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**Contact: [gala@gre.ac.uk](mailto:gala@gre.ac.uk)**

**Title** Response of *Aphidius colemani* to aphid sex pheromone varies depending on plant synergy and prior experience

**Short Title** *A. colemani* response to aphid sex pheromone

**Authors** GM Fernández-Grandon & GM Poppy

### **Abstract**

A critical stage in the success of a parasitoid is the ability to locate a host within its habitat. It is hypothesized that a series of olfactory cues may be involved in altering the parasitoid's movement patterns at this stage of foraging. This paper focuses specifically on host habitat location and host location and the olfactory stimuli necessary to mediate the transition between these stages. Firstly we confirm the ability of the parasitoid *Aphidius colemani* to detect the aphid sex pheromone at an electrophysiological level. Following this we investigate the effect of the sex pheromone component (4a*S*,7*S*,7a*R*)-nepetalactone on the movement patterns of *A. colemani* and its retention within an area. The key findings of this work are that *A. colemani* is able to detect the sex pheromone components, that parasitoid retention is increased by a synergy of nepetalactone and other host-associated cues and that foraging patterns are augmented by the presence of nepetalactone or experience associated with nepetalactone.

### **Introduction**

One of the main challenges facing a parasitoid is locating a host in a stimulus rich environment. For parasitoids, like many other insects, olfaction plays an important role in host location and host-habitat location (Wäschke *et al.*, 2014). Having an acute sense of smell is critical for the success of a parasitoid in locating its host but means that any given environment will appear populated with a wide range of stimuli. Part of the challenge for the foraging parasitoid will be to distinguish the odours which relate to host presence and adjust their behaviour accordingly to minimize the energy expenditure in locating the host.

The process of host location in a multitrophic environment is frequently viewed as a series of stages. Divisions of the stages of host selection are often defined either by a hierarchy of cues, or the order in which they are encountered (van Alphen & Vet, 1986; Wellings, 1991). One of the most widely adopted categorisation systems is that proposed by Vinson (1976). Vinson describes five distinct stages in host selection: host habitat location, host location, host acceptance, host suitability and regulation. Our focus in this study is the transition between the initial two stages: host habitat location and host location.

To understand the transition between host habitat location and host location we must also consider the reliability-detectability theory (Vet & Dicke, 1992). This theory identifies that while odours more distantly associated with the host may be more abundant, those most closely related with the host, while more discrete, will offer greater reliability. We speculate that less reliable host habitat odours (plant volatiles) will be the principal cue used in host habitat location but more reliable host cues will be required to initiate host location. For this reasons we selected the use of the aphid sex pheromone in this study. Aphid sex pheromones from a range of species have been shown to comprise (1*R*,4*aS*,7*S*,7*aR*)-nepetalactol and (4*aS*,7*S*,7*aR*)-nepetalactone (for full list see Hardie & Minks, 1999). In a natural environment

female oviparae will produce the pheromone from glands on the metathoracic tibia (Marsh, 1975) and wave their legs to increase exposure of the glands for the male aphids to detect (Pettersson, 1970). The odour is detected by a range of aphid parasitoids (see Powell and Pickett, 2003) in addition to lacewings (Zhang *et al.*, 2006) and ladybirds (Leroy *et al.*, 2012) which predate on aphids. Although aphid populations are often asexual, it is hypothesized that the strong response seen by parasitoids is because the critical timing at which aphid populations produce the pheromone which may be the final chance for the parasitoid to find an overwintering host (Hardie *et al.*, 1991; Wadhams *et al.*, 1999).

Integral to parasitoid foraging success is the learning plasticity displayed throughout their lifecycle. Although odour preferences may be acquired in the early stages of the parasitoid lifecycle they are frequently adjusted by experiences of associative learning which the adult will gain through successful oviposition (Storeck *et al.*, 2000). Odours which already exist in the wasp's chemical catalogue may elicit an increased responsiveness (Turlings *et al.*, 1993) such as seen in alpha conditioning. It has previously been hypothesized that nepetalactone, which is considered a highly reliable cue, will not be subject to alpha conditioning (Glinwood *et al.*, 1999). Using a stimulus to which the parasitoid potentially already holds an innate response offers an exciting opportunity to explore the extent of associative learning and address the reliability-detectability hypothesis with regard to changes in foraging behaviour. Experience in this study is provided via associative learning with the sex pheromone which was not present in any previous stage of the individual's lifecycle.

The high reliability of aphid sex pheromone makes it a good candidate for the study of behavioural changes during foraging but it could also have application in biological control systems. Work has previously shown that the spatial distribution of aphid parasitoids is

altered by the presence of nepetalactone (Powell, 2004). Although the compounds are detected by asexual aphids (Fernandez-Grandon *et al.*, 2013), there is no evidence that it would alter their behaviour or population dynamics. Laboratory bioassays have shown an innate behavioural responses to nepetalactone for the parasitoid *Aphidius ervi* (Poppy *et al.*, 1997). The prevalence of this behavioural response is supported by field work by Glinwood *et al.*, (1998) which has shown that parasitism by *Praon volucre* and *Aphidius rhopalosiphii* can be increased around vials of nepetalactone. Despite being one of the most commonly used parasitoids in the biological control of aphids, it is not known whether the aphid parasitoid *Aphidius colemani* Viereck (Hymenoptera: Aphidiinae) responds to components of the sex pheromone.

*Aphidius colemani* is a generalist parasitoid frequently employed in the control of a range of glasshouse pests. It is a favourable control agent due to its short life cycle, high reproductive potential, ability to parasitize a several aphid species and the relative ease with which they can be mass reared. As members of Hymenoptera they display a high aptitude for learning, this ability to learn combined with their need to oviposit in a suitable host to ensure reproduction make them an ideal candidate for studying foraging behaviours. A study by Ameixa & Kindlmann (2011) has shown that the behaviour of the parasitoid can be altered by *Nepeta cataria* oil, which contains the sex pheromone components, however no confirmation of a response to the specific compounds was provided.

The aims of this research were to determine the ability of *A. colemani* to detect pheromone components, if there are synergies with the pheromone which alter their behaviour and if the pheromone plays a role in transitioning from host habitat location to host location.

To achieve this we implemented electroantennography to test *A. colemani* response to the compounds (1*R*,4*aS*,7*S*,7*aR*)-nepetalactol and (4*aS*,7*S*,7*aR*)-nepetalactone which comprise the aphid sex pheromone. Following this we explored, through retention of the parasitoid, if there are any synergies with the component nepetalactone and plant or host volatiles. Finally, using tracking software, we identified the exact locomotary response of the parasitoid in various conditions to reveal changes associated with host foraging.

## Materials and Methods

### Insects

Peach-potato aphids, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), were reared on Chinese cabbage *Brassica rapa* spp. *pekinensis* cv. Wong Bok as asexual virginoparae morphs. The generalist parasitoid *Aphidius colemani* were maintained on these *M. persicae* colonies for use in electrophysiological work. While reproducing asexually, *M. persicae* do not produce any sex pheromone (Hardie *et al.*, 1990), therefore any treatments used in this study represent the parasitoids' first encounter with the aphid sex pheromone. For all behavioural assays, *A. colemani* were provided by Koppert (Koppert B.V., Netherlands) from a different host/plant rearing background (K. Bolckmans, pers. comm.). *A. colemani* were used directly from supply meaning they had no experience on our host/plant system prior to the assays.

### Electrophysiology

Electroantennogram (EAG) recordings from female *A. colemani* were made using Ag-AgCl glass electrodes filled with saline solution (composition as in Maddrell, 1969 but without glucose). The parasitoid was anaesthetized by chilling after which the head was excised and

the most distal antenna tips removed. The excised head was placed within the indifferent electrode, with the antennae protruding. Both antennae were inserted into the recording electrode. The signals were passed through a high impedance amplifier (UN-06, Syntech, Netherlands) and analysed using a customized software package (Syntech). The delivery system, which employed a filter paper in a disposable Pasteur pipette cartridge, has been described previously (Wadhams *et al.*, 1982). The stimulus (2s duration) was delivered into a purified air stream (1 l/min) flowing continuously over the preparation.

Samples (10  $\mu$ l) of the nepetalactone and nepetalactol, diluted in hexane (50  $\mu$ g/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 a disposable Pasteur pipette cartridge. Hexane was used as a control stimulus. Fresh cartridges were prepared immediately prior to use. EAG responses to the two sex pheromone components were standardized to the response to hexane, applied at the beginning and end of each run, following the methodology used by Park *et al.* (2001) to account for signal deterioration. A total of 5 individuals were tested, each representing an independent replicate.

### **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, vibrational and visual cues that it receives. The on-leaf retention time assay allowed an evaluation of latency to leaving the leaf under different conditions. This assay allowed us to investigate potential synergies between plant volatiles, aphid associated odours and nepetalactone in addition to the standardized visual stimulus of a leaf surface.

Leaves were excised from 3 week-old Chinese cabbage. Each leaf was placed in a small plastic 'Blackman box' measuring 7.5 x 4.4 x 2.2 cm. These boxes allow for the leaf stem to pass into another area where it is kept moist by a damp sponge, allowing a greatly improved longevity for excised leaves (for full description see Blackman, 1971). The box was rested in a shallow basin of water, allowing the leaf to receive moisture through a sponge in the base. Treatments described as empty had no leaf in the box and no odour stimuli present. Those described as previously infested had 10 aphids (3<sup>rd</sup> instar to adult) placed on the leaf using a size 0 paintbrush and allowed to feed. After feeding for 16h, when the experiment was to be conducted, the aphids were gently removed from the leaves but any exuviae and honeydew was allowed to remain. The treatment involving only 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 24h with no aphid attack to allow volatile production to diminish (Du *et al.*, 1996, 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 different leaf from the same plant was then used in the assay.

A female *Aphidius colemani* naïve to the sex pheromone and the plant/host complex was placed on the centre of the leaf surface and latency to leaving the area was recorded. It was important that a replicate for all treatments in a set could be completed within a day to reduce temporal and cohort behavioural variation. For this reason the treatments were split into two distinct sets with the first covering a broader range of potential cues and the second focusing on those showing the greatest parasitoid retention. In Set 1 the parasitoid was allowed 15 minutes before the assay was completed; in Set 2 each parasitoid was allowed 20 minutes. The extended time allowance was used because the odours present in Set 2 were considered



to be of greater reliability therefore increasing the time before the parasitoid would leave the area. If the parasitoid did stay longer as a result of these odours, 15 minutes would not have been sufficient to distinguish treatments. The assay was stopped if the parasitoid walked or flew out of the Blackman box. The time of leaving the leaf or central area, when no leaf was present, was also recorded giving an accurate measure of retention time, or latency of period before leaving the leaf/area. Treatments using nepetalactone had the compound 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 providing an approximate release rate of 0.3 µg/min and 3 µg/min respectively. In both sets of the assay 35 replicates were completed, each representing a separate individual leaf, Blackman box and female aged 2-4 days old. The bioassay room was maintained at  $20 \pm 1^\circ\text{C}$ .

### **Motivation assay**

The motivation assay arena was constructed of a Petri dish 9 cm in diameter (Sterilin Limited, UK) 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. One centimetre to the side of the square, a circle was cut of approximately 0.5 cm diameter which provided an entry point for the parasitoid from below the assay arena (Fig. 1). The larger upper dish formed the assay arena while the smaller dish on the underside was the odour source chamber. The parasitoids were provided with a wick of 20% honey solution and kept in a glass vial in the controlled environment 30 minutes before the start of the experiment to allow them to acclimate. 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 using a TK-C1360B colour camera (JVC). Experiments were conducted during the most active part of the insect's

photophase (08.00-12.00) under ~ 1.7 kilolux of lighting using a lamp with a diffusion light filter. The bioassay room was maintained at  $20 \pm 1^\circ\text{C}$ . The lid of a vial containing a parasitoid was placed in the 0.5 cm diameter hole in the base of the arena, once the lid was affixed to the entry point the recording commenced. The behaviour of the parasitoid was recorded for 8 minutes with the intention of identifying differences in movement speeds and frequency of turning to indicate changes in foraging behaviour. Particular differences in behaviour which may indicate a switch to foraging would be a reduction in locomotion and increase in turning. Our null hypothesis would be that none of the treatments used would alter the movement speed or pattern of the parasitoids when compared to values seen in the control group.

Recordings were not analysed in real time but were recorded onto DVD and tracking software Ethovision software (v 3.1 Noldus Information Technology) was applied later during playback to track the movement of the insect within the arena. Oviposition experience was provided by giving each parasitoid 5 minutes to freely oviposit in a Petri dish on a leaf containing 10 aphids of mixed instars. At least one oviposition attempt had to be observed for the wasp to be used in the study. All parasitoids were naïve in that they emerged entirely in an isolated laboratory environment and therefore had no previous adult encounter with aphids or the sex pheromone prior to the assays. A full listing of the treatments used can be seen in Table 1. Pre-treatments were administered immediately before commencing the assay in a separate Petri dish. Treatments were applied only 30s before the wasp entered the arena. The still-air nature of this assay and passive movement of the odour into the arena allowed odour saturation. This was intentional as the design was not aimed at testing the response to locating the odour source but behaviours when the parasitoid is in close proximity to the odour source. All replicates were completely independent with a new wasp, leaf and treatment applied. All equipment was cleaned between each replicate.

## **Statistical analysis**

### *Electrophysiology*

Antennal response to the aphid sex pheromone components was compared to the hexane control using a Mann-Whitney rank sum test conducted using Minitab software (Minitab 16, 2010).

### *Retention time*

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

### *Motivation assay*

Differences between treatments and the control group were identified using Kruskal-Wallis tests with Minitab statistical software (Minitab 16, 2010). Due to the repeated analysis, the Bonferroni correction was applied posthoc.

## **Results:**

### **Electrophysiology**

EAG was conducted using (4a*S*,7*S*,7a*R*)-nepetalactone and (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol comparing these treatments against hexane as a control (Fig. 2). EAG responses of the parasitoid antenna indicated that the 0.05 mg/ml of both isomers failed to elicit a response within the antenna. However, a concentration of 1 mg/ml the nepetalactone did elicit an

electrophysiological response significantly greater than the control ( $T_4 = 4.09$ ,  $P = 0.026$ ) which was also seen in the higher concentration of 10 mg/ml ( $T_4 = 12.6$ ,  $P < 0.001$ ). A response to nepetalactol was only seen in the highest concentration of 10 mg/ml ( $T_4 = 3.72$ ,  $P = 0.02$ ).

### **On-leaf retention time**

Retention of *A. colemani* in a specific area was tested using a range of treatments. This approach allowed the identification of any synergies or additive effects of the cues available. A comparison of the retention time between treatments also reveals the hierarchy of cues which may be utilized by the foraging wasp.

In the first set of trials 5 treatments were tested, each with a maximum running time of 15 minutes. The negative control (an empty arena) was found to have a significantly lower retention time than all treatments containing leaf material ( $42 \pm 12s$ ) ( $F_{4,159} = 15.55$ ,  $P < 0.001$ ). Between treatments containing a leaf there was no distinction between a leaf alone, a previously infested leaf or nepetalactone presented alongside the leaf (Fig. 3). The only treatment showing a greater retention time than the leaf alone was a previously aphid-infested leaf with nepetalactone.

In the second set of retention time assays the maximum permitted time was increased to 20 minutes. A significant difference was observed between treatments ( $F_{4,160} = 21.42$ ,  $P < 0.001$ ). As with Set 1, the negative control shows a significantly lower retention time than all other treatments (Fig. 4). Retention time for a high concentration of nepetalactone (10 mg/ml) alongside a leaf resulted in a retention time equal to that of the systemically damaged plant leaf alongside nepetalactone. A leaf containing aphid-related

cues alongside nepetalactone led the wasp to spend more than three times as long in the area than on the leaf with a high concentration of nepetalactone or a systemically damaged leaf and a more than six times longer than the negative control. This value was comparable to the previously infested leaf alongside nepetalactone which, as with set 1, led to the parasitoid being retained in the area for the longest period of time, significantly longer than all treatments except that containing aphid-related cues with nepetalactone.

### **Motivation assay**

The motivation assay was conducted to determine behavioural changes induced by exposure to nepetalactone and/or successful oviposition experience. It was found that learning experience with nepetalactone led to a decreased velocity ( $H = 7.32$ ,  $P = 0.007$ ) (Table 2). Naïve parasitoids (those without oviposition experience) exposed to a high concentration of nepetalactone showed both a decrease in velocity ( $H = 11.78$ ,  $P = 0.001$ ) and an increase in the mean turn angle during movement ( $H = 12.78$ ,  $P < 0.001$ ). A trend was observed that meander varied with treatment in a fashion concurrent to that seen for increasing turn angle and decreased velocity. The greatest disparity is seen in a comparison of treatments with the high concentration of nepetalactone leading to meander of more than twice the value ( $-653.9 \pm 113.75$ ) of the stimulus-free control ( $-294.28 \pm 72.15$ ) however with the sample variance no significant difference was observed between these treatments ( $H = 5.19$ ,  $P = 0.023$ , not significant following Bonferroni correction).

### **Discussion**

Electroantennography confirmed that the aphid sex pheromone components were both sufficient to elicit a response in *A. colemani*. This was expected as an electrophysiological

response has previously been shown to be detected by *Aphidius rhopalosiphi*, *Aphidius ervi*, *Aphidius matricariae*, *Diaeretiella rapae*, *Praon volucre* (Wadhams *et al.*, 1999) and *Aphidius gifuensis* (Dong *et al.*, 2008). Its effect on parasitoid behaviour as a kairomone is established for a range of parasitoid species (for more details see Powell & Pickett, 2003). Although both nepetalactol and nepetalactone are sufficient in eliciting an electrophysiological response, it is nepetalactone to which a response was seen at a lower concentration, which may offer a greater opportunity to exploit parasitoid behaviour. In exploring the potential use for nepetalactone in the field it has to be recognized that it may not be a simple case of attracting natural enemies to an area. It has already been noted that differences in behavioural response to nepetalactone may exist depending on the extent to which the parasitoid is a specialist or a generalist (Glinwood *et al.*, 1998; Wadhams *et al.*, 1999). Previous work has suggested that generalists may have a higher threshold for the pheromone, our work supports these findings but takes a wider view in recognising that related-host and plant cues may be important for the insect to reach, what is not necessarily a threshold relating to one compound, but rather the reliability of the signal as a whole. The subtle behavioural changes seen in this series of experiments (increased retention, altered foraging patterns) may also explain why the application of nepetalactone in field can reduce aphid populations and alter spatial dynamics but is not consistently observed to attract wasps into the area (Powell *et al.*, 2004).

Although nepetalactone alone was not found to increase the retention time of the parasitoids, a synergy was found to exist when presented with a previously infested leaf or when aphid-related cues are presented alongside the pheromone. Reliability-detectability theory (Vet & Dicke, 1992) recognizes the trade-off that exists in a natural environment with reliability and detectability. It is implicit in this theory that for the foraging individual a host-related signal

of lesser detectability will provide greater reliability in the presence of that host. In a multitrophic context, a plant may benefit from advertising to parasitoids the presence of a pest feeding on it (van Loon *et al.*, 2000; Hoballah & Turlings, 2001). This broadcasting of the signal is in contrast to pest species where selection pressures exist for them to remain discreet and avoid predation or parasitism. As a consequence of this, and their greater biomass, plant odours are often more frequently available within the environment and may initially be utilized by the parasitoid to locate the habitat of the aphid, however, once the habitat is located parasitoids may require more host-specific cues to encourage search of that area. These changes in foraging behaviour are ultimately linked to the optimisation of the foraging strategy employed by the parasitoid. By evaluating the cues associated with host presence, the parasitoid is able to reduce the energy expenditure involved in locating new sources of hosts. This study supports this hypothesis by demonstrating that more directly host-related cues are necessary to increase the retention of the natural enemy. Reception of the pheromones provides accurate temporal information regarding the presence of the host, conversely aphid-related cues provide exact spatial evidence pertaining to the location of hosts but may not provide evidence of when it was occupied. Whether or not this is the evolutionary rationale for such a response, the combination is sufficient to cause a substantial increase in the retention time of *A. colemani*. This may also be indicative of the natural enemy having a preference for the multimodal reception of host cues. While a difference is recognized between the control and any treatment involving leaves, this may not entirely be a consequence of volatiles as we recognize that the visual cue may also have affected the retention of the wasp. The on-leaf retention assay outlined may be considered a new and simple method of evaluating the retention time of insects with the caveat that visual stimuli also form part of this response.

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 before reverting to a general foraging pattern (Luck *et al.*, 1979). This time is also similar to that used in tracking studies of the more closely related *Aphidius uzbekistanicus* (Micha & Wyss, 1996). The temporal scale for these changes is also an important consideration for our assays. It was following this timescale that we chose an 8 minute recording for the motivation assays. Work by Hassell and Southwood (1978), has shown that invertebrate foraging behaviour at a local scale is characterized primarily by a decreased rate of movement (orthokinesis) but an increased rate of turning (klinokinesis). It was found that this foraging pattern would cease if the insect failed to find prey and it would revert to its previous pattern of searching (Hassell & May, 1974). In concurrence with these descriptions, it was found that *A. colemani* velocity was reduced following successful oviposition experience with nepetalactone. Although orthokinesis was reduced this did not correspond to any significant increase in the klinokinesis. It is speculated that with reduced error such a difference may have been observed as the values for meander (an indication of klinokinesis) are more than double that seen for the control.

The description of behavioural changes provided by our study and that of older work (Hassell and Southwood, 1978) can also be framed in the context of modern foraging theory which frequently examines foraging in a larger spatial scale. Lévy flight foraging theory represents an optimal method of foraging and expects that selection pressure in a natural environment will have led to a selection for behaviours which reflect this (Viswanathan *et al.*, 2008). The Lévy flights described in the theory are ostensibly random but distinct periods of isotropic movements originally described by Benoit Mandelbrot (1983). These Lévy flights are thought to occur during periods in which the foraging organism has little reliable information



regarding the desired resource (in our case the aphid host) and will allow them to efficiently explore a greater range of patches. These flights are interspersed by periods of foraging in a Brownian motion. In these terms we hypothesize that the initiation of Brownian motion from a Lévy flight pattern will be achieved through identifying signals relating to a host, signals of greater reliability will elicit a more exaggerated or rapid transition to the Brownian motion. This transition is characterized by increasing meander and a decrease in the total distance moved in a given time.

It is speculated that this reintroduction of nepetalactone was sufficient to elicit a foraging response in the parasitoid which it associated with successful location of the host. This behaviour is also compliant with the optimal foraging theory and will potentially reduce the time spent by the parasitoid foraging for another host habitat. It is noted that the stimulus used was the aphid sex pheromone, to which it is shown in this paper *A. colemani* has an innate response. In this context it is feasible that the choice of odour was irrelevant and may simply be a demonstration of classic or 'Pavlovian' conditioning. In classical conditioning a stimulus elicits behavioural or physiological change as an unrelated stimulus is linked to a recognized stimulus, in this series of experiments this is shown not to be the case as nepetalactone is also active in altering *A. colemani* behavioural patterns when presented in isolation. Results from the analysis of retention and for movement patterns of *A. colemani* are not only concurrent with searching patterns previously described (Hassell & May, 1974; Hassell & Southwood, 1978) but also support hypotheses proposed in reliability-detectability theory, optimal foraging theory and models of host location. From these experiments we provide evidence that a transition in foraging behaviour is characterized by reduced orthokinesis (to ensure the wasp does not leave the habitat) and increased klinokinesis (to increase the probability of encountering the host in the environment). The initiation of this

change in foraging strategy is likely to be linked to the reliability of the stimuli that it receives. During the habitat location a parasitoid will be reliant on cues of greater detectability but as it approaches the habitat it will also begin to receive more discrete stimuli which provide greater reliability of host presence. Once in this foraging mode the wasp will be retained for a greater time period, increasing its 'give-up time' (Croze, 1970) to reduce energy expenditure on repeating the initial habitat location stage.

This work specifically focussed on the behaviour response of the female *Aphidius colemani*, primarily because of their importance in biological control. However it is recognized that an interesting question is left unanswered of how the male parasitoids respond to this odour; although aphids are not a host for male *A. colemani* it may still be possible that they exploit the sex pheromone as a kairomone, to increase the likelihood of encountering females. It has been observed *Psytalia concolor* Braconid males will readily exploit the host pheromones to raise their chances of encountering receptive females (Benelli *et al.*, 2014). An interesting avenue for future work would be to examine the same behaviours in male Aphidiinae parasitoids where we might expect a similar foraging pattern to be observed.

In conclusion, for the first time we have identified the electrophysiological response of the aphid parasitoid *A. colemani* to the aphid sex pheromones (4a*S*,7*S*,7a*R*)-nepetalactone and (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol. We also identified that a synergy exists with nepetalactone and other host cues causing an increased retention of the parasitoid *A. colemani* in an area. The behavioural patterns of *A. colemani* were also shown to be altered by learning or through the presence of nepetalactone, changes which are thought to reflect a transition of the parasitoid from general habitat search to specific host searching.

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Figure 1. Motivation assay arena. A 9 cm diameter Petri dish forms the upper area of the arena which is rested on a 3 cm diameter Petri dish (1). Odour moves passively into the arena through the 2 x 2 cm gauze-covered opening (2). *Aphidius colemani* are introduced via the 0.5 cm circular opening which accommodates a small insect storage vial (3). The smaller Petri dish is used (4) to contain the treatment while preventing the parasitoid from contacting the source. A glass covering is placed over the apparatus to prevent escape of the insects while allowing recording.

Figure 2. Electroantennogram response of *Aphidius colemani* to nepetalactone and nepetalactol. All responses are standardized to the baseline hexane response shown as 100%. Five independent replicates were completed with each individual compared to the full complement of treatments. \* $P < 0.05$ , \*\*\* $P < 0.001$  when compared to the baseline.

Figure 3. On-leaf retention time Set 1. Differences in the retention time of the parasitoid *Aphidius colemani* within the arena for Set 1 where treatments are Nothing = empty arena, Lf + n = nepetalactone alongside an intact leaf, Lf = leaf, Prev. Inf. Lf = previously infested leaf, Prev. Inf. Lf + n = previously infested leaf with nepetalactone. Those not sharing a letter are significantly different ( $F_{4,159} = 15.55$ ,  $P < 0.001$ ). Thirty-five replicates were completed for each treatment.

Figure 4. On-leaf retention time Set 2. Differences in the retention time of the parasitoid *Aphidius colemani* within the arena for Set 2 where treatments are Nothing = empty arena, Lf + N = 10 mg/ml nepetalactone alongside an intact leaf, Syst. + n = nepetalactone alongside systemically damaged leaf, ARC + n = aphid-related cues on leaf with nepetalactone, Prev.



Inf. Lf + n = previously infested *Brassica rapa* leaf with nepetalactone. Those not sharing a letter are significantly different ( $F_{4,160} = 21.42$ ,  $P < 0.001$ ). Thirty-five replicates were repeated for each treatment.

Table 1. Treatment sets used in motivation assay. Treatments in the arena were presented from a chamber below, odours passively entered the arena but contact with the parasitoid was prevented by gauze. Nepetalactone was administered on filter paper and aphid-infested leaf describes a *Brassica rapa* leaf with 10 *Myzus persicae* feeding. N represents the number of independent replicates completed for each treatment.

Pre-treatment	Treatment in arena	N
None	None	18
None	1 ng of nepetalactone	18
None	Aphid-infested leaf	17
None	10 ng of nepetalactone	18
<b>Oviposition experience</b>	None	18
<b>Oviposition experience</b>	1 ng of nepetalactone	17
<b>with 1 ng of nepetalactone</b>		

Table 2. Movement patterns of female *Aphidius colemani* recorded for 8 minutes using a series of treatments. All parasitoids had no previous encounter with the host-plant complex or the sex pheromone nepetalactone prior to the bioassay. Pre-treatments were those administered directly before commencing the assay while treatments in the arena were

presented from below with the wasp unable to contact them. \*P < 0.05 when compared to the control of no pre-treatment and no treatment in arena.

Pre-treatment	Treatment in arena	Velocity (cm/s) ± SE	Mean Turn angle ± SE	Meander (Degrees per cm) ± SE
None	None	0.33 ± 0.034	49.20 ± 4.55	-294.28 ± 72.15
None	Nepetalactone (1 ng)	0.32 ± 0.039	59.3 ± 8.94	-351.29 ± 121.21
None	Aphid-infested leaf	0.28 ± 0.054	47.57 ± 8.56	-283.86 ± 89.24
None	Nepetalactone (10 ng)	0.20 ± 0.08*	86.18* ± 7.75	-653.9 ± 113.75
Oviposition experience	None	0.28 ± 0.042	63.08 ± 9.57	-535.1 ± 154.99
Oviposition experience with 1 ng of nepetalactone	Nepetalactone (1 ng)	0.17 ± 0.043*	72.35 ± 11.88	-629.42 ± 156.56