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Orientation behavior of *Propylaea japonica* toward visual and olfactory cues from its prey–host plant combination

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Abstract

The lady beetle *Propylaea japonica* (Thunberg) (Coleoptera: Coccinellidae) is an important predator of aphids in agroecosystems. The inundative release of coccinellid beetles can be an effective biological control strategy. An understanding of how biological control agents perceive and use stimuli from host plants is the key to successfully implement commercially produced predators. Here, we studied the relative role of visual and volatile cues. Dual-choice assays using foraging-naïve and foraging-experienced *P. japonica* adults were conducted using cotton plants [*Gossypium hirsutum* L. (Malvaceae)] with or without infestation by the cotton aphid, *Aphis gossypii* (Glover) (Hemiptera: Aphididae). Overall, experienced beetles were more attracted than naïve beetles toward cues associated with aphid-infested plants. Experienced beetles were also more responsive to olfactory cues compared with naïve beetles. Both foraging-naïve and -experienced lady beetles integrate olfactory and visual cues from plants infested with aphids, with an apparently greater reliance on olfactory cues. The results suggest that foraging experience may increase prey location in *P. japonica*.

Introduction

Coccinellid beetles are important predators of terrestrial arthropods and have been widely used as biological control agents, especially of aphids (Obrycki & Kring, 1998; Ouyang et al., 2012). The ladybeetle *Propylaea japonica* (Thunberg) (Coleoptera: Coccinellidae) is a common natural predator of aphid pests in China (Ge & Ding, 1996; Zhang et al., 2007), including the cotton aphid, *Aphis gossypii* (Glover) (Hemiptera: Aphididae).

The inundative release of coccinellid beetles can be an effective control strategy (Tedders & Schaefer, 1994). Lady beetles respond to herbivore-induced plant volatiles (HIPVs) (Sloggett et al., 2011) and/or visual cues (Obata, 1997; Ninkovic et al., 2001), which increase the efficiency of prey location. An understanding of how biocontrol agents perceive and use stimuli from host plants of their prey is the key to successfully implement commercial mass production of predators.

Experience may play an important role in foraging of lady beetles (Mondor & Warren, 2000). It has been shown that *Harmonia axyridis* (Pallas) reared on artificial diets is less efficient when foraging for prey (Ettifouri & Ferran, 1993; Lambin et al., 1996). Prey handling time of *P. japonica* reared on cotton aphids was clearly shorter than when reared on artificial diet (Zhang et al., 2008), indicating that experience affects foraging of this ladybeetle, possibly also its behavioral response toward foraging-related cues. In this manuscript, we evaluated the response of foraging-naïve and foraging-experienced *P. japonica* lady beetles to volatile and visual cues associated with aphid-infested host plants.

Materials and methods

Cotton [*Gossypium hirsutum* L. (Malvaceae)] seeds (var. GK12) were supplied by the Biotechnology Research Institute of the Chinese Academy of Agricultural Sciences (Beijing, China). Eighty cotton plants were planted outdoors in plastic pots filled with a 1:1 (vol/vol) mixture of nutrient soil and vermiculite. Eight-week-old plants were divided in two groups (n = 40 for each group) spaced >20 m apart. One week later, plants in one group were infested with *A. gossypii* (80 aphids per plant) for 1 week, after which period the plants were individually transferred into mesh cages (50 × 50 × 100 cm). To minimize the possible HIPVs adhered on the surface of uninfested

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cotton, the plants were rinsed using distilled water and allowed to air dry before experimentation. The aphid-infested cotton was, however, not washed to preserve any HIPVs.

Foraging-naïve and -experienced beetles were reared in the laboratory from first instar to adulthood on artificial diet (Song et al., 1988) and on cotton plants infested with *A. gossypii* respectively. The adults used in the experiments were 5–10 days old and had been starved for 12 h prior to the experiment.

A dual-choice behavioral assay system (Figure 1A) consisted of an I-tube arena, two chambers, a gas supply device, and polytetrafluoroethylene (ptfe; Teflon) tubing (Bahlai et al., 2008; Milet-Pinheiro et al., 2012). The ptfe-tube air entry port in the I-tube arena had 40 fine holes (1 mm diameter) opened to the center, and the ptfe tube air egress port had 40 similar holes opened to the two ends. The system was set up as follows for the various experiments. (1) Visual attraction was tested by passing purified air directly into a gas flow meter and the arena, bypassing the plant odor chamber (Figure 1B); (2) Olfactory attraction was tested by passing purified air through the plant odor chamber with the side abutting the arena covered with a 45-cm-side white square paperboard (Figure 1A-11) to prevent the beetle from seeing the tested plant (Figure 1C); (3) Purified air entered an unmodified chamber, and the system was used to test the attraction to olfactory and visual cues together (Figure 1D).

Each test was conducted outdoors on sunny days to optimize the amount and composition of volatile organic compound (VOC) (Baldwin et al., 2006). HIPVs in cotton follow a diurnal cycle, with a peak emission of volatiles in the early afternoon (Loughrin et al., 1994). The bioassays were carried out during the peak activity of foraging *P. japonica* between 10:00 and 14:00 hours. Considering the positive phototaxis of the ladybeetle (Kawai, 1976), the direction of the dual-choice arena was adjusted at noon to keep it vertical against sunlight to minimize the influence of light direction on the ladybeetle.

Forty to 50 foraging-naïve and foraging-experienced adults (sex ratio = 1:1) were tested in randomized order for each treatment. Each individual beetle was used only once for a given experiment and its behavioral response was recorded within 5 min as either (1) no response (i.e., not crossing a 'choice line' at 20 cm from the end), or (2) response (crossing the choice line and flying or climbing toward one end). The position of the plant material was exchanged, the I-tube arena was cleaned with ethanol, and the ptfe tubes were renewed every five tests. Dual-choice bioassays were performed on foraging-naïve and -experienced *P. japonica* lady beetles. The three experiments each included two treatments: (1) cotton plants with vs. without infestation with *A. gossypii*; (2) one infested plant vs. a blank control; and (3) two infested plants to compare visual vs. volatile cues. Experiments 1 and 2 tested combinations of stimuli in the following order: (1) visual cues, (2) olfactory cues, and (3) olfactory + visual cues. In the third experiment, the attractiveness of cues from infested cotton plants was

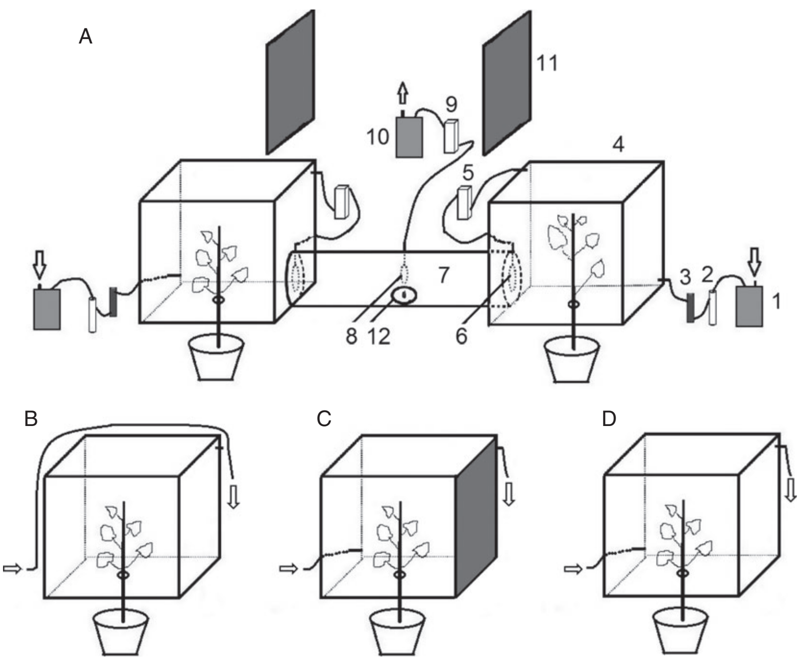

Figure 1 Diagram of the dual-choice bioassay system. (A) Plant odor chambers and test arena; 1, air sampler; 2, glass column with distilled water; 3, glass filter with activated charcoal; 4, one of the dual chambers; 5, gas flow meter; 6, Teflon tube air entry port; 7, I-tube arena; 8, Teflon tube air egress port; 9, gas flow meter; 10, air sampler; 11, white board; 12, access/egress port. Conditions for (B) vision test, (C) olfaction test, and (D) olfaction + vision test. The arrows indicate the air flow.

compared in the following order: (1) olfactory vs. visual cues; (2) olfactory + visual vs. olfactory cues; and (3) olfactory + visual vs. visual cues.

All data analyses were performed using SPSS v.17 (SPSS, Chicago, IL, USA). Non-responders were recorded but excluded from analysis. To assess the differences in response of foraging-naïve and -experienced lady beetles between the paired treatments in each dual-choice bioassay, observed vs. expected χ^2 tests were conducted (Milet-Pinheiro et al., 2012). In order to evaluate the effect of foraging-experience on the ladybeetle's orientation behavior, Fisher's exact tests were used as post hoc tests for 2 × 2 χ^2 contingency tables (based on behavioral responses between foraging-naïve and -experienced lady beetles in the bioassay with infested and uninfested cotton plants). The responses of male and female lady beetles were pooled because no obvious differences were found between the two sexes.

Results

Different response modes were exhibited by foraging-naïve and -experienced lady beetles. Depending on the treatment pair, 70–86% of beetles made a choice (Table 1).

Infested cotton plants were less attractive than uninfested plants to foraging-naïve *P. japonica* with both olfactory and visual cues. However, significantly more foraging-experienced lady beetles were attracted to infested than to uninfested cotton plants when olfactory and olfactory + visual cues could be used. No difference in response was observed based on visual cues alone. The difference in response between foraging-naïve and -experienced beetles was not significant in the vision test. However, significant differences were found in the olfaction test and olfaction + vision test (vision test: $\chi^2 = 0.547$, $P = 0.62$; olfaction test: $\chi^2 = 5.145$, $P = 0.03$; olfaction + vision test: $\chi^2 = 4.712$, $P = 0.043$; all d.f. = 1).

Olfactory and combined cues from infested cotton plants were more attractive than the blank control. There was, however, no role for visual cues in attracting lady beetles in the blank control. Significantly more foraging-experienced adults preferred the visual, olfactory, and combined cues from infested cotton over the blank control.

Olfactory cues were more attractive for foraging-naïve lady beetles than visual cues when tested against each other, and significantly more adults preferred the mixed cues over visual cues, but not over olfactory cues. Foraging-experienced lady beetles preferred olfactory over visual cues when tested against each other, and both cues together attracted more adults than either one alone.

Table 1 Behavioral responses of foraging-naïve and foraging-experienced *Propylaea japonica* in dual-choice bioassays

Treatment pair	Cue		Foraging-naïve						Foraging-experienced					
Odor source					% choice						% choice			
T1 vs. T2	T1	T2	n	% response	T1	T2	χ^2	P	n	% response	T1	T2	χ^2	P
Uninfested vs. infested cotton	Visual	Visual	44	70	45	55	0.290	0.59	44	79	46	54	0.257	0.61
	Olfactory	Olfactory	48	75	47	53	0.111	0.79	50	80	23	77	12.100	0.001
	Olfactory + visual	Olfactory + visual	48	77	40	60	1.324	0.25	48	81	18	82	17.780	<0.001
Blank control vs. infested cotton	Blank control	Visual	40	73	40	60	1.690	0.19	46	70	28	72	5.121	0.024
	Blank control	Olfactory	40	73	28	72	5.828	0.016	44	77	21	79	11.765	0.001
	Blank control	Olfactory + visual	50	80	28	72	8.100	0.004	48	81	21	79	14.400	<0.001
Infested vs. infested cotton	Visual	Olfactory	44	73	31	69	4.500	0.034	50	78	26	74	9.256	0.002
	Olfactory	Olfactory + visual	48	75	42	58	1.000	0.32	46	83	32	68	5.518	0.026
	Visual	Olfactory + visual	48	79	32	68	5.518	0.023	50	86	26	74	10.256	0.001

Discussion

As generalist natural enemies of insect pests, coccinellid beetles may orient themselves to the most suitable microhabitat using foraging experience in a relatively complex prey–host habitat (Vet & Dicke, 1992). Although our results suggest that *P. japonica* does not have an innate preference for the cues from infested over uninfested prey–host plants, our results also demonstrate that the lady beetles became more responsive to olfactory cues as well as combined olfactory + visual cues from infested cotton plants after gaining foraging experience, thus displaying stronger taxis compared to the weaker responses exhibited by foraging-naïve beetles. Exploration of the effect of forage experience may help understanding the difference in response between the lady beetles reared on a non-aphid artificial diet and those living under natural conditions. Conducting diet acclimation on foraging-naïve lady beetles before releasing them in massive number in the field may facilitate their function as a biological control agent. Similar studies were reported in 'training' parasitoids (Ngumbi et al., 2012), a predatory mite, and an anthocorid predator (Sabelis et al., 1999; Drukker et al., 2000a,b).

HIPVs offer an adaptive advantage to both the plant and natural enemies by shortening the prey-search time required to find and kill pest insects (Hattingh & Samways, 1995) and thus function as indirect defenses for prey–host plants (Baldwin et al., 2002). *Harmonia axyridis* preferred the volatile compounds of aphid-infested leaves over those of uninfested leaves (Obata, 1997; Ninkovic et al., 2001), and the ladybeetle did not discriminate between visual stimuli from apple and buckthorn leaves until olfactory cues were also included (Bahlai et al., 2008). Our study suggests that olfactory cues from aphid-infested cotton play a key role in mediating the orientation behavior of *P. japonica*. Furthermore, it is directly and indirectly demonstrated that olfactory cues alone provide more meaningful information than visual cues alone at a certain spatial scale in the ladybeetle's orientation behavior. However, the presentation of a single synthetic HIPV did not elicit any significant response in *P. japonica* in a previous study (Yu et al., 2008). We conjecture that VOC mixtures, designed and prepared to mimic the natural VOC production environment, may aid in preconditioning mass reared predators prior to their inundative release in the field. The next step in our work will be to analyze the aphid-induced cotton volatiles and test the response (e.g., orientation behavior) of *P. japonica* to the compounds identified.

To understand why foraging-naïve and -experienced ladybeetle preferred olfactory over visual cues from infested cotton requires further analysis. In this study, we hypothesized that visual cues may function in searching for a suitable habitat, but that the ladybeetle could not determine at long distances whether aphids inhabit the host plants using only visual cues. In our supplementary test, adult *P. japonica* visually perceived cotton aphid only at 5 mm (P Wang, unpubl.). Similarly, the ladybeetle *Coccinella septempunctata* L. did not identify aphid prey at even 7 mm distance (Nakamuta, 1984). Chemicals derived from infested plants and the prey itself are generally reliable in showing herbivore presence, accessibility, and suitability (Vet & Dicke, 1992). Therefore, when encountering a choice of olfactory and visual cues in a certain range, starved adults of *P. japonica* were more attracted to the olfactory than to the visual cues.

In conclusion, our investigations show that *P. japonica* does not express an innate ability to differentiate the cues from infested from those of uninfested prey–host plants. Diet acclimation could improve ladybeetle's orientation efficiency and value as a biological control agent if beetles are reared on artificial diet. Olfactory cues have a major role on a biotope scale, whereas visual cues play a secondary role. The combination of both cues contributes to identify prey–host plants in prey-finding activity of foraging-naïve and -experienced *P. japonica*.

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