

# Behavioural responses of the seven-spot ladybird *Coccinella septempunctata* to plant headspace chemicals collected from four crop Brassicas and *Arabidopsis thaliana*, infested with *Myzus persicae*

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- Abstract**
- 1 Insects using olfactory stimuli to forage for prey/hosts are proposed to encounter a ‘reliability–detectability problem’, where the usability of a stimulus depends on its reliability as an indicator of herbivore presence and its detectability.
  - 2 We investigated this theory using the responses of female seven-spot ladybirds *Coccinella septempunctata* (Coleoptera: Coccinellidae) to plant headspace chemicals collected from the peach-potato aphid *Myzus persicae* and four commercially available Brassica cultivars; *Brassica rapa* L. cultivar ‘turnip purple top’, *Brassica juncea* L. cultivar ‘red giant mustard’, *Brassica napus* L. cultivar ‘Apex’, *Brassica napus* L. cultivar ‘Courage’ and *Arabidopsis thaliana*. For each cultivar/species, responses to plants that were undamaged, previously infested by *M. persicae* and infested with *M. persicae*, were investigated using dual-choice Petri dish bioassays and circular arenas.
  - 3 There was no evidence that ladybirds responded to headspace chemicals from aphids alone. Ladybirds significantly preferred headspace chemicals from *B. napus* cv. Apex that were undamaged compared with those from plants infested with aphids. For the other four species/cultivars, there was a consistent trend of the predators being recorded more often in the half of the Petri dish containing plant headspace chemicals from previously damaged and infested plants compared with those from undamaged ones. Furthermore, the mean distance ladybirds walked to reach aphid-infested *A. thaliana* was significantly shorter than to reach undamaged plants. These results suggest that aphid-induced plant chemicals could act as an arrestment or possibly an attractant stimulus to *C. septempunctata*. However, it is also possible that *C. septempunctata* could have been responding to aphid products, such as honeydew, transferred to the previously damaged and infested plants.
  - 4 The results provide evidence to support the ‘reliability–detectability’ theory and suggest that the effectiveness of *C. septempunctata* as a natural enemy of aphids may be strongly affected by which species and cultivar of Brassica are being grown.

**Keywords** *Arabidopsis thaliana*, arrestment/attractant stimuli, *Brassica* spp., *Coccinella septempunctata*, *Myzus persicae*, tritrophic interactions, volatiles.

## Introduction

It has been suggested that insects which utilize olfactory stimuli in foraging for their insect herbivore hosts/prey face a ‘reliability–detectability problem’ (i.e. that the usability of a

stimulus depends on both its reliability in indicating herbivore presence and also its detectability). In host/prey habitat location, herbivore-derived semiochemicals are highly reliable but their low concentration, due to the small biomass of the herbivores, may make them difficult to detect at longer distances. A proposed solution to this detectability problem is to use plant volatiles, produced as a response to infestation by herbivores, as indicators of herbivore presence (Vet &

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Dicke, 1992). There is a great deal of evidence to suggest that natural enemies can use herbivore-induced plant volatiles as foraging cues to locate herbivore prey/host patches (Dicke, 1999). These volatiles may be more detectable because plants have a relatively larger biomass than the herbivores, and are also reliable because they are induced by attack by the herbivore (Vet & Dicke, 1992).

To date, most studies of reactions of natural enemies to herbivore-induced plant headspace chemicals have focused on the responses of parasitoid wasps and acarine predators (Dicke & Sabelis, 1988; Dicke *et al.*, 1990a, b; Turlings *et al.*, 1990; Steinberg *et al.*, 1992; Du *et al.*, 1996; Dicke *et al.*, 1998; Takabayashi *et al.*, 1998; Dicke *et al.*, 1999; van Poecke *et al.*, 2001; Girling *et al.*, 2006). In addition, a small number of studies have investigated the responses of other predatory insects species (Drukker *et al.*, 1995; Shimoda *et al.*, 1997; Royer & Boivin, 1999; Weissbecker *et al.*, 1999; Zhu *et al.*, 1999; van Loon *et al.*, 2000).

The responses of predatory coccinellids to herbivore-induced plant headspace chemicals has received little attention (Hodek & Honěk, 1996; Dixon, 2000), even though the coccinellid family contains predators that feed on a number of important pest insect species. Furthermore, its members have been widely used in biological control attempts of both aphid and coccid (scale insects) species, with varying levels of success (Dixon, 2000).

Until recently, most authors have concluded that coccinellids are guided by innate behaviour patterns, rather than by long distance olfactory or visual orientation (Hodek & Honěk, 1996). A number of recent studies, however, have suggested that species of both aphidophagous and coccidophagous coccinellids may be able to utilize plant headspace chemicals from undamaged host plants and from plants infested by herbivores to help locate plants potentially infested with their herbivore prey (Obata, 1986; Hamilton *et al.*, 1999; Zhu *et al.*, 1999; Birkett *et al.*, 2000; Le Rü & Makosso, 2001; Ninkovic *et al.*, 2001).

In the present study, we investigate whether the polyphagous aphid predator *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) can exploit plant headspace chemicals as foraging cues, when the headspace chemicals are collected and applied to a surface. Headspace chemicals were collected from four commercially grown Brassica cultivars: *Brassica rapa* L. cultivar 'turnip purple top', *Brassica juncea* L. cultivar 'red giant mustard', *Brassica napus* L. cultivar 'Apex', *Brassica napus* L. cultivar 'Courage', and another member of the Brassicaceae, *Arabidopsis thaliana* Columbia. Dual-choice experiments were used to compare the preferences of female *C. septempunctata* to headspace chemicals from undamaged plants, plants infested with *Myzus persicae* Sulzer (Homoptera: Aphididae) and plants previously damaged by *M. persicae* but from which all the aphids were removed, and also the responses of *C. septempunctata* to headspace chemicals collected from *M. persicae* alone.

The responses of female *C. septempunctata* were investigated because they are most likely to respond to chemical stimuli that indicate location of prey. 'The fitness of female coccinellids depends on their locating and ovipositing in high-quality

patches of nursery prey, whereas that of males is dependant on their success in finding and fertilizing females' (Dixon, 2000).

We tested three null hypotheses: (i) female *C. septempunctata* will not show an attraction to headspace chemicals from *M. persicae* alone; (ii) female *C. septempunctata* will not show a preference to plant headspace chemicals from members of the Brassicaceae infested with *M. persicae* over plant headspace chemicals from undamaged plants; and (iii) female *C. septempunctata* will not show a preference to plant headspace chemicals from members of the Brassicaceae damaged by *M. persicae*, but without *M. persicae* still present, over plant headspace chemicals from undamaged plants.

## Materials and methods

### Plants

*Brassica rapa* L. cultivar 'turnip purple top' (supplied by Johnsons Seeds, U.K.), *B. juncea* L. cultivar 'red giant mustard' (supplied by Thompson & Morgan, U.K.), *B. napus* L. cultivar 'Apex' and *B. napus* L. cultivar 'Courage' (both supplied by Advanta, U.K.) were grown in Levington multi-purpose compost (The Scotts Co. U.K. Ltd, U.K.) in controlled temperature cabinets at  $23 \pm 2.5^\circ\text{C}$  under an LD 16:8 h photoperiod at approximately  $29.28\text{ W m}^{-2}$ .

*Arabidopsis thaliana* Col-*gl* (wild-type) seeds were provided by J. G. Turner (University of East Anglia). Seeds were grown in Levington multi-purpose compost at  $20 \pm 2.5^\circ\text{C}$  under an LD 8:16 h photoperiod to stimulate vegetative growth and prevent flowering. Seedlings were separated and transferred to individual pots (diameter 8 cm) of compost approximately 2 weeks after sowing.

### Insects

*Myzus persicae* were reared on greenhouse grown *Brassica rapa* L. ssp. *pekinensis* (Lour.) Hanelt (Brassicaceae) in Perspex cages ( $50 \times 35 \times 45$  cm) under an LD 16:8 h photoperiod.

*Coccinella septempunctata* used in Brassica experiments were collected from nettles on the University of East Anglia campus (Norwich, Norfolk) (Grid Reference  $52^\circ37'N$ ,  $1^\circ14'E$ ) during June 2003 and those used in *A. thaliana* experiments were collected from the ground layer vegetation of Thetford Forest (Grid Reference  $52^\circ28'N$ ,  $0^\circ40'E$ ) during May 2002. In both cases, females were separated from males and kept in Perspex cages ( $50 \times 35 \times 45$  cm) at room temperature under an LD 16:8 h photoperiod. To reduce the effect of associative learning directed to the aphid species used in the bioassays (*M. persicae*), *C. septempunctata* were fed on *Acyrtosiphon pisum* Harris (Homoptera: Aphididae), reared on greenhouse grown, *Vicia faba* L. (Fabaceae) plants, which were replaced twice weekly.

### Petri dish arena experiments

Plant headspace chemicals were collected for use in dual-choice experiments (Table 1). Collections were made from

**Table 1** Design of dual-choice bioassay experiments for investigating responses of *Coccinella septempunctata* to a range of potential sources of volatile chemicals collected in hexane

Null hypothesis experiment no.	Spp. and cultivar of potential volatile source	Pairwise choice experiments				Control treatments
		No. of aphids	Length of infestation prior to volatile collection (days)	Aphids present or removed when volatiles collected	Experimental treatments	
Control <sub>1</sub>	Hexane	–	–	–	Hexane	
Control <sub>2</sub>	<i>Brassica rapa</i>	0	0	–	Undamaged <i>B. rapa</i>	
Control <sub>3</sub>	<i>Brassica juncea</i>	0	0	–	Undamaged <i>B. juncea</i>	
Control <sub>4</sub>	<i>Brassica napus</i> cv. Courage	0	0	–	Undamaged <i>B. napus</i> cv. Courage	
Control <sub>5</sub>	<i>Brassica napus</i> cv. Apex	0	0	–	Undamaged <i>B. napus</i> cv. Apex	
Supplementary 1	<i>Arabidopsis thaliana</i>	0	0	–	Hexane	
1	<i>Myzus persicae</i> (aphids)	100	–	Present	Hexane	
2a	<i>Brassica rapa</i>	100	3	Present	Undamaged <i>B. rapa</i>	
2b	<i>Brassica juncea</i>	100	3	Present	Undamaged <i>B. juncea</i>	
2c	<i>Brassica napus</i> cv. Courage	100	3	Present	Undamaged <i>B. napus</i> cv. Courage	
2d	<i>Brassica napus</i> cv. Apex	100	3	Present	Undamaged <i>B. napus</i> cv. Apex	
2e	<i>Arabidopsis thaliana</i>	40	3	Present	Hexane	
2f	<i>Arabidopsis thaliana</i>	500	1	Present	Hexane	
3a	<i>Brassica rapa</i>	100	3	Removed	Undamaged <i>B. rapa</i>	
3b	<i>Brassica juncea</i>	100	3	Removed	Undamaged <i>B. juncea</i>	
3c	<i>Brassica napus</i> cv. Courage	100	3	Removed	Undamaged <i>B. napus</i> cv. Courage	
3d	<i>Brassica napus</i> cv. Apex	100	3	Removed	Undamaged <i>B. napus</i> cv. Apex	
3e	<i>Arabidopsis thaliana</i>	40	3	Removed	Hexane	
3f	<i>Arabidopsis thaliana</i>	500	1	Removed	Hexane	

single potted plants in a controlled temperature cabinet at  $23 \pm 2.5^\circ\text{C}$ , under an LD 16:8 h photoperiod, for *Brassica* collections and at  $20 \pm 2.5^\circ\text{C}$ , under a constant photoperiod, for *A. thaliana* collections. Headspace volatiles were collected on an ARS Filter Inc., volatile collection trap (P/N# VCT-1/4X3-SPQ), made from the porous polymer Porapak Q. Before collections were made, the volatile traps were conditioned by repeated washing with hexane, until a few mL of hexane had been washed through each tube. The tubes were then placed in a heating block at  $200^\circ\text{C}$  with nitrogen passing through them for at least 2 h.

Headspace volatile entrainment was performed by pumping air through a flow meter, to regulate airflow to 600 mL/min, then through an activated charcoal filter and via Teflon tubing into a sealed 2-L glass vessel containing the volatile source. Air was drawn from the vessel via another tube by another pump; a flow meter regulated the exit flow rate to 500 mL/min. The different flow rates ensured that there was always a positive pressure within the vessel so that external air could not enter the glass flask and contaminate the sample. The air was drawn from the glass vessel and passed through an ARS filter that trapped any volatiles given off by the sample. Collections were made for 24 h, after which the filter was removed and sealed temporarily in a glass ampoule until the plant headspace chemicals were eluted with 600  $\mu\text{L}$  hexane into a darkened glass vial, to form a hexane extract. The glass vessel was cleaned thoroughly between entrainments by washing with 90% ethanol, then rinsing with distilled water.

For each dual-choice experiment (Table 1), two plant headspace chemical collections were made for every treatment. The hexane extracts from these two collections were mixed so each extract contained plant headspace chemicals collected from two different plants, treated in the same way. Headspace chemicals were also collected twice from 100 *M. persicae* only. This was performed by restraining the aphids by themselves, with no plant or leaf, inside a clip cage, and the two collections mixed.

All plants used for collections had fully developed adult leaves. *Brassica juncea* used for headspace chemical collections were between 23 and 33 days old; *B. rapa* between 16 and 25 days old; *B. napus* cv. Courage between 20 and 35 days old; *B. napus* cv. Apex between 19 and 34 days old. All *A. thaliana* plants were 5–7 cm in diameter.

Dual-choice bioassays were conducted in arenas made from sterile Petri dishes (diameter 9 cm) on which a line was drawn on the outside of the base, dividing it into two equal sized semi-circles. The inside walls of the dish were coated with a thin layer of white petroleum jelly to prevent coccinellids from climbing them.

Thirty  $\mu\text{L}$  of each of the different hexane extracts being tested (Table 1) was applied to each half of the Petri dish and each spread evenly using a sterile glass spreader. The extract that was applied first was alternated for each replicate to avoid any bias that may have occurred due to the length of time volatiles were allowed to evaporate before a coccinellid was introduced into the Petri dish. The hexane was allowed to evaporate for approximately 20–30 s before experiments

were begun. A 5-cm tall cylinder of 1-mm in diameter gauze mesh was wrapped around the outside of the Petri dish and the lid of the Petri dish placed on top of this cylinder. This ensured that animals could not fly away and that there was not a build up of harmful volatiles during experiments. Petri dishes were placed on green card to provide a more realistically leaf coloured surface for *C. septempunctata* to forage on.

Experiments were conducted in a constant temperature room at circa 23 °C. Petri dish arenas were lit from above by two 15-W fluorescent lamps, fitted with a prismatic filter, to ensure a completely even distribution of light. This illuminated the arenas with a light intensity of 0.67 W/m<sup>2</sup>. *Coccinella septempunctata* were placed in the room under the light at least 1 h before any experiments were conducted to allow them to acclimatize to the light level and temperature within the room. Experiments were started by releasing a female *C. septempunctata* into the centre of the dish and recording the half of the dish it was in at 30-s intervals, for 10 min.

The dual-choice bioassays were used to compare the preferences of *C. septempunctata* to a number of plant headspace chemical sources (Table 1). Control experiments were conducted to investigate whether individuals showed any inherent bias to one or other half of the arenas. Therefore, the responses of *C. septempunctata* to two clean hexane samples on either side of the arena were tested (Table 1, Experiment Control<sub>1</sub>). Further control experiments, to investigate whether *C. septempunctata* displayed any preference between different undamaged plants of the same species for Brassica cultivars, were conducted by testing the responses of *C. septempunctata* to headspace chemicals from undamaged plants versus headspace chemicals from other undamaged plants (Table 1, Experiments Control<sub>2</sub>–Control<sub>5</sub>). In addition to these control experiments, a supplementary experiment was also conducted, to test whether *C. septempunctata* showed a preference for headspace chemicals from undamaged *A. thaliana* over clean hexane (i.e. whether there was any attraction to the undamaged plant) (Table 1, Supplementary Experiment).

To test the first null hypothesis, that female *C. septempunctata* will not respond to headspace chemicals from *M. persicae* alone, headspace chemicals collected from 100 *M. persicae* were tested versus a clean sample of hexane (Table 1, Experiment 1).

To test the second null hypothesis, that female *C. septempunctata* will not respond differently to plant headspace chemicals from members of the Brassicaceae infested with *M. persicae* compared with plant headspace chemicals from undamaged plants, the responses of *C. septempunctata* to plant headspace chemicals from infested plants were tested versus either undamaged plants, for Brassica cultivars, (Table 1, Experiments 2a–d) or clean hexane, for *A. thaliana*, (Table 1, Experiments 2e–f). Brassica cultivars were infested with approximately 100 *M. persicae* for 3 days prior to headspace chemical collection because Guerrieri *et al.* (1999) showed that broad beans (*Vicia faba*) must be infested with pea aphids (*Acyrtosiphon pisum*) for 60–72 h to become attractive to the parasitoid wasp *Aphidius ervi*. *Arabidopsis*

*thaliana* were infested with approximately 40 aphids (due to the smaller size of the plant) for 3 days prior to collection (Experiment 2e) and approximately 500 aphids for 1 day prior to collection (Experiment 2f) to investigate responses of *C. septempunctata* to more heavily infested *A. thaliana* plants.

To test the third null hypothesis, that female *C. septempunctata* will not respond differently to plant headspace chemicals from members of the Brassicaceae damaged by *M. persicae*, but without them still present, compared with plant headspace chemicals from undamaged plants, the responses of *C. septempunctata* to plant headspace chemicals from aphid damaged plants were tested versus either undamaged plants, for Brassica cultivars (Table 1, Experiments 3a–d), or clean hexane, for *A. thaliana* (Table 1, Experiments 3e–f). Infestation levels and lengths for damaged plants were the same as for infested plants, except *M. persicae* were removed from plants immediately prior to volatile collection.

For the experiments with *M. persicae*, *B. rapa* and *B. juncea*, 20 different individual *C. septempunctata* were tested, with the same individuals being used for each of the three pairwise comparisons to ensure full comparability between control and experimental choices. For the two *B. napus* cultivars, the same 20 individuals were used for all six experiments to ensure full comparability between the two lines. For the experiments with undamaged *A. thaliana* and plants infested with 500 aphids, the same 18 individuals were used. In experiments with *A. thaliana* infested with 40 aphids and the hexane versus hexane control, the same 15 individuals were used. Different cultivars and species were tested on different days and all three dual-choice experiments for each cultivar were performed on the same day (for *A. thaliana*, tests with 18 individuals were performed on 1 day and 15 individuals on a different day), the order of the pairwise comparison each beetle was tested in was randomized. All individuals were starved for approximately 24 h before experiments. Starving beetles for 18 h results in maximum search activity (Frazer & Gilbert, 1976).

### Circular arena experiments

Arenas consisted of circular flat plastic dishes (diameter 55 cm), with a 7-cm high wall and a Perspex lid. The internal walls were coated with Fluon to stop coccinellids from climbing on them. Thirty minutes prior to commencement of an experiment, one 9-cm plant pot, containing the test plant, was fitted into a circular hole in the centre of the base of the arena floor so that the top of the pot was flush with the arena floor, to allow build up of a headspace volatile gradient within the arena. Experiments were conducted in a darkened constant temperature room at approximately 20 °C and the arena was lit directly from above using a unidirectional spot lamp.

One female *C. septempunctata* was inserted into the arena through a small hatch in the sidewall. The behaviour of the animal was monitored by tracing its path, in pen, on a sheet of acetate on top of the Perspex lid of the arena. The observer sat directly above the arena to avoid parallax. Animals were

monitored for 7 min because, in trial experiments, this was shown to be sufficient time for individuals to be able to find the plant, without making the drawing on the acetate too intricate. Each animal was monitored for 7 min and, at 10-s intervals, a mark was made on the trace. Arenas were divided into two zones, the plant and the arena. From the acetate sheets, it was possible to later calculate three parameters: (i) mean distance walked to reach the plant; (ii) mean time taken to find the plant; and (iii) percent of individuals finding the plant.

To test null hypotheses two and three; that *C. septempunctata* will not respond differently to plant headspace chemicals from aphid infested plants compared with undamaged plants and aphid damaged plants compared with undamaged plants, the responses of ten female *C. septempunctata* to each of three plant treatments were recorded: (i) undamaged *A. thaliana*; (ii) *A. thaliana* infested by approximately 500 *M. persicae* for 1 day; and (iii) *M. persicae* damaged *A. thaliana* (aphids removed immediately prior to experiment) by approximately 500 aphids for 1 day.

Each treatment was carried out over a number of days to eliminate any random daily bias. All *A. thaliana* were 5–7 cm in diameter. All coccinellids were starved for approximately 24 h before testing.

### Statistical analysis

Results were first checked for normality using a series of Shapiro–Wilk tests. For dual-choice experiments, data were then analysed using a series of one-sample *t*-tests. The mean number of occurrences that each *C. septempunctata* was recorded in one half of the Petri dish was compared against a test value of 10 (half the total number of recordings made for each individual coccinellid). If the mean number of occurrences of *C. septempunctata* on one side of a Petri dish was significantly greater than 10, it was scored as a significant ‘preference’ for the volatile chemicals on that half of the Petri dish. For circular arena experiments, data were analysed

using a series of one-way analysis of variance and chi-square tests. Statistical tests were carried out using SPSS, version 10 (SPSS Inc., Chicago, IL).

## Results

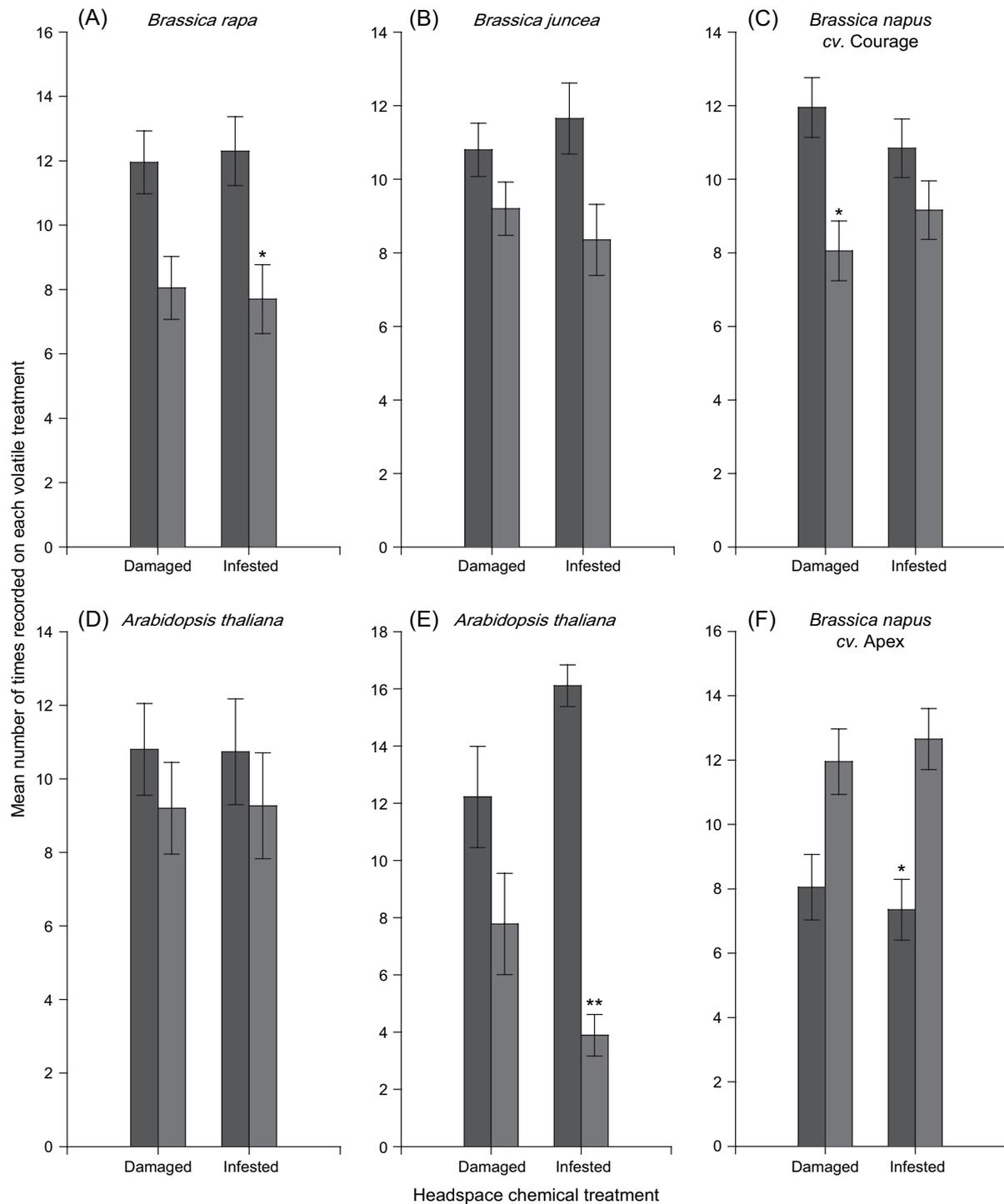
### Petri dish dual-choice bioassays

In control experiments, *C. septempunctata* showed no significant differences in time spent on either side of the Petri dishes, where both sides were coated either with pure hexane or with plant headspace chemicals collected from undamaged plants (Table 2, Experiments Control<sub>1</sub>–Control<sub>5</sub>). This confirms that the distribution of *C. septempunctata* on either side of the Petri dish, in control experiments, was not significantly different from 50:50, validating the use of a statistical test value of 10 for use in one sample *t*-tests with experimental treatments. Additionally, *C. septempunctata* showed no preference for headspace chemicals from undamaged *A. thaliana* over clean hexane, indicating there was no attraction to undamaged plants alone ( $t_{18} = 0.84$ ,  $P = 0.41$ ). *Coccinella septempunctata* also showed no significant responses to headspace chemicals from *M. persicae* alone ( $t_{19} = 0.76$ ,  $P = 0.46$ ).

For *B. rapa*, *B. juncea*, *B. napus* cv. Courage and both densities of infestation of *A. thaliana*, in all ten experiments, *C. septempunctata* were recorded more times on the side of the Petri dish with headspace chemicals collected from the damaged and infested plants than the controls (Fig. 1A–E). For *B. rapa* and *A. thaliana* infested with 500 aphids, *C. septempunctata* were recorded significantly more times on the infested treatments whereas, for *B. napus* cv. Courage, they were recorded significantly more times on the damaged treatment. By contrast, *C. septempunctata* were recorded significantly fewer times on the infested *B. napus* cv. Apex treatment and were also recorded fewer times on the damaged than on the undamaged treatment (Fig. 1F), although the difference was not significant ( $t_{19} = -1.918$ ,  $P = 0.07$ ).

**Table 2** Control experiments: mean number of times *Coccinella septempunctata* were recorded in dual-choice tests in one or another half of a Petri dish coated with different hexane extract treatments

Null hypothesis experiment no.	Control experiments	No. of individuals tested	Treatments	Mean ± SE number of times recorded on each volatile treatment	<i>t</i> -value	d.f.	<i>P</i>
Control <sub>1</sub>	Hexane	15	Hexane	8.4 ± 1.86	0.86	14	0.404
Control <sub>2</sub>	<i>Brassica rapa</i>	20	Hexane	11.6 ± 1.86	1.36	19	0.189
			Undamaged	9.1 ± 0.66			
Control <sub>3</sub>	<i>Brassica juncea</i>	20	Undamaged	10.9 ± 0.66	0.42	19	0.679
			Undamaged	9.7 ± 0.71			
Control <sub>4</sub>	<i>Brassica napus</i> cv. Courage	20	Undamaged	10.3 ± 0.71	0.58	19	0.568
			Undamaged	9.5 ± 0.86			
Control <sub>5</sub>	<i>Brassica napus</i> cv. Apex	20	Undamaged	10.5 ± 0.86	0.39	19	0.705
			Undamaged	9.6 ± 1.04			
			Undamaged	10.4 ± 1.04			



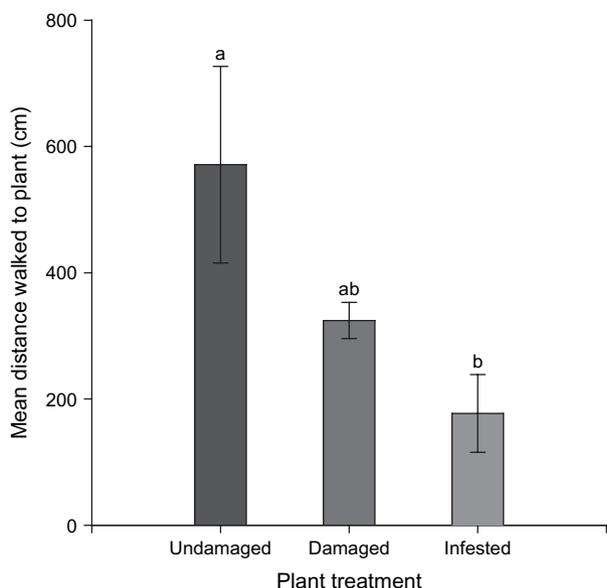
**Figure 1** Mean number of times *Coccinella septempunctata* were recorded in dual-choice Petri dish tests with different plant headspace chemicals on the two halves of the arenas. Light gray bars indicate a control treatment, either: in experiments with *Brassica* cultivars, volatiles collected from undamaged plants; in experiments with *Arabidopsis thaliana*, pure hexane. Dark gray bars indicate hexane extracts of headspace chemicals from either plants damaged by *Myzus persicae* (but with the aphids removed before testing) or *M. persicae* infested plants: (A) *Brassica rapa* ( $n=20$ ) (significant difference between infested and undamaged;  $t_{19}=2.152$ ,  $P=0.04$ ); (B) *Brassica juncea* ( $n=20$ ); (C) *Brassica napus* cv. Courage ( $n=20$ ) (significant difference between damaged and undamaged;  $t_{19}=-2.400$ ,  $P=0.03$ ); (D) *A. thaliana* infested with 40 aphids for 3 days ( $n=15$ ); (E) *A. thaliana* infested with 500 aphids for 1 day ( $n=18$ ) (significant difference between infested with 500 aphids for 1 day and hexane;  $t_{17}=8.405$ ,  $P<0.001$ ); (F) *B. napus* cv. Apex ( $n=20$ ) (significant difference between infested and undamaged;  $t_{19}=-2.799$ ,  $P=0.01$ ). All data are the mean  $\pm$  SE.

### Circular arena bioassay

No significant differences were found in the time to reach the plant or the number of individuals that found plants between all three plant treatments. There were significant differences between plant treatments in the distance female *C. septempunctata* travelled to reach the plant (Fig. 2). *Coccinella septempunctata* walked a significantly shorter distance before finding infested *A. thaliana* than undamaged *A. thaliana*.

### Discussion

The results show that *C. septempunctata* is capable of both detecting and altering its behaviour in response to plant headspace chemicals emitted from plants damaged or infested by aphids. In ten out of the 12 plant/aphid Petri dish dual-choice experiments, *C. septempunctata* were recorded more times in the half of the Petri dish with headspace chemicals from plants damaged or infested by *M. persicae* than in the half with headspace chemicals from undamaged plants. In only three of these instances, however, were the data significantly different (Fig. 1). In addition, in two of these 12 tests, the opposite results were recorded. We suggest that, because there was a significant response in three of the ten tests and a trend in the same direction in the other seven, these experiments do provide evidence against the null hypothesis that female *C. septempunctata* will not respond



**Figure 2** Responses of *Coccinella septempunctata*, in circular arena experiments, to undamaged, *Myzus persicae* damaged and *M. persicae* infested *Arabidopsis thaliana*. Mean distance travelled to the plant by *C. septempunctata* that found the plant (significance of difference between plant treatments; one-way analysis of variance:  $F_{2,13} = 3.677$ ,  $P = 0.05$ ). Undamaged,  $n = 6$ ; damaged,  $n = 3$ ; infested,  $n = 7$ . All data are the mean  $\pm$  SE. Different letters show significant differences between treatments in Tukey test's ( $P < 0.05$ ).

differently to plant headspace chemicals from members of the Brassicaceae infested with or damaged by *M. persicae* compared with headspace chemicals from undamaged plants. Instead, these results suggest that *C. septempunctata* can detect and respond to plant headspace chemicals induced by aphid feeding. The responses in these dual-choice experiments were to volatile extracts at a higher concentration than normally found in nature. However, the preference for damaged/infested plants was also confirmed at lower more 'normal' volatile concentrations in the circular arena assays, which showed that *C. septempunctata* moved more directly to plants when they were damaged/infested with aphids (Fig. 2).

It is not clear, from these experiments, whether individuals respond to airborne plant volatiles evaporating from the Petri dish using their antennae, or to chemicals adhered to the surface of the Petri dishes using their palps or tarsal receptors. Therefore, these chemicals could be functioning either as an arrestment stimulus, which would keep coccinellids within a patch, and/or possibly as an attractant stimulus, which would attract coccinellids to an aphid patch. Because there was no air flow in these experiments, it cannot be confirmed that the plant headspace chemicals are acting as an attractant. However, a similar experiment, where headspace volatiles were collected from barley, showed that *C. septempunctata* responded to olfactory stimuli (Ninkovic *et al.*, 2001).

Furthermore, is it also not possible to fully establish whether the differences in preferences of female *C. septempunctata* to volatile headspace chemicals from the different treatments may have been a result of innate preferences by *C. septempunctata*, or due to associative learning from previous experience (Vet & Dicke, 1992). Associative learning appears to be less likely in these experiments because none of the test plants were growing in the field in the location from which the coccinellids were collected, and furthermore the two-spot ladybird *Adalia bipunctata* does not acquire learnt olfactory preferences as a result of adult experience (Raymond *et al.*, 2000). In addition, because the same individuals of *C. septempunctata* were used for each of the three pairwise comparisons for each plant cultivar/species, there is a possibility that coccinellids may have been conditioned to a certain volatile blend from the first pairwise comparison that they were tested with. However, because the order of pairwise comparison each coccinellid was tested in was randomized, any effect conditioning may have had on the coccinellids preferences should have been negated.

The responses to chemicals in these experiments are unlikely to be to headspace chemicals from the aphids themselves because the aphids-alone treatment elicited no significant response from *C. septempunctata* (Table 2). Furthermore, *C. septempunctata* were recorded consistently, but often not significantly, more times in the half of the Petri dishes with headspace chemicals from plants that had been damaged by the aphids, but with aphids no longer present, than those of undamaged plants (Fig. 1). Our results are in accordance with studies that have shown coccinellids are unable to detect chemicals from aphids alone (Nakamuta, 1984; Schaller & Nentwig, 2000; Ninkovic *et al.*, 2001), as is the case for the larvae of *A. bipunctata*, which are not

attracted by the odour of aphids (Hemptinne *et al.*, 2000). Similarly, aphid alarm pheromone [(*E*)- $\beta$ -farnesene] does not elicit searching behaviour in *C. septempunctata* (Nakamura, 1991). However, other studies do suggest that some coccinellids are able to use aphid volatiles as cues to find their prey (Obata, 1986; Sengonca & Liu, 1994; Raymond *et al.*, 2000). There is the possibility in these experiments that ladybirds could have been responding to aphid honeydew because honeydew was not removed from the plants due to its removal being likely to cause mechanical damage to the leaves, thus resulting in the emission of a different set of plant volatiles. However, if the coccinellids were responding to honeydew alone, this would not explain why coccinellids showed a preference for headspace chemicals from some cultivars when infested/damaged, but with *B. napus* cv. Apex showed a preference for headspace chemicals from undamaged plants over those from damaged/infested plants (Fig. 1).

In the Petri dish experiments, there were generally relatively low responses by coccinellids to the volatile cues and, although there was a trend for *C. septempunctata* individuals to spend longer on the infested/damaged treatment side of the arena, this trend was not often significant (Fig. 1). The current experiments assumed that coccinellids will respond to the plant headspace chemicals at a short range when walking around on a plant surface. However, this may not be the case and coccinellids may only respond strongly to these volatile cues at a longer range (e.g. when in flight). This may explain some of the lower and nonsignificant responses.

Previously, five coccinellid species, other than *C. septempunctata*, have been demonstrated to detect and respond to plant-derived chemicals as host location cues (Obata, 1986; Rapusas *et al.*, 1996; Hamilton *et al.*, 1999; Zhu *et al.*, 1999; Le Rü & Makosso, 2001). Additionally, *C. septempunctata* has been shown to be attracted to aphid-induced plant volatiles from barley (Ninkovic *et al.*, 2001). Attraction of coccinellids to volatiles from aphid-infested cruciferous plants has only been described for one species, *Hippodamia convergens*, which is attracted to radish leaves infested with *M. persicae* (Hamilton *et al.*, 1999). Responses to herbivore-induced volatiles from *Brassica* spp., by other insect groups, have been recorded. For example, the braconid parasitoid *Cotesia plutellae* is attracted to plant volatiles induced from *B. napus* by its host *Plutella xylostella* in its in-flight searching behaviour (Potting *et al.*, 1999). Similar responses have been shown by the parasitoid, *Cotesia rubecula*, to volatiles induced by its host *Pieris rapae* from Brussels sprouts (Geervliet *et al.*, 1994) and *Cotesia glomerata* to volatiles induced by its host *Pieris brassicae* from cabbage (Steinberg *et al.*, 1993). Our results indicate that *C. septempunctata* is capable of detecting and altering its behaviour in response to plant headspace chemicals emitted from members of the Brassicaceae damaged by or infested with *M. persicae*. However, it is unclear from the current experiments whether *C. septempunctata* is utilizing these chemicals over a short or long range.

There were two exceptions to the general trend in responses of *C. septempunctata* to damage/infestation in the Petri dish experiments. These were both of the responses to

*B. napus* cv. Apex cultivar treatments (Fig. 1F), where *C. septempunctata* were recorded more often on the side with the plant chemicals from the control treatments than those from plants infested or damaged by aphids. This suggests that something in the volatile blend from aphid damaged plants for this cultivar made the plants less preferable than undamaged plants to *C. septempunctata*, in contrast to the potential arrestment/attractant stimuli emitted from infested/damaged plants of the other cultivars and species tested. Furthermore, there were variations in the level of response of *C. septempunctata* to those plants where infestation or damage did act as a potential arrestment/attractant (Fig. 1A–E). Some parasitoids show preferences between volatiles from uninfested plants of different cultivars or species (Elzen *et al.*, 1986; Kaas *et al.*, 1992; Rapusas *et al.*, 1996). In addition, different plant species are known to produce different induced volatile blends in response to attack by the same herbivore, between which natural enemies can discriminate (Turlings *et al.*, 1990; Dicke *et al.*, 1990b; McCall *et al.*, 1993; Takabayashi *et al.*, 1994). For example, when both corn and soybean plants are infested with beet armyworm, they emit qualitatively different volatile blends (Turlings *et al.*, 1993). Potential differences in volatile production by the different cultivars in the current experiments could also have been due to possible variations in feeding rates of *M. persicae* on each cultivar, which could have quantitative effects on volatile production. If there are differences in responses to different cultivars by *C. septempunctata*, caused by variations in types of headspace chemicals produced, this may have important implications for the prey foraging behaviour of *C. septempunctata*. Furthermore, whether a cultivar has repellent or arrestment/attractant properties when wounded, and how the level of any arrestment/attractant properties varies between cultivars, could influence the effectiveness of natural enemies as components of integrated control programmes.

It has been suggested that natural enemies encounter a 'reliability–detectability problem' where host-produced chemicals will be highly reliable and taxonomically specific host location cues for natural enemies but may have low detectability because of a need for the host to remain inconspicuous. Instead, it is proposed that natural enemies may utilize herbivore-induced signals from plants as more detectable, and still reliable, prey location cues (Vet & Dicke, 1992). These results provide some support for this theory, particularly in demonstrating a high degree of specificity in responses to different cultivars of the same species, with obvious potential commercial significance in relation to pest control.

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