, Ragsdale DW & Flanders KL (1993) Management of aphids and leafhoppers. St. Pileups Press.

Rains GC, Tomberlin JK, D'alessandro M & Lewis WJ (2004) Limits of volatile chemical detection of a parasitoid wasp, *Microplitis croceipes*, and an electronic nose: A comparative study. *Transactions of the Asae,* 47, 2145-2152.

Rand TA, Van Veen FJ & Tscharntke T (2011) Landscape complexity differentially benefits generalized fourth, over specialized third, trophic level natural enemies. *Ecography,* 35, 97-104.

Reddy GVP & Guerrero A (2004) Interactions of insect pheromones and plant semiochemicals. *Trends in Plant Science,* 9, 253-261.

Rehman A & Powell W (2010) Host selection behaviour of aphid parasitoids (Aphidiidae: Hymenoptera). *Journal of Plant Breeding and Crop Science,* 2, 299-311.

Resh VH & Cardé RT (2009) *Encyclopedia of insects*, Academic Press.

Reuter OM (1913) *Lebensgewohnheiten und Instinkte der Insekten bis zum erwachen der sozialen Instinkte geschildert*, R. Friedländer & Sohn.

Roschewitz I, Hücker M, Tscharntke T & Thies C (2005) The influence of landscape context and farming practices on parasitism of cereal aphids. *Agriculture, ecosystems & environment,* 108, 218-227.

Rose USR, Manukian A, Heath RR & Tumlinson JH (1996) Volatile semiochemicals released from undamaged cotton leaves (a systemic response of living plants to caterpillar damage). *Plant Physiology,* 111, 487-495.

Rosenheim JA & Rosen D (1991) Foraging and oviposition decisions in the parasitoid *Aphytis lingnanensis*: distinguishing the influences of egg load and experience. *The Journal of Animal Ecology*, 873-893.

Ruther J, Meiners T & Steidle JLM (2002) Rich in phenomena-lacking in terms. A classification of kairomones. *Chemoecology,* 12, 161-167.

Ruther J, Reinecke A, Thiemann K, Tolasch T, Francke W & Hilker M (2000) Mate finding in the forest cockchafer, *Melolontha hippocastani*, mediated by volatiles from plants and females. *Physiological Entomology,* 25, 172-179.

Rutledge CE (1996) A survey of identified kairomones and synomones used by insect parasitoids to locate and accept their hosts. *Chemoecology,* 7, 121-131.

Saïd I, Torre RA, Morin JP & Rochat D (2006) Adaptation of a four-arm olfactometer for behavioural bioassays of large beetles. *Chemoecology,* 16, 9-16.

Sanders D & Van Veen FJF (2012) Indirect commensalism promotes persistence of secondary consumer species. *Biology Letters*, Available online 15/08/2012.

Sequeira R & Mackauer M (1992a) Nutritional ecology of an insect host-parasitoid association: the pea aphid-*Aphidius ervi* system. *Ecology,* 73, 183-189.

Sequeira R & Mackauer M (1992b) Covariance of adult size and development time in the parasitoid wasp *Aphidius ervi* in relation to the size of its host, *Acyrthosiphon pisum*. *Evolutionary Ecology,* 6, 34-44.

Shu-Sheng L & Hughes RD (1984) Effect of host age at parasitization by *Aphidius sonchi* on the development, survival, and reproduction of the sowthistle aphid, *Hyperomyzus lactucae*. *Entomologia experimentalis et applicata,* 36, 239-246.

Starý P (1970) Biology of aphid parasites (Hymenoptera: Aphidiidae) with respect to integrated control. *Series entomologica.,* 6, 643.

Starý P (1975) *Aphidius colemani* Viereck: its taxonomy, distribution and host range (Hymenoptera, Aphidiidae). *Acta Entomologica Bohemoslovaca,* 72, 156-163.

Stewart-Jones A, Dewhirst SY, Durrant L, Fitzgerald JD, Hardie J, Hooper AM, Pickett JA & Poppy GM (2007) Structure, ratios and patterns of release in the sex pheromone of an aphid, *Dysaphis plantaginea*. *Journal of Experimental Biology,* 210, 4335-4344.

Storeck A, Poppy GM, Van Emden HF & Powell W (2000) The role of plant chemical cues in determining host preference in the generalist aphid parasitoid *Aphidius colemani*. *Entomologia experimentalis et applicata,* 97, 41-46.

Sutherland ORW & Mittler TE (1971) Influence of diet composition and crowding on wing production by the aphid *Myzus persicae*. *Journal of Insect Physiology,* 17, 321-328.

Takasu K & Lewis WJ (1993) Host and Food-Foraging of the Parasitoid *Microplitis croceipes*: Learning and Physiological State Effects. *Biological Control,* 3, 70-74.

Tanaka C, Kainoh Y & Honda H (2001) Host frass as arrestant chemicals in locating host *Mythimna separata* by the tachinid fly *Exorista japonica*. *Entomologia experimentalis et applicata,* 100, 173-178.

Thacker JRMD (2002) *An introduction to arthropod pest control*, Cambridge University Press.

Trionnaire G, Hardie J, Jaubert-Possamai S, Simon JC & Tagu D (2008) Shifting from clonal to sexual reproduction in aphids: physiological and developmental aspects. *Biology of the Cell,* 100, 441-451.

Turlings TCJ & Benrey B (1998) Effects of plant metabolites on the behavior and development of parasitic wasps. *Ecoscience,* 5, 321-333.

Turlings TCJ, Loughrin JH, Mccall PJ, Röse U, Lewis WJ & Tumlinson JH (1995) How caterpillar-damaged plants protect themselves by attracting parasitic wasps. *Proceedings of the National Academy of Sciences,* 92, 4169.

Turlings TCJ, Tumlinson JH & Lewis WJ (1990) Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science,* 250, 1251-1253.

Turlings TCJ, Wäckers FL, Vet LEM, Lewis WJ & Tumlinson JH (1993) Learning of host-finding cues by hymenopterous parasitoids. *Insect learning: ecological and evolutionary perspectives*, 51-78.

Van Alphen JJM & Vet LEM (1986) An evolutionary approach to host finding and selection. In: Waage JK & Greathead DJ (eds.) *Insect parasitoids: 13th symposium of the Royal Entomological Society of London.* London.

Van Emden HF, Douloumpaka S, Poppy GM & Powell W (2008) Plant chemistry and aphid parasitoids (Hymenoptera: Braconidae): Imprinting and memory. *European Journal of Entomology,* 105, 477-483.

Van Emden HF, Eastop VF, Hughes RD & Way MJ (1969) The ecology of *Myzus persicae*. *Annual Review of Entomology,* 14, 197-270.

Van Emden HF, Eletherianos I, Rose J, Douloumpaka S & Pettersson J (2002) Aphid parasitoids detect that an alien plant was present nearby during their development. *Physiological Entomology,* 27, 199-205.

Van Emden HF, Sponagl B, Wagner E, Baker T, Ganguly S & Douloumpaka S (1996) Hopkins' 'host selection principle', another nail in its coffin. *Physiological Entomology,* 21, 325-328.

Van Huynh N, Birch ANE, Songa J, Vanđinh N, Andow D, Hillbeck A & Tuat N (2008) Potential effects of transgenic cotton on non-target insect parasitoids in Vietnam. *Environmental Risk Assessment of Genetically Modified Organisms: Vol. 4: Challenges and Opportunities with Bt cotton in Vietnam,* 4, 212.

Van Loon JJA, Boer JG & Dicke M (2000) Parasitoid-plant mutualism: parasitoid attack of herbivore increases plant reproduction. *Entomologia experimentalis et applicata,* 97, 219-227.

Van Steenis MJ (1995) Evaluation and application of parasitoids for biological control of *Aphis gossypii* in glasshouse cucumber crops. University of Wageningen.

Verheggen FJ, Haubruge E, De Moraes CM & Mescher MC (2009) Social enviroment influences aphid production of alarm pheromone. *Behavioral Ecology,* 20, 283-288.

Vet LEM & Dicke M (1992) Ecology of infochemical use by natural enemies in a tritrophic context. *Annual Review of Entomology,* 37, 141-172.

Vet LEM, Lenteren JC, Heymans M & Meelis E (1983) An airflow olfactometer for measuring olfactory responses of hymenopterous parasitoids and other small insects. *Physiological Entomology,* 8, 97-106.

Vet LEM, Lewis WJ, Papaj DR & Van Lenteren JC (1990) A variable-response model for parasitoid foraging behavior. *Journal of Insect Behavior,* 3, 471-490.

Villagra CA, Pennacchio F & Niemeyer HM (2007) The effect of larval and early adult experience on behavioural plasticity of the aphid parasitoid *Aphidius ervi* (Hymenoptera, Braconidae, Aphidiinae). *Naturwissenschaften,* 94, 903-910.

Vinson SB (1976) Host selection by insect parasitoids. *Annual Review of Entomology,* 21, 109-133.

Vinson SB & Iwantsch G (1980) Host suitability for insect parasitoids. *Annual Review of Entomology,* 25, 397-419.

Wäckers FL & Bruin J (2005) *Plant-provided food for carnivorous insects: a protective mutualism and its applications*, Cambridge University Press.

Wäckers FL & Lewis WJ (1994) Olfactory and visual learning and their combined influence on host site location by the parasitoid *Microplitis croceipes* (Cresson). *Biological Control,* 4, 105-112.

Wadhams LJ, Angst ME & Blight MM (1982) Responses of the olfactory receptors of *Scolytus scolytus* (F.)(Coleoptera: Scolytidae) to the stereoisomers of 4-methyl-3-heptanol. *Journal of chemical ecology,* 8, 477-492.

Wajnberg É (2006) Time allocation strategies in insect parasitoids: from ultimate predictions to proximate behavioral mechanisms. *Behavioral Ecology and Sociobiology,* 60, 589-611.

Wang HL, Svensson GP, Rosenberg O, Bengtsson M, Jirle EV & Löfstedt C (2010) Identification of the sex pheromone of the spruce seed moth, *Cydia strobilella* L. *Journal of chemical ecology,* 36, 305-313.

Wang KY, Guo QL, Xia XM, Wang HY & Liu TX (2007) Resistance of *Aphis gossypii* (Homoptera: Aphididae) to selected insecticides on cotton from five cotton production regions in Shandong, China. *Journal of Pesticide Science,* 32, 372-378.

Webster B, Bruce TJA, Dufour S, Birkemeyer C, Birkett MA, Hardie J & Pickett JA (2008) Identification of volatile compounds used in host location by the black bean aphid, *Aphis fabae*. *Journal of chemical ecology,* 34, 1153-1161.

Wei JN, Bai BB, Yin TS, Wang Y, Yang Y, Zhao LH, Kuang RP & Xiang RJ (2005) Development and use of parasitoids (Hymenoptera: Aphidiidae & Aphelinidae) for biological control of aphids in China. *Biocontrol Science and Technology,* 15, 533-551.

Wellings PW (1991) Host location and oviposition on animals. In: Bailey WJ & Ridsdill-Smith J (eds.) *Reproductive behavior of insects: individuals and populations.* Chapman & Hall.

Wertheim B, Van Baalen EJA, Dicke M & Vet LEM (2005) Pheromone-mediated aggregation in nonsocial arthropods: an evolutionary ecological perspective. *Annu. Rev. Entomol.,* 50, 321-346.

Wiskerke JSC, Dicke M & Vet LEM (1993) Drosophila parasitoid solves foraging problem through infochemical detour: the role of adult fly pheromone. *Proceedings of the Section Experimental and Applied Entomology of teh Netherlands Entomological Society.*

Wohlers P (1981) Effects of the alarm pheromone (E)-β- farnesene on dispersal behaviour of the pea aphid *Acyrthosiphon pisum*. *Entomologia experimentalis et applicata,* 29, 117-124.

Wyatt IJ & White P (1977) Simple estimation of intrinsic increase rates for aphids and tetranychid mites. *Journal of Applied Ecology,* 14, 757-766.

Wyatt TD (2003) *Pheromones and animal behaviour: communication by smell and taste*, Cambridge University Press.

Yu XD, Pickett JA, Ma YZ, Bruce TJA, Napier J, Jones HD & Xia LQ (2012) Metabolic Engineering of Plant-derived (E)-β-farnesene Synthase Genes for a Novel Type of Aphid-resistant GM Crop Plants. *Journal of Integrative Plant Biology,* 54, 282-299.