



Short communication

The olfactory responses of coccinellids to aphids on plants

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Introduction

Our understanding of the foraging behaviour of predaceous coccinellids is limited, despite the importance of this information to the use of coccinellids in the biological control of insect pests (Obrycki & King, 1988). Laboratory studies have revealed that coccinellids use multiple cues, especially olfaction and vision, to locate prey (e.g. Hattingh & Samways, 1995; Hamilton et al., 1999; Harmon et al., 1998; Obata, 1997) and that their responsiveness to prey is influenced by intrinsic factors, especially relating to hunger, age and sex (e.g., Sengonca & Liu, 1994; Sengonca et al., 1995), and extrinsic factors, including prey species, temperature and light intensity (e.g., Ferran & Dixon, 1993; Kalushkov, 1999). Feeding experience can also affect the searching activity and prey preference of coccinellids. For example, *Harmonia axyridis* larvae changed from 'extensive' to 'intensive' search mode only when the larvae encountered familiar prey (Ettifouri & Ferran, 1993) or their odour tracks (Ferran et al., 1997); and *Adalia decempunctata* larvae that encountered the toxic aphid *Hyalopterus pruni* (including penetration of the aphid cuticle) subsequently rejected this aphid (Dixon, 1958).

In the field, coccinellid-prey interactions generally occur on plants. Coccinellids forage on many different plant species and their foraging success is influenced by the architecture and surface features of the plant (e.g., Carter et al., 1984; Clark & Messina, 1998; Eigenbrode et al., 1996). Little consideration, however, has been given to the effect of the plant and feeding experience on the olfactory responses of foraging coccinellids. To address this last issue, we investigated the olfactory responsiveness of the aphidophagous coccinellid *Adalia bipunctata* L. to the

prey/plant complex and how this may be affected by experience. *Aphis fabae* Scop. was used as the prey item. Although *A. fabae* has been reported as a food of relatively low suitability for *A. bipunctata* in the laboratory (Blackman, 1967; Kalushkov, 1999), wild populations of this coccinellid forage extensively on *A. fabae* colonies on various plant species in the field (Banks, 1955; unpub. obs. of authors).

Materials and methods

Two plant species, *Vicia faba* cv. The Sutton and *Tropaeolum majus* cv. Alaska, were raised to 4 weeks post-sowing under glass in coco-fibre compost. Aphid-infested plants bore vigorous cultures (>100 aphids) of laboratory stocks of *Aphis fabae* (subsp. *fabae* on *V. faba* and subsp. *mordwilkoii* on *T. majus*).

A. bipunctata from two separate collections were used. The first (termed 'unfed') were derived from pupae collected from *Acer* sp. trees in Oxford (July 1998), maintained in the dark at 8 °C, and used for experiments within 1 day of reaching adulthood without prior access to any prey. The second (termed 'fed') were collected as adults from the University of York campus (June 1997), maintained for 6 days on aphid-infested *V. faba* or *T. majus* at 20 °C with L18:D6 and starved for 1 day prior to experiments.

The olfactory responses of individual *A. bipunctata* were tested in a four-arm olfactometer, following Vet et al. (1983), with test chamber of maximum dimensions 34 × 34 cm. Air (1 l min⁻¹) was drawn through a hole at the centre of the test chamber to create four discrete odour plumes, one from each odour source contained within a 3 l bottle. Tests with carbon dioxide vapour confirmed the absence of turbulence between

odour fields. To protect against bias, all experiments were conducted under diffuse illumination at 20°C with the olfactometer shielded with white card to exclude visual cues, similar/identical treatments were placed in opposite sectors, the olfactometer was regularly rotated by 90°, and the apparatus was routinely washed in detergent (Decon) and rinsed in distilled water and ethanol. In preliminary experiments with identical treatments in all sectors, the coccinellid responses did not differ from random, confirming that the apparatus was unbiased.

Each coccinellid was sexed without anaesthesia, placed in the centre of the olfactometer and observed for 5 min. The total time spent in each odour field and the location of the insect at the end of the trial ('final choice') were recorded. To investigate the olfactory cues used by foraging coccinellids, two experiments were conducted on the unfed group of coccinellids: (1) response to odour of aphid-infested and aphid-free *V. faba* and (2) response to aphid-free *V. faba* and *V. faba* from which the aphid infestation had recently been removed (the aphids and their honeydew and exuviae were brushed from the plants with a fine paintbrush and the plants were washed in water at room temperature; the aphid-free control plants were subjected to the same treatment). The impact of foraging experience of *V. faba* or *T. majus* on the olfactory responses of the coccinellids was explored with fed group of coccinellids: (1) response to aphid-free *V. faba* and *T. majus*, with two controls (moist compost to ensure uniform humidity of airflows), to test for associative learning of plant cues, and (2) response to aphid-infested and aphid-free *V. faba* and *T. majus* to test for preference learning (*sensu* Turlings et al., 1993) of familiar aphid/plant combinations.

The data were analysed with nonparametric tests, using statistical package SPSS 7.1. The data on time spent in each odour sector was analysed using Friedman's two-way analysis of variance by ranks for experiments with four different odour treatments and by Wilcoxon's analysis of paired matched samples for two odour treatments (sectors with the same treatment were pooled). 'Final choice' data were analysed by the χ^2 test, with pooling as above. The difference between sexes in 'time spent' data were compared with the Mann-Whitney U test.

Results and discussion

When females of the unfed group of *A. bipunctata* were exposed to the choice between aphid-infested and aphid-free *Vicia faba* in the olfactometer, they exhibited an olfactory response to aphid-infested plants. Over the 5 min test, they spent significantly more time in the odour plumes of aphid-infested plants than of aphid-free plants ($n = 30$, $Z = -2.13$, $df = 1$, $P < 0.05$) (Figure 1a). At the end of the trials, a higher frequency of coccinellids was recorded in the sectors of aphid-infested plants than aphid-free plants (Figure 1b), but the difference was not statistically significant ($n = 30$, $\chi^2 = 3.33$, $df = 1$, $0.1 > P > 0.05$). Males spent significantly less time than females in the sectors of the aphid-infested plants (Table 1a) and displayed no discernible olfactory response to the aphid-infested plants ($n = 30$; time spent: $\chi^2 = -0.63$, $df = 1$, $P > 0.1$; final choice, $\chi^2 = 0.02$, $df = 1$, $P > 0.10$).

The unfed female *A. bipunctata* did not display a significant olfactory response to *V. faba* plants from which aphids had recently been removed, as assessed by mean time spent in different sectors of the olfactometer ($n = 31$, $Z = -1.19$, $df = 1$, $P > 0.1$) and location at the end of the trial ($\chi^2 = 1.6$, $df = 1$, $P > 0.1$). These results suggest that presence of the aphids or their products (e.g., honeydew) is required for the olfactory response of unfed *A. bipunctata*. Although the chemical identity of the olfactory stimulus remains to be established, these data are in broad agreement with the behavioural, electrophysiological and ultrastructural evidence that other coccinellid species can respond to prey-related odours (Hamilton et al., 1999; Zhu et al., 1999). However, our results differ from the finding of Hamilton et al. (1999) that *Hippodamia convergens* exhibit an olfactory response to radish plants, whether they are aphid-free or infested with the aphid *Myzus persicae*; differences in coccinellid, aphid and plant species could have contributed to this discrepancy.

Adult *A. bipunctata* previously cultured on aphid-infested *Vicia faba* or *Tropaeolum majus* for 6 days were used to test whether insects which had foraged successfully on one plant species preferentially used the olfactory cues of that plant or that aphid/plant species combination. In these experiments there were no significant differences in the olfactory responses of male and female coccinellids to aphid-infested plants in experiments testing for preference learning of familiar aphid/plant combinations (Table 1a), or to

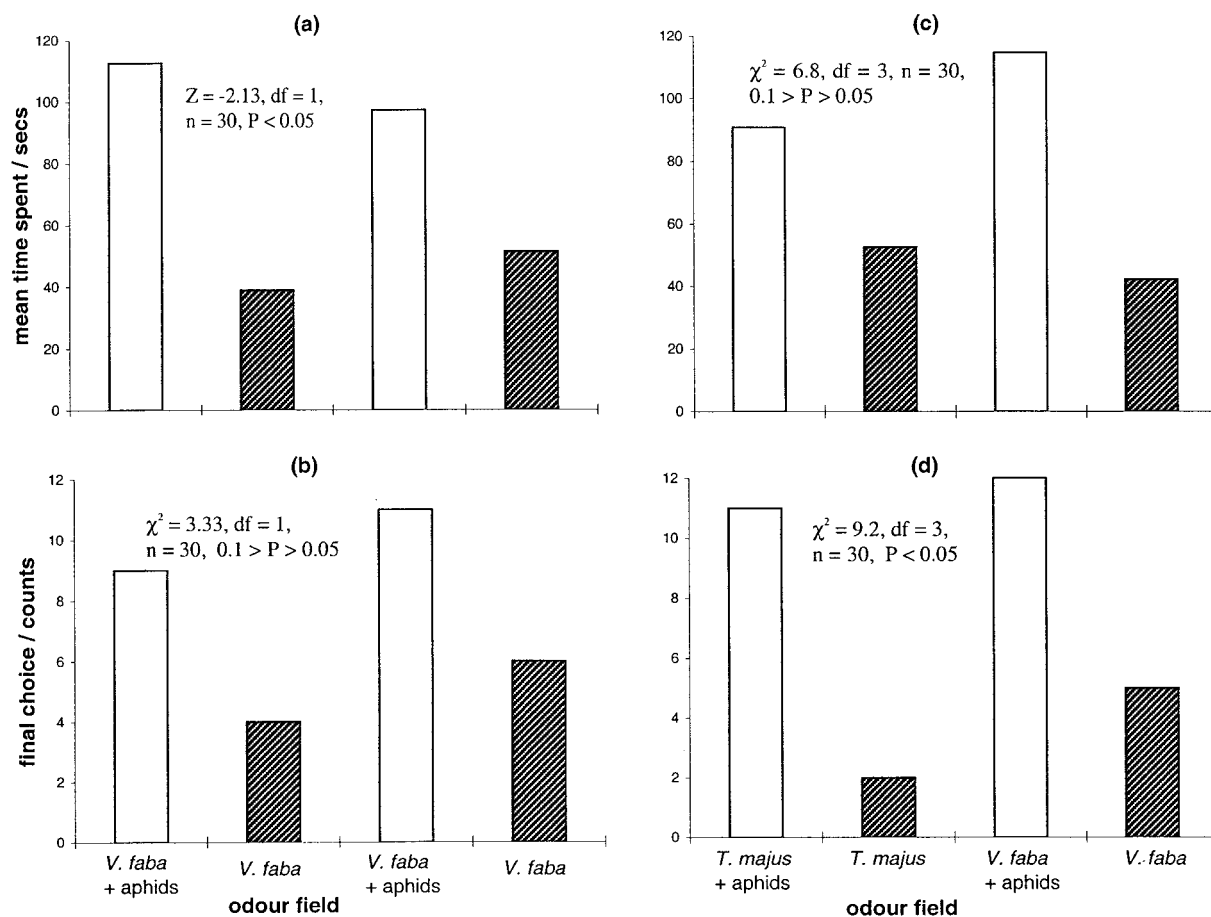


Figure 1. The olfactory responses of adult *A. bipunctata*. (a) and (b) unfed females exposed to aphid-infested and aphid-free *V. faba*; (c) and (d) adults (sexes pooled) which had fed on aphids on *V. faba* exposed to aphid-infested and aphid-free *V. faba* and *T. majus*

aphid-free plants in experiments testing for associative learning of plants cues (Table 1b) and data for the two sexes were pooled. The coccinellids from the *V. faba*-*A. fabae* culture did not show a significant olfactory response to aphid-free *V. faba* or *T. majus* ($n = 30$, time spent: $\chi^2 = 2.1$, $df = 3$, $P > 0.1$; final choice: $\chi^2 = 2.5$, $df = 3$, $P > 0.1$). They did, however, respond to the odour plumes of aphid-infested plants of both species. The time spent by the coccinellids in the sectors of aphid-infested plants was elevated, albeit not significantly ($n = 30$, $\chi^2 = 6.8$, $df = 3$, $0.10 > P > 0.05$) relative to aphid-free plants (Figure 1c) and, at the end of the trials, their frequency in the sectors of aphid-infested plants was significantly higher than in sectors of aphid-free plants ($\chi^2 = 9.2$, $df = 3$, $P < 0.05$) (Figure 1d). These results suggest that the experience of successful foraging on *V. faba* did not influence the olfactory response of *A. bipunctata*. In other words, the coccinellids had neither learnt

to associate the odour of *V. faba* with the presence of prey nor acquired a learnt olfactory preference for the familiar aphid/*V. faba* combination. Further research, however, is needed to establish whether these conclusions can be generalized to either *A. bipunctata* feeding on other prey species and to other coccinellids.

The behaviour of *A. bipunctata* which had fed on aphid-infested *T. majus* was different from that of individuals from aphid-infested *V. faba*. Their olfactory responses did not differ significantly from random in experiments comparing the response to aphid-free *T. majus* and *V. faba* (time spent: $\chi^2 = 6.41$, $df = 3$, $P > 0.1$; final choice: $\chi^2 = 2.4$, $df = 3$, $P > 0.1$) or in experiments comparing responses to aphid-infested and aphid-free *T. majus* and *V. faba* ($n = 30$; time spent: $\chi^2 = 1.8$, $df = 3$, $P > 0.1$; final choice: $\chi^2 = 1.83$, $df = 3$, $P > 0.10$).

A notable feature of the results of this study was the variability in responsiveness of *A. bipunctata* to

Table 1. Impact of sex of adult *A. bipunctata* on behaviour in the olfactometer

Coccinellids	Time spent (s)		Mann–Whitney test
	Mean \pm s.e. (n)		
	Male	Female	
a. Impact of sex on the time spent in the odour plumes of aphid-infested plants in experiments testing responses to the odour of aphid-infested and aphid-free plants			
Unfed	160 \pm 17 (31)	210 \pm 19 (30)	W = 1077, P < 0.05
Fed on:			
Aphid-infested <i>V. faba</i>	210 \pm 14 (15)	200 \pm 22 (15)	W = 245, P > 0.05
Aphid-infested <i>T. majus</i>	150 \pm 20 (19)	160 \pm 11 (11)	W = 173, P > 0.05
b. Impact of sex on time spent in the odour plumes of aphid-free plants in experiments testing responses to the odour of aphid-free plants and blank controls			
Fed on:			
Aphid-infested <i>V. faba</i>	162 \pm 20 (17)	132 \pm 19 (13)	W = 176, P > 0.05
aphid-infested <i>T. majus</i>	170 \pm 28 (18)	122 \pm 17 (13)	W = 262, P > 0.05

olfactory cues from the *A. fabae*/plant combinations. Olfactory responses were evident for only females among the unfed group and for both sexes of the fed group of coccinellids (Table 1a); different behavioural indices were statistically significant for the two groups (Figure 1); and, among the fed group, olfactory responses were obtained only for coccinellids that had foraged on *V. faba* but not on *T. majus*. The coccinellids may have had greater foraging success on *T. majus* than on *V. faba* because *T. majus* plants are architecturally simpler than *V. faba* and, as a consequence, the coccinellids on the *A. fabae*/*T. majus* pre-treatment may not have been sufficiently hungry to respond to prey-associated olfactory cues. Both intrinsic differences between coccinellids of different provenance (year and location) and differences in pre-treatments may have contributed to the discrepancies between the olfactory responses of the fed and unfed groups of coccinellids. One implication of this behavioural variability is that it may not be appropriate for conclusions based on tightly-defined and uniform experimental protocols to be generalized beyond those conditions. The variability of coccinellid foraging responses (e.g., Frazer, 1988) is one factor to be taken into account when the results of laboratory-based analyses are used to predict behaviour under field conditions, in relation to both the ecology of coc-

cinnellids and their management as biological control agents.

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