Influence of aphid honeydew on the foraging behaviour of *Hippodamia convergens* larvae

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Abstract. 1. Environmental cues associated with prey are known to increase predator foraging efficiency. Ladybird larvae are major predators of aphids. The sugary excretion of aphids (honeydew) has been proposed to serve as a prey-associated cue for ladybird larvae.

2. Ladybird larvae are regularly found on the ground moving between plants or after falling off plants. The use of prey-associated cues would be particularly beneficial for ladybird larvae on the ground in that such cues would help them to decide which plants to climb because aphids are patchily distributed within as well as amongst plants and, as a result, many plants are either not infested with aphids or do not host an aphid species of high nutritional value for ladybird larvae.

3. Laboratory experiments with larvae of *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae) were carried out to explore whether honeydew accumulated on the ground is used as a foraging cue. The study also investigated whether, if honeydew is a foraging cue, larvae show differential responses to honeydew of high-quality prey *Acyrthosiphon pisum* Harris compared with that of low-quality prey *Aphis fabae* Scopoli (both: Homoptera: Aphididae).

4. *Hippodamia convergens* larvae stayed longer in areas containing honeydew but did not engage in longer bouts of searching. Furthermore, larvae did not distinguish between honeydew from high- and low-quality aphid prey.

Key words. *Acyrthosiphon pisum*, *Aphis fabae*, foraging behaviour, *Hippodamia convergens*, honeydew, ladybird beetle, patch residence time, prey-associated cue, prey quality.

Introduction

Environmental cues often facilitate the location of prey and thus increase foraging efficiency. Such cues are particularly important if prey distribution is aggregated because prey aggregation increases the variance in foraging success. Aphids (Homoptera: Aphididae) are aggregated both within host plants (not every leaf has aphids) and between plants (many plants have no aphids). Aphids feed on phloem that is rich in sugars but poor in amino acids. As a result, aphids must feed continuously to ingest phloem in large amounts and then excrete excess sugars in the form of honeydew (Dixon, 1998). Natural enemies of aphids are known to use honeydew as part

of their diet (Hogervorst *et al.*, 2008; Lundgren, 2009a) and as a cue in host/prey location (Budenberg, 1990; Romeis & Zebitz, 1997; Ide *et al.*, 2007) because honeydew typically accumulates in the vicinity of aphid aggregations. This knowledge has led to research into the effectiveness of spraying sugar solutions on crop fields to attract and retain natural enemies (Lundgren, 2009b; Seagraves *et al.*, 2011). However, the benefit of providing sugar to increase the effectiveness of natural enemies is not clear because non-prey food including sugars can also divert predators from predation (Spellman *et al.*, 2006). Ladybird beetles are major predators of aphids and some species are commonly used as biological control agents to control aphid populations. Both the adult and larval stages of ladybird beetles consume aphids. The present study explored whether the foraging behaviour of predatory larvae of the ladybird species *Hippodamia convergens* Guérin-Méneville (Colleoptera: Coccinellidae) is influenced by honeydew.

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At a minimum, honeydew composition varies with the host plant, aphid species, aphid age and level of ant tending (Fischer & Shingleton, 2001; Fischer *et al.*, 2002). Thus, honeydew may provide not only information on the presence of prey, but also information on prey suitability and vulnerability. Most research on the use of honeydew as an environmental cue has focused on parasitoids, which are more specialised foragers than ladybird beetles. Parasitoids are more reliant on environmental cues to find suitable aphid hosts. In the past, researchers concluded that ladybird larvae search for prey randomly and are unable to detect prey prior to physical contact (Banks, 1957; Dixon, 1959). However, later evidence suggests that they respond to visual and olfactory cues (Stubbs, 1980; Nakamuta, 1984; Jamal & Brown, 2001).

Few studies have evaluated the effect of honeydew on the foraging behaviour of ladybird larvae (Carter & Dixon, 1984; Ide *et al.*, 2007). Generally, prey-associated cues act as attractant stimuli and cause foragers to bias their movement towards areas containing hosts or prey. Alternatively, they can act as arrestant stimuli which reduce movement rate (the distance or area covered per unit of time) and thereby increase the likelihood of prey encounter (Fellows *et al.*, 2005). Carter and Dixon (1984) demonstrated that *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) larvae were more likely to return to wheat ears that were covered with *Sitobion avenae* Fabricius honeydew compared with clean wheat ears. Repeat searching of the ears with honeydew resulted in an increased number of aphids consumed compared with that on ears without honeydew.

Larvae move frequently from one plant to another via touching leaves of neighbouring plants (Banks, 1957). However, sometimes they must walk on the soil to reach a plant that is further away or because they have been dislodged from a plant by wind or water, or have dropped in response to predators. It would be beneficial for ladybird larvae on the ground to recognise which plants are infested with aphids because aphids have a clumped distribution and, as a consequence, only a few plants in a field may be infested with aphids. Experiments by Ide *et al.* (2007) suggest that honeydew that accumulates on the ground beneath aphid-infested plants might be used by *C. septempunctata* larvae as a cue for locating aphid-infested plants. Furthermore, groups of plants can be infested with many different aphid species (either on the same plant or on different plant species) that vary in suitability for ladybird larvae as a result of aphid abundance, size, escape ability and nutritional quality (Dixon, 2000). Thus, it would be additionally advantageous for ladybird larvae to be able to distinguish among honeydew from different aphid–plant systems in order to choose plants hosting the most profitable aphid species. Ide *et al.* (2007) showed that *C. septempunctata* larvae stayed longer in areas containing honeydew of prey that was easy to catch and so more profitable. We might expect a similar response from ladybird larvae if the difference in prey profitability reflects a difference in the nutritional value of aphids.

The objective of the present study was to test the generality of Ide *et al.*'s (2007) findings by using a different ladybird species and honeydew from two aphid species that differ in nutritional value. Specifically, we asked: does honeydew on the ground act as a prey-associated cue? If so, does honeydew from low-quality aphid species act as a deterrent?

Materials and methods

The experiments were designed to increase our understanding of the behaviour of ladybird larvae that are searching on the ground for plants that are infested with aphids. We used honeydew of two aphid species, *Acyrthosiphon pisum* Harris (Homoptera: Aphididae) and *Aphis fabae* Scopoli (Homoptera: Aphididae) that vary considerably in nutritional value. Survival of *H. convergens* from first instar to adult stage was reduced by 81%, developmental time increased by 55%, and adult mass decreased by 49% on an *Ap. fabae* diet relative to an *Ac. pisum* diet (T. Hinkelman and B. Tenhumberg, unpublished data). The experimental arena was similar to that described in Ide *et al.* (2007). Each foraging arena contained two patches that differed in foraging cues (Fig. 1). Each patch consisted of a Petri dish lid with a climbing structure (either a plant or a stick) in the middle of it.

Hypothesis 1: honeydew on the ground acts as a prey-associated cue

To test the hypothesis that honeydew on the ground acts as a prey-associated cue, the foraging behaviours of ladybird larvae on Petri dish lids with and without honeydew were compared (Experiments 1–3; Table 1). Ladybird larvae use visual and olfactory cues from plants and aphids to locate prey (Stubbs, 1980; Jamal & Brown, 2001). In order to isolate the effect of honeydew on foraging behaviour, aphids were not included in the trials and sticks (bamboo skewers, diameter 4 mm, height 15 cm) were used as climbing structures instead of plants (Experiments 2–4). To ensure that using a stick as a climbing structure would not conceal the effect of honeydew, one experiment with 3-day-old *Vicia faba* L. plants was conducted (Experiment 1). The plants had two leaves of similar sizes and were approximately 10 cm high. The aim of this experiment was to exclude the possibility that ladybird larvae recognise that sticks cannot contain aphids and thus do not respond to honeydew as a cue. If *H. convergens* larvae use honeydew as a cue, we would predict that, compared with patches without honeydew, patches with honeydew would attract a larger proportion of larvae, a higher proportion of the larvae would climb sticks, the patch residence time would be longer, the times until encountering a stick would be shorter, and larvae would spend more time searching than resting or consuming honeydew.

Hypothesis 2: honeydew from low-quality aphids acts as a deterrent

To test the hypothesis that honeydew from low-quality aphids acts as a deterrent, we conducted Experiment 4, in which one foraging patch included honeydew of low-quality
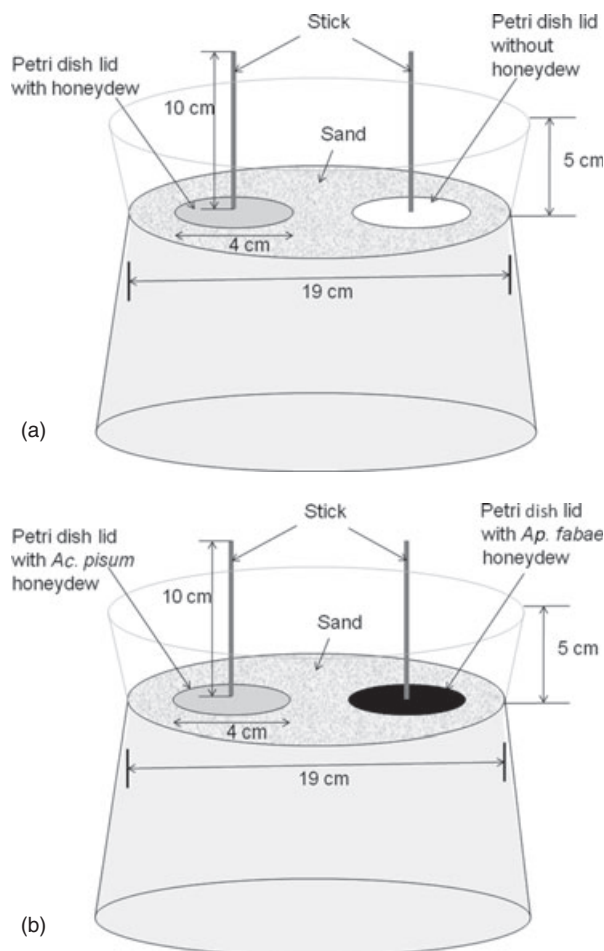
Fig. 1. Experimental arena. (a) Set-ups for Experiments 2 and 3; in Experiment 1, the sticks were replaced with small *Vicia faba* plants. (b) Set-up for Experiment 4. *Ac. pisum*, *Acyrthosiphon pisum*; *Ap. fabae*, *Aphis fabae*.

Table 1. Overview of experiments.

Experiment				Aphids used for honeydew collection, n	
No.	Treatment	CS	n	*Ac. pisum*	*Ap. fabae*
1	*Ac. pisum*/none	Plants	31	30	—
2	*Ac. pisum*/none	Sticks	63	30	—
3	*Ap. fabae*/none	Sticks	68	—	30
4	*Ac. pisum/Ap. fabae*	Sticks	66	12	30

The treatment indicates whether one or both Petri dishes contained honeydew (*Ac. pisum* or *Ap. fabae*), and if one of the Petri dishes was clean (none).
CS, climbing structure; *Ac. pisum*, *Acyrthosiphon pisum*; *Ap. fabae*, *Aphis fabae*.

Ap. fabae and the other patch contained honeydew of high-quality *Ac. pisum*. If larvae can distinguish between honeydew types, we would expect that the difference in respective responses to *Ac. pisum* honeydew and *Ap. fabae* honeydew

would be qualitatively similar to the difference in responses to honeydew and no honeydew. Compared with Petri dish lids with *Ap. fabae* honeydew, patches with honeydew from high-quality *Ac. pisum* would be expected to attract a larger proportion of larvae, a higher proportion of the larvae would climb sticks, patch residence time would be longer, time until encountering a stick would be shorter, and larvae would spend more time searching than resting or consuming honeydew.

General experimental procedure

An overview of the different experiments is shown in Table 1. The arena was uniformly covered with white desert sand so that the sand was flush with the edges of the Petri dish lids. The sand in the experimental arena was rinsed five times with water after each experimental trial; all plants, sticks and larvae were used only once. In all experiments honeydew quantity was recorded to detect any bias resulting from differences in honeydew quantity. The experiments were carried out at 25 °C under a fluorescent light (27 W) on a laboratory table. To acclimatise the ladybird larvae to the experimental arena, a single individual was placed under a Petri dish lid in the centre of the experimental arena equidistant from both Petri dish lids. After 3 min, the Petri dish lid was carefully removed and the behaviour of the released ladybird larva was videotaped using two cameras (Sony models HDR-SR11 and SR5). The cameras were positioned to cover behaviour on the Petri dish lids and both sides of the plant or stick. As a consequence, the resolution of the video-recordings was insufficient to distinguish between detailed behavioural categories such as being still and consuming honeydew. Therefore, we merged both behaviours into a 'resting/feeding' category. The trial was terminated after the larva left the first encountered Petri dish lid or after 30 min. If a larva did not start searching within the first 10 min of removing the Petri dish lid, the trial was discarded. The videos were replayed and the behaviour of ladybird larvae was scored using an event recorder (Jwatcher, Version 1.0 for Windows XP Blumstein & Daniel, 2007). All behavioural categories are listed in Table S1 (online); an example of a behavioural sequence is shown in Fig. S1 (online).

Honeydew collection. Honeydew was collected on 4-cm Petri dish lids placed inside clip cages (diameter 6 cm, depth 3 cm). Thirty adult aphids were transferred to each clip cage using a paint brush. Then the clip cages were fastened to leaves of intact *V. faba* plants. The clip cages restricted the aphids to feeding in a confined area and to dropping honeydew on Petri dish lids in the bottom of the clip cages. The aphids were allowed to feed and deposit honeydew for 24 h. Each Petri dish lid was weighed before and after honeydew collection to measure the quantity of honeydew deposited (Adventurer Pro AV64C, reading to 0.0001 g; Ohaus Corp., Parsippany, New Jersey). For the experiments with plants and the experiments investigating the influence of honeydew presence, equal numbers of aphids were used per clip cage (30 of each of *Ac. pisum* and *Ap. fabae*). The amount of

honeydew produced in this way varied between the two aphid species (*Ac. pisum*: mean 13.04 mg, range 6.10–22.60 mg; *Ap. fabae*: mean 5.26 mg, range 2.03–12.37 mg). Thus, in the last experiment, 12 *Ac. pisum* and 30 *Ap. fabae* per clip cage were used to obtain approximately equal amounts of honeydew (Table 1).

Providing experience. Prior to the experiments, *H. convergens* larvae were fed exclusively on *Ac. pisum* rather than on a mixed diet because of their extremely low survival on an *Ap. fabae* diet [13% on *Ap. fabae*, 70% on *Ac. pisum* (T. Hinkelman and B. Tenhumberg, unpublished data)]. To ensure that a single aphid diet would not bias our results in any way [e.g. learning has been demonstrated in ladybird larvae (Boivin *et al.*, 2010)], we provided all experimental larvae with an opportunity to make an association between honeydew type and aphid species. Specifically, prior to the experimental trials, all ladybird larvae were provided with 2 h of experience with each aphid species and its honeydew (e.g. they were allowed to forage for *Ac. pisum* aphids in the presence of *Ac. pisum* honeydew, and for *Ap. fabae* aphids in the presence of *Ap. fabae* honeydew). The sequence of honeydew type experience was randomised. To ensure that the ladybird larvae made the association between honeydew type and aphid species, only larvae that consumed at least one aphid of each species were used for the trials. Leaves of *V. faba* covered with honeydew were cut from aphid-infested plants and all aphid exuviae were removed. Then each leaf was placed on the bottom of a clip cage with four first-instar aphids of the species that produced the honeydew. First instars were used because they are sufficiently small in size to not satiate the ladybird larvae and thus not to affect their motivation to search for food. A single fourth-instar ladybird larva was transferred to the honeydew-covered leaf in the clip cage and the clip cage was fastened to an intact leaf of a *V. faba* plant.

Insect rearing. Acrythosiphon pisum, Ap. fabae and *H. convergens* cultures were reared in growth chambers at approximately 27 °C under an LD 16:8 h cycle. Both aphid species had been maintained in the laboratory for 3 years using *V. faba* as the host plant. The aphids and their host plants were kept in Dacron chiffon-netted aluminium cages (31 × 31 × 61 cm; Bioquip Products, Rancho Dominguez, California). Adult *H. convergens* were purchased from commercial suppliers (Carolina Biological Supply Co., Burlington, North Carolina) and reared on an *Ac. pisum* diet in larger chiffon-netted aluminium cages (44 × 51 × 61 cm). Clay pots were provided as oviposition substrate and, once eggs were found, the pots were transferred to a hatching cage to avoid egg cannibalism by adults. Within 24 h of hatching, individual larvae were transferred to glass vials (diameter 2.5 cm, length 9.5 cm) with foam stoppers. The larvae were fed an excess amount of fresh *Ac. pisum* daily until they reached the fourth-instar stage. Fourth-instar ladybird larvae (within 24 h of molting from the third-instar stage) were used in all experiments as this is the most voracious juvenile stage (Dixon, 2000).

Statistical analysis

All analyses were performed using R Version 2.10.0 (R Development Core Team, 2009).

Binomial tests were used to analyse whether the proportion of larvae arriving at a Petri dish lid was influenced by the presence and type of honeydew (Experiments 1–4). Tests of equal or given proportions were used to test the null hypothesis that the proportion of larvae climbing a stick or plant is independent of the presence or type of honeydew (Experiments 1–4). Patch residence times (Experiments 1–4), times to encountering the stick (Experiments 2–4) and bout duration data (Experiments 2–4) were analysed with Cox's proportional hazards models using the presence or type of honeydew, and honeydew quantity, as covariates. If a larva did not stop a particular behaviour until the end of the trial (30 min), the observation was censored. Transition rates were calculated as follows: for example, if 'searching on a Petri dish' is state A, 'resting/feeding on a Petri dish' is state B, 'searching on sand (off the Petri dish)' is state C, and 'climbing a stick' is state D, then the rate of transitioning from searching to resting/feeding (α_{AB}) was calculated as:

$$\alpha_{AB} = N_{AB}/(N_{AB} + N_{AC} + N_{AD})\bar{x} \qquad (1)$$

where N indicates the total number of observed transitions between two states, and $\bar{x}$ is the mean search duration (Haccou & Meelis, 2002).

Results

In all experiments, the covariate honeydew quantity had no significant effect on patch residence time, time to encountering a stick, or search and rest bout durations (results not shown). Thus only the results of Cox proportional hazard models that included a single covariate z_1 [i.e. the presence (Experiments 1–3) or type (Experiment 4) of honeydew] are shown.

Hypothesis 1: honeydew on the ground acts as a prey-associated cue

Arrival at a Petri dish lid. Hippodamia convergens larvae were equally likely to arrive at Petri dish lids with or without honeydew (Table S2; Experiments 1–3; in all three binomial tests, $P \gg 0.05$), suggesting that the arrival of ladybird larvae at a Petri dish lid is independent of the presence of honeydew.

Climbing response. The presence of honeydew had no effect on the proportion of larvae climbing plants or sticks at least once during a patch visit (Table S3; Experiments 1–3; in all three tests of equal or given proportions, $P \gg 0.05$).

Patch residence time. Ladybird larvae stayed significantly longer on Petri dish lids containing *Ac. pisum* or *Ap. fabae* honeydew compared with Petri dish lids without honeydew

Table 2. Results of Cox's proportional hazards analysis of patch residence time.

| Experiment | | | | | | T50, s | | |
No.	Treatment	CS	β	SE	Pr $(>|z|)$	None	Ac. pisum	Ap. fabae
1	*Ac. pisum*/none	Plant	−0.99	0.42	0.017	223	712	—
2	*Ac. pisum*/none	Stick	−1.34	0.29	<0.001	30	443	—
3	*Ap. fabae*/none	Stick	−0.87	0.27	0.002	10	—	160
4	*Ac. pisum/Ap. fabae*	Stick	−0.16	0.25	0.53	—	252	355

The treatment indicates whether one or both Petri dishes contained honeydew (*Ac. pisum* or *Ap. fabae*), and if one of the Petri dishes was clean (none).
CS, climbing structure; SE, standard error; T50, time until 50% of the ladybird larvae have left the patch; *Ac. pisum*, *Acyrthosiphon pisum*; *Ap. fabae*, *Aphis fabae*.
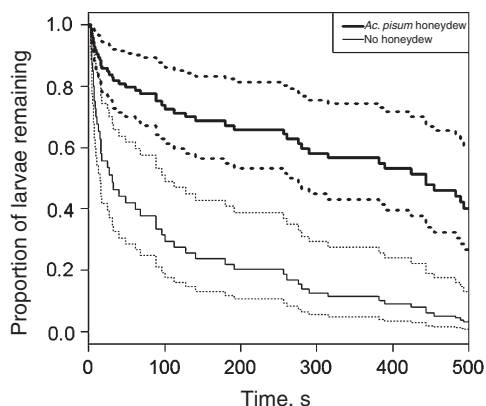

Fig. 2. Effect of honeydew presence on patch residence time. Solid lines represent how the proportion of ladybird larvae remaining in the patch changes over time; dotted lines show 95% confidence intervals. *Ac. pisum*, *Acyrthosiphon pisum*.

(Table 2, Experiments 1–3). Figure 2 illustrates how the proportions of ladybird larvae remaining in the patch changed over time (Experiment 2). In the presence of *Ac. pisum* honeydew, this proportion changed more slowly compared with its change in the absence of honeydew. The time to when 50% of the ladybird larvae left a patch (T50) can be interpreted as the average patch residence time. In the absence of honeydew, 50% of the larvae left after only 30 s (T50, Table 2), whereas ladybird larvae stayed an average of 443 s in patches with *Ac. pisum* honeydew. Using plants as climbing structures produced qualitatively similar results: the presence of *Ac. pisum* honeydew significantly increased patch residence time (Experiment 1, Table 2). However, the average patch residence time in both treatments was longer when plants were the climbing structure.

The β-value of the Cox's proportional hazards model quantifies how much the leaving tendency of the baseline hazard changes as a result of covariates. In Experiments 1–3, the baseline hazard was the model without honeydew; in Experiment 4, the baseline hazard was leaving tendency on Petri dish lids with *Ap. fabae* honeydew. The β-value of −0.99 in Experiment 1 indicated that, in the presence of *Ac. pisum* honeydew, the leaving tendency was about one third of that in the absence of honeydew ($e^{-0.99} = 0.37$). A low leaving tendency produced long patch residence times; thus the more negative the β-value, the longer the average patch residence time (Table 2, no honeydew: T50 = 223 s; *Ac. pisum* honeydew: T50 = 712 s).

Time until first stick encounter. After arriving at a Petri dish lid, ladybird larvae took significantly longer to encounter the stick when *Ac. pisum* or *Ap. fabae* honeydew was present compared with when honeydew was absent (negative β-values, Table 3).

Behavioural pattern. The duration of individual rest bouts was significantly influenced by the presence of honeydew (Table 4). If there was honeydew on a Petri dish lid, *H. convergens* larvae rested for longer periods (negative β-values, Table 4) than they did on clean Petri dish lids.

Table 3. Results of Cox's proportional hazards analysis of time until first stick encounter.

| Experiment | | | | | | T50, s | | |
No.	Treatment	CS	β	SE	Pr $(>|z|)$	None	Ac. pisum	Ap. fabae
2	*Ac. pisum*/none	Stick	−1.45	0.54	0.007	25	405	—
3	*Ap. fabae*/none	Stick	−1.42	0.47	0.002	25	—	368
4	*Ac. pisum/Ap. fabae*	Stick	0.15	0.30	0.61	—	265	208

The treatment indicates whether one or both Petri dishes contained honeydew (*Ac. pisum* or *Ap. fabae*), and if one of the Petri dishes was clean (none).
CS, climbing structure; SE, standard error; T50, time until 50% of the ladybird larvae have encountered a stick; *Ac. pisum*, *Acyrthosiphon pisum*; *Ap. fabae*, *Aphis fabae*.

Table 4. Results of Cox's proportional hazards analysis of rest bout duration.

Experiment						T50, s		
No.	Treatment	CS	β	SE	Pr (>|z|)	None	*Ac. pisum*	*Ap. fabae*
2	*Ac. pisum*/none	Stick	−2.12	0.48	<0.001	3	24	—
3	*Ap. fabae*/none	Stick	−0.91	0.29	<0.001	6	—	16
4	*Ac. pisum/Ap. fabae*	Stick	−0.10	0.14	0.44	—	27	24

The treatment indicates whether one or both Petri dishes contained honeydew (*Ac. pisum* or *Ap. fabae*), and if one of the Petri dishes was clean (none).
CS, climbing structure; SE, standard error; T50, time until 50% of the ladybird larvae have transitioned to another behaviour; *Ac. pisum*, *Acyrthosiphon pisum*; *Ap. fabae*, *Aphis fabae*.

Table 5. Results of Cox's proportional hazards analysis of search bout duration.

Experiment						T50, s		
No.	Treatment	CS	β	SE	Pr (>|z|)	None	*Ac. pisum*	*Ap. fabae*
2	*Ac. pisum*/none	Stick	0.15	0.14	0.31	7	6	—
3	*Ap. fabae*/none	Stick	0.44	0.14	0.003	6	—	4
4	*Ac. pisum/Ap. fabae*	Stick	− 0.06	0.11	0.58	—	5	5

The treatment indicates whether one or both Petri dishes contained honeydew (*Ac. pisum* or *Ap. fabae*), and if one of the Petri dishes was clean (none).
CS, climbing structure; SE, standard error; T50, time until 50% of the ladybird larvae have transitioned to another behaviour; *Ac. pisum*, *Acyrthosiphon pisum*; *Ap. fabae*, *Aphis fabae*.

The presence of *Ap. fabae* honeydew significantly decreased the duration of search bouts compared with the absence of honeydew (positive β-values, Table 5), but the effect was very small (in the absence of honeydew, T50 = 6 s; in the presence of *Ap. fabae* honeydew, T50 = 4 s). This difference was no longer significant ($P = 0.117$) when three unusual data points that referred to unusually long search bouts in the absence of *Ap. fabae* honeydew were removed. By contrast, the presence of *Ac. pisum* honeydew had no effect on search bout duration. However, when honeydew was present, ladybird larvae were more likely to transition from searching to resting/feeding than to any other behaviour (Fig. 3a). By contrast, in the absence of honeydew, the transition from searching to resting/feeding was smallest and *H. convergens* larvae were more likely to transition to climbing or to leaving the Petri dish lid (Fig. 3b). High transition rates to resting/feeding and long resting/feeding bouts resulted in patch residence times that were longer in the presence than in the absence of honeydew (Table 2). (Note that the resting/feeding category included the consuming of honeydew.) *Aphis fabae* honeydew had similar effects on behavioural transitions as *Ac. pisum* honeydew (Fig. S2).

Hypothesis 2: honeydew from low-quality aphids acts as a deterrent

The foraging behaviour of ladybird larvae was not influenced by the type of honeydew. There was no difference in the proportions of larvae arriving at Petri dish lids with *Ac. pisum* honeydew and Petri dish lids with *Ap. fabae* honeydew, respectively (binomial test, $P \gg 0.05$), which is consistent with the hypothesis that the arrival of ladybird larvae at a Petri dish lid is independent of the type of honeydew. Having arrived
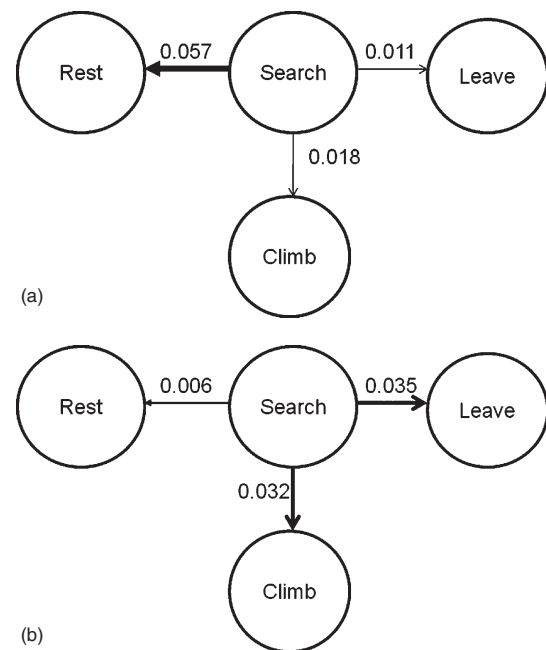
Fig. 3. Rates at which *Hippodamia convergens* larvae transition from searching on Petri dish lids to resting/feeding, climbing or leaving a patch in Experiment 2. The thickness of the arrow is proportional to the transition rates. (a) Presence of *Acyrthosiphon pisum* honeydew. (b) Absence of honeydew.

on a Petri dish lid, the tendency to leave was not influenced by the type of honeydew (Table 2). The time until encountering a stick was not influenced by the type of honeydew (Table 3), and honeydew type had no effect on the proportion of larvae

climbing sticks (test of equal or given proportions, $P \gg 0.05$). Finally, the durations of rest and search bouts were not influenced by honeydew type (Tables 4 and 5), and behavioural transitions were similar on Petri dish lids containing *Ac. pisum* and *Ap. fabae* honeydew (Fig. S3).

Discussion

Foraging cues from the environment generally increase the foraging efficiency of insect natural enemies. This study explored whether honeydew on the ground acts as a foraging cue for *H. convergens* larvae. If honeydew acts as an attractant stimulus, we would expect that the proportion of *H. convergens* larvae arriving at Petri dish lids containing honeydew would be higher than that arriving at Petri dish lids with no honeydew. However, in our experiment, *H. convergens* larvae arrived at each Petri dish lid in equal proportions. This is consistent with the findings of Ide *et al.* (2007), although these authors used larvae of a different ladybird species. The experiments of this study and those of Ide *et al.* (2007) were not conducted in a wind tunnel because the main purpose was to examine changes in behaviour after encountering honeydew. Thus, it is possible that the experimental design was not suitable for detecting responses to olfactory cues. There is evidence that *H. convergens* larvae respond to olfactory volatile chemicals associated with the aphid *Myzus nicotianae* Blackman (Homoptera: Aphididae). Jamal and Brown (2001) found that, in a wind tunnel, *H. convergens* larvae responded to aphids feeding on leaves as well as to leaves that had been previously exposed to aphids. However, these authors did not isolate the effect of *M. nicotianae* honeydew in their experiments.

It has been suggested that honeydew encounter elicits an intensive search mode in mealybug predators [ladybird species *Cryptolaemus montrouzieri* Mulsant (Heidari & Copland, 1993)] and psyllid parasitoids [*Psyllaephagus pistaciae* Ferrière (Hymenoptera: Encyrtidae) (Mehrnejad & Copland, 2006)]; this may explain why *C. septempunctata* larvae consumed a higher number of aphids on wheat ears that were covered with honeydew compared with clean wheat ears that had only recently been colonised by aphids (Carter & Dixon, 1984). However, the ladybird larvae also spent a much longer time on honeydew-covered ears, so that the number of aphids consumed per minute (consumption rate) was actually lower. In our experiments, *H. convergens* larvae responded to the presence of honeydew by staying longer in patches containing honeydew than in clean patches. However, our experimental design did not allow us to examine the aphid encounter rate as we did not include aphids in the trials.

If aphid honeydew encounter elicits an intensive search mode in *H. convergens* larvae, then larvae searching on Petri dish lids with honeydew would encounter a stick (and climb it) more frequently compared with the rate at which they would do so on clean Petri dish lids. By contrast, in our experiments the time until encountering a stick was longer on Petri dish lids with honeydew than on Petri dish lids without honeydew. Almost all stick encounters were followed by climbing the

stick as a consequence of negative geotaxis. The slow rate of stick encounter in the presence of honeydew was related to the frequent resting/feeding of *H. convergens* larvae (they were more likely to transition from searching to resting/feeding than to any other behaviour) and their remaining in a resting/feeding state for a very long time (Table 4). (Note that the resting category included the consuming of honeydew.) By contrast, Ide *et al.* (2007) found that the proportion of ladybird larvae (*C. septempunctata*) climbing a stick was higher if honeydew was present at the base of Petri dish lids than in clean Petri dish lids. Ide *et al.* (2007) also suggested that the climbing response of the larvae is influenced by the vulnerability of the aphid species excreting the honeydew. They reported that more larvae climbed the sticks in the presence of *Aphis craccivora* Koch honeydew than in the presence of honeydew from *Ac. pisum*, a species that is difficult to catch. In our experiments, although *Ap. fabae* is a lower-quality prey than *Ac. pisum*, we did not find any effect of honeydew type on the foraging behaviour of *H. convergens* larvae (Experiment 4).

There are several potential reasons for the discrepancies between the results of the present study and those of Ide *et al.* (2007). Firstly, Ide *et al.* (2007) used *C. septempunctata* larvae, whereas we used *H. convergens* larvae. It is possible that different coccinellid species respond differently to honeydew and that findings in one species cannot be generalised to all other aphidophagous coccinellid species.

Secondly, the honeydew of the aphid species in the study by Ide *et al.* (2007) differed in quantity and distribution (*Ap. craccivora* produced more honeydew and excreted it closer to the plant stem than *Ac. pisum*). *Coccinella septempunctata* larvae returned and re-searched areas with honeydew more frequently than they did areas without honeydew (Carter & Dixon, 1984) and it is possible that this response to honeydew is stronger if honeydew occurs in larger amounts. This response to honeydew might have resulted in a higher probability of stick encounter in trials with either more honeydew or in which most of the honeydew was located closer to the stick, independently of the aphid species that produced the honeydew. Thus, the differences in the distribution and quantity of honeydew of *Ac. pisum* and *Ap. craccivora*, rather than differences in honeydew composition, might explain differences in the climbing response of *C. septempunctata* larvae. By contrast, we used clip cages to collect honeydew, which allowed us to control honeydew quantity by using different numbers of aphids (30 *Ap. fabae*, 12 *Ac. pisum*) per clip cage. Furthermore, because the clip cages were fastened to the leaves and the collection area was small, we did not see any obvious differences in honeydew distribution between the two aphid species.

Thirdly, in Ide *et al.* (2007), the predatory larvae had a longer experience with both prey species compared with larvae in our study. *Coccinella septempunctata* were reared on approximately equal amounts of *Ap. craccivora* and *Ac. pisum*, whereas, in the current study, *H. convergens* larvae were reared on *Ac. pisum* exclusively because their survival on *Ap. fabae* is poor; thus their experience with *Ap. fabae* was restricted to a 2-h exposure prior to the experiment. However, insufficient experience with *Ap. fabae* would have biased *H. convergens*

larvae to respond more strongly to the more familiar prey (Ettifouri & Ferran, 1993). By contrast, *H. convergens* larvae were impartial to the two honeydew types, and the likelihood of larvae climbing a stick or plant was not influenced by prior *Ac. pisum* honeydew encounter. This suggests the short experience was sufficient for *H. convergens* larvae to become familiar with *Ap. fabae* and its honeydew. The results of the present study are also consistent with the observation that *H. convergens* larvae did not discriminate against *Ap. fabae* when reared on a mixed diet of *Ap. fabae* and *Ac. pisum* (in glass vials in the laboratory; T. Hinkelman and B. Tenhumberg, unpublished data).

Why did larvae not use honeydew on the ground as a foraging cue indicating the presence of aphids on the plants above?

It is possible that cue use may vary among different predator and prey systems. For instance, *H. convergens* larvae may not climb a plant or stick in response to honeydew of *Ap. fabae* or *Ac. pisum* because its evolutionary history with each of these species is relatively short. Both aphid species originated in Europe and were introduced to North America before 1880 (Foottit *et al.*, 2006), whereas *H. convergens* is native to North America. It is possible that predators in Ide *et al.* (2007) used honeydew as a cue because the predator and prey species used in that study have a longer shared evolutionary history. Ladybird beetles are generalist predators that feed on a large number of different aphid species (Hodek & Honek, 1996), resulting in a relatively low encounter rate with the introduced aphid species, which would slow the selection pressure for recognising cues from introduced prey species.

Furthermore, honeydew on the ground may not be a reliable indicator of aphid density on surrounding plants, and the usefulness of cues to increase foraging efficiency depends on how reliable cues are (Vet *et al.*, 1991). If the benefit of using honeydew as a cue is small, it is possible that not all aphidophagous predator species have evolved a response to honeydew. It is possible that under field conditions, honeydew evaporates quickly, is washed away by rain, or that the volatile components of honeydew lose kairomonal activity in a short time. For instance, the kairomonal activity of *Brevicoryne brassicae* L. (Hemiptera: Aphididae) honeydew has been reported to decrease over time and to be lost completely after 72 h (Shaltiel & Ayal, 1998). Furthermore, when ladybird larvae encounter honeydew on the ground, the aphids that produced the honeydew may no longer be present because they may have been preyed upon or dispersed (Li *et al.*, 1997).

Conclusions

Our experiments illustrate that *H. convergens* larvae are not more likely to climb a stick or plant in the presence of either *Ac. pisum* or *Ap. fabae* honeydew and nor do they seem to distinguish between the honeydew of aphids that differ in profitability. We speculate that our results could be explained by the short shared evolutionary history between predator and prey.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/j.1365-2311.2012.01351.x

Fig. S1. Bar plot of behaviors scored during one example visit of a Petri dish lid.

Fig. S2. Rates at which *H. convergens* larvae transition from searching on the Petri dish to resting, climbing or leaving a patch in the presence and absence of honeydew in Experiment 3. The thickness of the arrow is proportional to the transition rates. (A) Presence of *A. fabae* honeydew; (B) Absence of honeydew.

Fig. S3. Rates at which *H. convergens* larvae transition from searching on the Petri dish lid to resting, climbing or leaving a patch (Experiment No 4). The thickness of the arrow is proportional to the transition rates. (A) Presence of *A. fabae* honeydew; (B) Presence of *A. pisum* honeydew.

Table S1. Description of behavioral categories (see Fig S1 for an example).

Table S2. Arrival at Petri dish lids. The treatment indicates whether one or both Petri dishes contained honeydew (*A. pisum* or *A. fabae*), and if one of the Petri dishes was clean (None). CT specifies the climbing structure. The binomial test evaluated if the proportion of larvae arriving at a Petri dish (number of successes) is influenced by the presence of honeydew. If the confidence intervals include 0.5 the presence of honeydew does not significantly influence the arrival response.

Table S3. Climbing Response. The treatment indicates whether one or both Petri dishes contained honeydew (*A. pisum* or *A. fabae*), and if one of the Petri dishes was clean (None). CT specifies the climbing structure. The test of equal or given proportions evaluated if the proportion of larvae climbing a

plant or a stick (number of successes) is influenced by the presence of honeydew. If the confidence intervals include 0.5 the presence of honeydew does not significantly influence the climbing response.

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