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Aphid Alarm Pheromone Component, (*E*)-β-farnesene, and Local Search by a Predatory Lady Beetle, *Coccinella septempunctata bruckii* MULSANT (Coleoptera: Coccinellidae)

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Responses of a predatory lady beetle, *Coccinella septempunctata bruckii*, to synthetic aphid alarm pheromone were studied to determine possible influences of the pheromone on the beetle's local search. Three search parameters—locomotory rate, turn rate and "meander"—were tested after consumption of an aphid prey and after contact with aphid alarm pheromone. The 2 sets of parameters were significantly different, suggesting that aphid alarm pheromone is not utilized as an orientation cue toward an additional prey item.

Key words: local search, (*E*)-β-farnesene, lady beetle, *Coccinella septempunctata*

INTRODUCTION

Area-concentrated or local search after location of an essential resource item occurs in predatory coccinellids (BANKS, 1957; DIXON, 1959), parasitoid wasps (WAAGE, 1978; STRAND and VINSON, 1982; BOUCHARD and CLOUTIER, 1984), the German cockroach (SCHAL et al., 1983), and flies (DETHIER, 1957; NELSON, 1977; WHITE et al., 1984). While executing local search, insects may use several kinds of information (such as idiothetic, genetic, learned, external sensory, internal sensory and internal stochastic (BELL, 1985)) for orientation toward an additional resource item.

A predatory coccinellid lady beetle, *Coccinella septempunctata bruckii* MULSANT, executes local search after finding an aphid prey (NAKAMUTA, 1982, 1985). Aphids, however, excrete alarm pheromone in response to attack by predators (NAULT et al., 1973). This pheromone causes siblings to disperse. The area where lady beetles execute local search, thus, might be contaminated with the aphid alarm pheromone. It would be not advantageous to coccinellids to spend time intensively searching alarm pheromone contaminated areas, since the pheromone causes aphids to disperse from such areas. Quickly moving away from alarm pheromone contaminated areas might therefore be advantageous to predatory beetles. We are intrigued to determine whether the lady beetle utilizes alarm pheromone as a cue to localize the searching or to disperse from the alarm pheromone contaminated area. In this paper, therefore,

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responses of lady beetles in the laboratory to a synthetic aphid alarm pheromone, (*E*)-*β*-farnesene, are described. (*E*)-*β*-farnesene is the principal component of the alarm pheromone utilized by several aphid species including the green peach aphid, *Myzus persicae* SULZER (NAULT and PHERAN, 1984).

MATERIALS AND METHODS

Lady beetles, *C. septempunctata bruckii*, and green peach aphids, *M. persicae* were reared as in NAKAMUTA (1983) at LD 16: 8 hr (light-on at 6: 00, light-off at 22: 00). Beetles reared on *M. persicae* at LD 16: 8 for 5–10 days after adult emergence were used for recording the search pathways. (*E*)-*β*-farnesene was synthesized by Dr. J. A. PICKETT of Rothamsted Experimental Station, U.K. (DAWSON et al., 1982) and provided by Dr. R. NISHIDA of the Pesticide Research Institute, Kyoto University, Japan. The percentage of (*E*)-*β*-farnesene in this product was 47% (DAWSON et al., 1982).

A lady beetle starved for 24 hr was placed beneath a 3.5-cm dia. inverted cup containing one of the following four stimuli; i) an apterous adult *M. persicae*, ii) an agar block coated with aphid (*M. persicae*) body fluid (NAKAMUTA, 1984), iii) a filter paper (1 cm × 1 cm) permeated with 200 ng (*E*)-*β*-farnesene and then dried, iv) a filter paper (1 cm × 1 cm) permeated with *n*-hexane, which was used as a solvent for (*E*)-*β*-farnesene, and then dried: control. Consumption of an agar block coated with aphid body fluid is known to elicit local search by lady beetles, but the search duration is shorter than those elicited by the consumption of an aphid (NAKAMUTA, 1985). (*E*)-*β*-farnesene and the agar block were compared to determine which elicits longer local searches.

The motions of lady beetles after exposure to stimulus were recorded in an experimental arena (30 cm dia.) on video tape for 3 min or until the beetle reached the edge of the experimental arena. This experiment was conducted in the laboratory from 10:00 to 15:00 when the beetles were normally active (NAKAMUTA, 1983). Standard conditions of 25 ± 1°C and light intensity of 2,900 lux were used for all assays.

Video records were analyzed, which the X-Y coordinate positions of the insect were recorded every 0.1 sec with a video position analyzer (VPA-1100, Nippon Jimu-Koki, Tokyo) and entered into a personal computer at 10 data points sec^{-1}. Coordinates were then aligned and scaled according to the recorded reference points. Computer programs were used to obtain locomotory rates, turn rates and the turns per unit distance ("meander"). Locomotory rate, or orthokinesis index, was calculated as mm $(0.1\ \text{sec})^{-1}$ and expressed as the distance (cm) moved during 1 sec. Turning rate, or klinokinesis index was calculated as degrees $(0.1\ \text{sec})^{-1}$ turned to left or right and expressed as the sum of the left and right turning components during 1 sec. Turning per unit distance, "meander", was calculated by dividing locomotory rate into turn rate, and expressed as degrees turned for each centimeter moved.

M. persicae was observed to disperse by falling when exposed to (*E*)-*β*-farnesene in the range of 5 to 1,000 ng (MONTGOMERY and NAULT, 1977). Accordingly, the amount of (*E*)-*β*-farnesene for assaying was determined to be 200 ng. Moreover, the pheromonal activity of 200 ng (*E*)-*β*-farnesene on the green peach aphid *M. persicae* was investigated through the following procedure. After the aphids on a cabbage leaf were counted (these numbers differed between experiments), a filter paper (20 mm dia.) permeated with 200 ng (*E*)-*β*-farnesene was held above the leaf. The number of aphids dropping or walking off the leaf within 1 min after this treatment was recorded and response (%) calculated by dividing the number of aphids leaf into the number of aphids dropping or walking off the leaf. The percentage of aphids dropping or walking off the leaf after being stimulated by either a filter paper permeated with solvent (*n*-hexane) and then dried or a crushed aphid were also recorded. Table 1 shows that the percentage of aphids dropping or walking off the leaf after the treatment with 200 ng (*E*)-β-farnesene and that with a crushed *M. persicae* are not different ($p>0.05$, MANN-WHITNEY *U* test), suggesting that the pheromonal activity of (*E*)-β-farnesene is similar to that of artificially released alarm pheromone from a crushed aphid cornicle.

RESULTS

Figure 1 shows the movements of lady beetles treated with different stimuli. After

Table 1. Comparison of pheromonal activity between synthetic (*E*)-β-farnesene and artificially released alarm pheromone from the green peach aphid, *Myzus persicae*

Stimulus used for the experiment	Aphids dispersed from a cabbage leaf by walking or falling (%)[1]	No. of aphid colonies
Filter paper permeated with 200 ng (*E*)-β-farnesene	32.2±8.1	5
Crushed apterous *Myzus persicae*	30.4±2.9	5
Solvent (*n*-hexane) on filter paper	0	6

[1] Values are represented with mean±S.D.

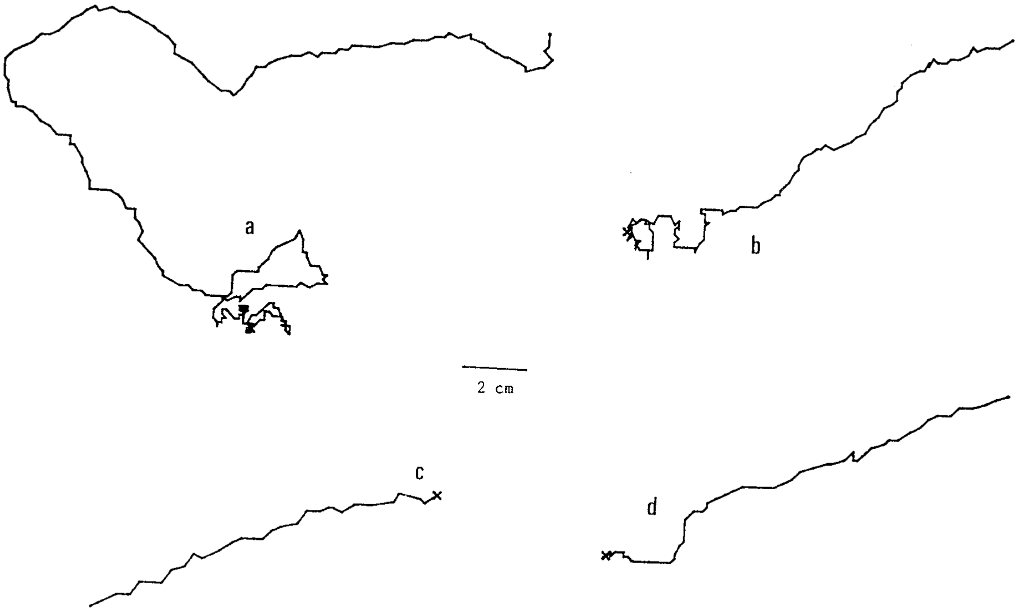

Fig. 1. Individual search pathways of *Coccinella septempunctata bruckii* until reaching an edge of an experimental arena. Pathways occurred after (a) consuming an adult apterous *Myzus persicae*, (b) consuming an agar block, (c) contacting filter paper permeated with 200 ng (*E*)-β-farnesene, (d) contacting filter paper permeated with solvent (*n*-hexane). × indicates the site where the lady beetle started searching after each stimulus presentation.

Table 2. Significance test of three search parameters[1)]—locomotory rate, turn rate and "meander"—among four stimuli exposed to a 24hr starved lady beetle

	Time (sec)					
	1	2	3	4	5	6
Locomotory rate (cm/sec)						
Consumption of an aphid	0.8±0.2 a (11)	1.2±0.2 a (11)	1.5±0.2 a (11)	1.5±0.3 a (11)	17.±0.3 a (11)	1.7±0.3 a (11)
Consumption of agar block	2.2±1.3 a (6)	2.8±1.7 a (6)	2.2±0.7 a (6)	2.7±0.7 ab(6)	1.9±0.8 ab(6)	3.1±1.3 a (6)
Contact with (*E*)-β-farnesene	2.7±0.4 b (9)	3.6±0.4 b (9)	3.8±0.3 b (8)	3.8±0.3 b (7)	3.3±0.2 b (3)	3.4±1.0 a (3)
Control	3.5±0.4 b (6)	4.1±0.2 b (6)	4.2±0.4 b (6)	4.2±0.4 b (4)	3.8±0.2 b (3)	
Turn rate (degrees/sec)						
Consumption of an aphid	58.1± 9.8 ab	71.9± 6.4 a	70.6± 8.2 a	75.1±13.6 a	70.6±10.9 a	56.2± 9.4 a
Consumption of agar block	86.0± 5.9 b	79.1±14.7 a	63.3±11.3 ab	85.1±11.9 a	87.0±13.4 a	67.3±15.4 a
Contact with (*E*)-β-farnesene	32.7± 4.9 a	28.5± 4.6 b	34.5± 9.1 b	40.9±10.7 a	43.2±22.7 a	47.6±17.7 a
Control	76.1±10.7 b	53.3±10.5 ab	60.7± 6.3 ab	55.9±12.0 a	68.6±22.2 a	
Meander (degrees/cm)						
Consumption of an aphid	24.3±5.0 ab	32.9±4.3 a	32.9±6.2 a	32.5±5.6 a	31.5±5.4 a	26.1±5.3 a
Consumption of agar block	29.8±6.2 b	28.8±5.6 ab	29.2±6.7 ab	32.7±7.7 ab	29.0±6.7 a	22.0±5.2 a
Contact with (*E*)-β-farnesene	11.8±3.2 a	9.1±1.8 c	10.0±2.8 c	11.7±3.2 b	11.1±5.9 a	17.5±5.2 a
Control	22.2±3.6 ab	16.1±3.4 bc	15.6±1.5 b	17.4±8.4 ab	22.3±5.0 a	

[1)] Means within columns of each parameter not followed by a common letter are significantly different at 5% level (MANN-WHITNEY *U* test). Values are represented with mean±standard error. Numerals in parentheses are the number of insects tested.

Table 3. Time spent before reaching an edge of the experimental arena by the lady beetle

Stimuli	Mean[1] ±S.D. (sec)	N
Consumption of an aphid (*Myzus persicae*)	17.6±10.8a	11
Consumption of an agar block coated with aphid body fluid	14.7±3.7a	6
Contact with (*E*)-*β*-farnesene	5.1±1.8b	9
Control	5.2±1.9b	6

[1] Values with different letters differ from each other at a 5% significance level (DUNCAN's multiple range test).

consumption of an aphid (a) or an agar block (b), the pathways became much more convoluted than those observed after contact with (*E*)-*β*-farnesene (c) or the control (d). The difference between search paths of (a) and (b) and those of (c) and (d) was very clear immediately after stimulus presentation.

Table 2 shows the significance test of locomotory rate, turn rate and meander among four stimuli exposed to a 24 hr starved lady beetle. Number of insects used in contact with (*E*)-*β*-farnesene or control decreased with time, since some individual beetles in these treatments reached edges of an experimental arena within a few sec.

Locomotory rate after contacting (*E*)-*β*-farnesene was significantly higher than that after consumption of prey for the first 5 sec (Table 2), or agar block for the first 3 sec, but not different from that after contact with filter paper (control). This suggests that the consumption of prey increases orthokinetic response of the lady beetle, while contact with (*E*)-*β*-farnesene does not.

The variability of turn rate after exposure to either stimuli was not as discernible as that of the locomotory rate. Mean turn rate after consumption of an aphid or agar block was not different from that after contacting the filter paper, suggesting that klinokinesis does not increase after consumption of prey. Although a significant difference was obtained between consumption of an aphid and contact with (*E*)-*β*-farnesene for the second and third sec and between consumption of an agar block and contact with (*E*)-*β*-farnesene for the first and second sec, it was not obtained between contact with (*E*)-*β*-farnesene and the control except within the first 1 sec. This suggests that contact with (*E*)-*β*-farnesene do not suppress the klinokinetic response more than the control.

"Meander" after contacting (*E*)-*β*-farnesene was significantly lower than that after consumption of prey for the first 4 sec except during the first 1 sec, but not different from that after contacting filter paper except during the 3rd sec (Table 2).

The time preceding the arrival of lady beetles to the edge of an experimental arena after contacting (*E*)-*β*-farnesene was significantly shorter than that after consumption of a prey, but not different from that of control (Table 3).

DISCUSSION

It would be advantageous to the coccinellids to move quickly away from aphid alarm pheromone-contaminated areas, since the alarm pheromone causes aphids to disperse from such areas. However, (*E*)-β-farnesene, a principal component of the aphid alarm pheromone, did not elicit the lady beetle to move faster or to turn much less than did the consumption of the aphid. At present it is unknown whether other components of the alarm pheromone may cause dispersal.

Since the pheromonal activity of 200 ng (*E*)-β-farnesene used in the present experiment was not different from that of naturally released alarm pheromone from the aphid cornicle released during attack by natural enemies (See Table 1), the fact that lady beetles do not initiate local search after contact with (*E*)-β-farnesene cannot be due to any difference of pheromonal activity of the synthetic and natural pheromones.

The time preceding the arrival of beetles to the edge of an experimental arena after contacting (*E*)-β-farnesene was not different from that of control (Table 3), suggesting that contact with (*E*)-β-farnesene does not constrain the beetle to search longer in the vicinity of previous prey capture site.

These results suggest that the lady beetles do not utilize (*E*)-β-farnesene as an eliciting or orientation cue of local search, and that the locomotory pattern of local search is probably generated idiothetically by an internal mechanism rather than allothetically by the perception of some change in an environmental cue (NAKAMUTA, 1985). Chemicals excreted from aphids other than (*E*)-β-farnesene, however, can cue local search by predators. For instance, CARTER and DIXON (1984) showed that the presence of honeydew produced by aphid increased the time spent to search for prey by larval *C. septempunctata* on plants. Adult *C. septempunctata* engaged in local search after the consumption of an aphid prey without honeydew or any other chemicals present (NAKAMUTA, 1982, 1985), suggesting that some cues other than honeydew are also utilized by lady beetles in local search.

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