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RESPONSE OF THE SEVEN-SPOT LADYBIRD TO AN APHID ALARM PHEROMONE AND AN ALARM PHEROMONE INHIBITOR IS MEDIATED BY PAIRED OLFACTORY CELLS

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Abstract—Electrophysiological responses of adult seven-spot ladybirds, *Coccinella septempunctata*, to (E)-β-farnesene, an aphid alarm pheromone, and (–)-β-caryophyllene, a plant-derived alarm pheromone inhibitor, were investigated by recording from single olfactory cells (neurons) on the antenna. Cells having high specificity for each of the two compounds were identified. Furthermore, these two cell types were frequently found in close proximity, with a larger amplitude consistently recorded for the cell responding specifically to (E)-β-farnesene. Preliminary behavioral studies in a two-way olfactometer showed that walking adults were significantly attracted to (E)-β-farnesene; this activity was inhibited with increasing proportions of (–)-β-caryophyllene. The possible ecological significance of colocation or pairing of olfactory cells for semiochemicals with different behavioral roles is discussed.

Key Words—Seven-spot ladybird, *Coccinella septempunctata*, Coleoptera, Coccinellidae, electrophysiology, single neuron recording, dose-response, behavior, olfactometer, aphid alarm pheromone, (E)-β-farnesene, (–)-β-caryophyllene.

INTRODUCTION

A wide range of insects, including ladybirds or ladybugs (Coleoptera: Coccinellidae) and parasitic wasps (Hymenoptera: Braconidae), are important beneficial

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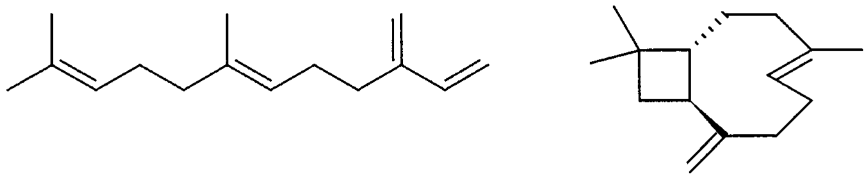

I II

FIG. 1. Structures of (E)-β-farnesene (I) and (−)-β-caryophyllene (II).

insects aiding the regulation of populations of aphid pests (Pickett et al., 1997; Obrycki and Kring, 1998; Wadhams et al., 1999). The chemical ecology of multitrophic interactions between plants, aphids, and parasitic wasps has been widely studied for exploitation of beneficial insects in biological control programs (Pickett et al., 1991; Takabayashi and Dicke, 1996; Bottrell and Barbosa, 1998). However, relatively little is known about the role of semiochemicals in interactions with aphidophagous ladybirds (Dicke et al., 1990). Studies on adult seven-spot ladybirds, *Coccinella septempunctata* L., have shown that olfactory and visual cues are used in prey location (Stubbs, 1980; Sengonca and Liu, 1994). One possible olfactory cue is the aphid alarm pheromone (E)-β-farnesene (Figure 1, **I**) (Nakatuma and Saito, 1985; Nakatuma, 1991), which is released by many aphid species, particularly in the subfamily Aphidinae, when attacked by natural enemies (Pickett et al., 1992; Hardie et al., 1999). (E)-β-Farnesene is also a ubiquitous plant volatile, but in this context the alarm response of aphids is inhibited by simultaneous detection of other plant chemicals, such as the biosynthetically related (−)-β-caryophyllene (Figure 1, **II**) (Dawson et al., 1984). Thus, for aphids, alarm response is stimulated by a rapid increase in the level of **I** relative to **II**.

The purpose of this study was to determine the nature of neurophysiological responses of adult *C. septempunctata* to the aphid alarm pheromone **I**. The presence of highly specific olfactory cells for this compound may relate to an important ecological role which could be exploited in aphid control (Pickett et al., 1998). Since the use of an aphid alarm pheromone as a kairomone in locating prey might be confounded by its cooccurrence in plants, the response of *C. septempunctata* to the aphid alarm pheromone inhibitor **II** was also investigated.

METHODS AND MATERIALS

Insects. *C. septempunctata* adults for electrophysiological studies were collected from the grounds surrounding IACR-Rothamsted, kept in ventilated sandwich boxes at 20°C, and provided with a diet of potato aphids, *Macrosiphum euphorbiae* (Thomas). For olfactometer studies, *C. septempunctata* adults were collected in Uppsala, Sweden, kept in cages in a glasshouse (18–22°C) and provided with bird-cherry-oat aphids, *Rhopalosiphum padi* L.

Chemicals. Solutions for electrophysiological studies (10^{-5}–10^{-9} g/10 μl) were prepared in purified hexane. Solutions (10^{-5} g/10 μl) for behavioral studies were prepared in purified diethyl ether, sealed in glass ampoules under nitrogen, and mailed to Sweden. (*E*)-β-Farnesene (**I**, 99% pure by gas chromatography) was synthesized from (*E,E*)-farnesyl chloride (Kang et al., 1987). (–)-β-Caryophyllene (**II**, 99%) was purchased from the Fluka Chemical Company, Gillingham, UK.

Electrophysiology. Single-cell recordings (SCR) from olfactory cells on the antennae of *C. septempunctata* adults were made with tungsten microelectrodes (Boeckh, 1962). The indifferent electrode was placed in the antennal scape, and the recording electrode was brought into contact with the surface of the antenna until impulses were recorded. Signals were amplified (UN-06, Syntech) and displayed on an oscilloscope, and permanent copies of the action potentials generated by the olfactory cells were obtained by standard methods (Wadhams et al., 1982). Impulse frequency was determined as the number of impulses elicited during the first 1 sec after stimulation.

Stimulus Delivery. The delivery system, which employed a filter paper strip in a disposable Pasteur pipet cartridge, has been described previously (Wadhams et al., 1982). The stimulus (2-sec duration) was delivered into a purified airstream (1 liter/min) flowing continuously over the preparation. Samples (10 μl) of the standard solutions of test compounds were applied to filter paper strips, and the solvent was allowed to evaporate (30 sec) before the paper strip was placed in the cartridge. Fresh cartridges were prepared prior to each stimulation. The solutions of **I** and **II** were presented once or twice to the preparation at intervals of 2–10 min, the exact interval being determined by the concentration of the previous stimulus.

Olfactometry. Behavioral assays with *C. septempunctata* adults were carried out in a two-way olfactometer as described previously (Al Abassi et al., 1998). For each experiment, one *C. septempunctata* adult was introduced into the center of the chamber and its position noted every 2 min for 20 min. The experiment was replicated 10 times. The results were analyzed by paired *t* test, the number of visits into the treatment arm being compared with the number of visits into the control arm. If the insect did not move between observations, the experiment was terminated and the data discarded. Stimuli were (*E*)-β-farnesene (**I**) and (–)-β-caryophyllene (**II**), either alone or in varying ratios: (A) **I** alone; (B) **I** : **II**, 3 : 1; (C) **I** : **II**, 1 : 1; (D) **I** : **II**, 1 : 3; (E) **II** alone. The control stimulus was diethyl ether (10 μl). All treatments were applied in 0.5-μl microcaps.

RESULTS AND DISCUSSION

Single-cell recordings from the antennae of *C. septempunctata* adults showed the presence of receptors specific for either (*E*)-β-farnesene (**I**) or (−)-β-caryophyllene (**II**). In this study, these two receptor types were by far the most abundant: of 36 cells responding to plant- or aphid-derived compounds, 21 were specific for **I** or **II**. In addition, they were frequently found in close proximity, with a large amplitude cell responding to **I** and a smaller amplitude cell responding to **II** (Figure 2). This pairing occurred on both male and female antennae and comprised 86% of the cells specific for the two compounds. Dose–response analysis of these two cell types showed similar sensitivities (Figure 3), and at the levels tested neither type showed any response to the compound for which it was not specific. To our knowledge, this is the first published report of paired olfactory cells in coccinellids.

In the olfactometer, adult *C. septempunctata* were significantly attracted to compound **I** ($P < 0.005$). This activity decreased with increasing amounts of **II**, i.e., there was significant attraction to 3 : 1 and 1 : 1 mixtures of **I** : **II**, but not to a 1 : 3 mixture nor to **II** alone (Table 1). Thus, the response of *C. septempunctata* to the aphid alarm pheromone **I** is inhibited by the presence of the plant volatile **II**, presumably as a mechanism by which the kairomonal effect is not induced merely by detection of ubiquitous **I** released by plants (Dawson et al., 1984). The ecological role of compounds **I** and **II** for *C. septempunctata* therefore appears to be analogous to that for aphids.

Perception of compounds **I** and **II** in aphids is mediated by specialized olfactory cells within separate organs (rhinaria) on the antenna (Campbell et

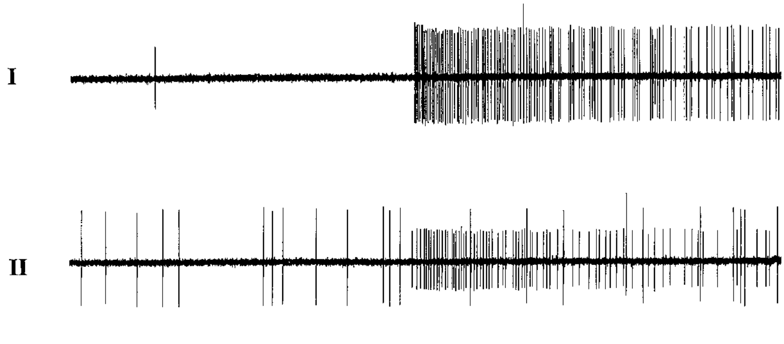

FIG. 2. Response of a pair of olfactory cells on the antenna of a female *Coccinella septempunctata* to compounds at 10^{-6} g/10 μl (bar = 2 sec stimulation). Upper trace: (*E*)-β-farnesene (**I**); lower trace, (−)-β-caryophyllene (**II**).

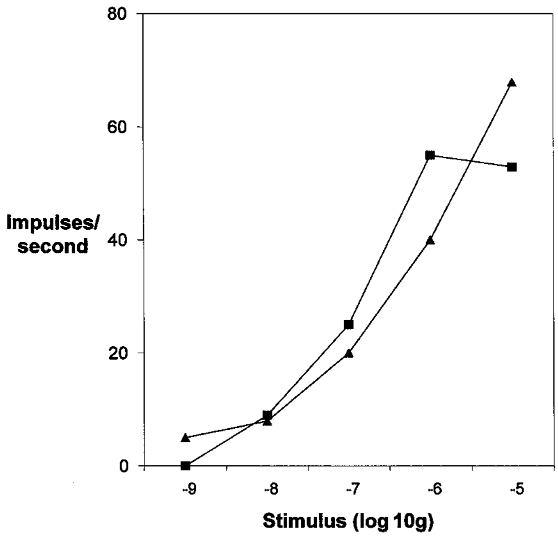

FIG. 3. Dose–response of a pair of olfactory cells on the antenna of a female *Coccinella septempunctata* to (*E*)-β-farnesene (■, large-amplitude cell) and (−)-β-caryophyllene (▲, small-amplitude cell). The three central points on each graph are the means of two applications.

al., 1993), as suggested previously (Nault et al., 1973; Shambaugh et al., 1978). In this study, specific olfactory cells for the two compounds were found to be colocated on the antenna of adult *C. septempunctata*. This pairing of olfactory cells may facilitate recognition of the ratios of compounds released, with a rapid release of **I** from an aphid being detected against a constant background for **II** from the plant. Similar pairings of olfactory cells have been found in other Coleoptera, for example, the cabbage seed weevil, *Ceutorhynchus assimilis* (Curculionidae) (Blight et al., 1995), but for this insect the significance of relative ratios of chemicals is associated with host-plant recognition. The role of cell pairing, and indeed the involvement of greater numbers of colocated olfactory cells as seen in the pea and bean weevil, *Sitona lineatus* (Curculionidae) (C. M. Woodcock, unpublished data), is being investigated in behavioral assays.

The foraging behavior of *C. septempunctata* adults involves extensive random searching followed by intensive (area-restricted) searching in response to encountering aphids or their honeydew (Carter and Dixon, 1984). Although it has been shown that the intensive searching behavior is not elicited by contact with the alarm pheromone **I** (Nakatuma, 1991), the results in this study strongly suggest that walking *C. septempunctata* adults utilize the compound as a volatile aphid cue. In a study describing electroantennogram responses of the

TABLE 1. RESPONSES OF ADULT SEVEN-SPOT LADYBIRDS, *Coccinella septempunctata* L., IN TWO-WAY OLFACTOMETER

Stimulus	Ratio	Observations[a] (*N*, mean ± SD) Treated arm	Control arm[b]	*P*
(*E*)-β-Farnesene		6.4 ± 2.0	3.0 ± 2.0	<0.005
(*E*)-β-Farnesene–(−)-β-caryophyllene	3 : 1	6.6 ± 2.5	3.5 ± 2.5	<0.005
	1 : 1	6.0 ± 1.8	4.0 ± 1.8	<0.05
	1 : 3	5.9 ± 2.0	4.1 ± 2.0	NS[c]
(−)-β-Caryophyllene		5.8 ± 3.5	4.2 ± 3.5	NS

[a] Cumulative counts over 20 minutes ($N = 10$).
[b] Control = solvent (diethyl ether).
[c] NS = not significantly different (paired *t* test).

12-spot ladybird, *Coleomegilla maculata*, it is noted that preliminary behavioral assays with two other ladybird species, *Hippodamia convergens* and *Harmonia axyridis*, also have shown attraction to (*E*)-β-farnesene (Zhu et al., 1999). Further investigations will evaluate the potential of employing stable formulations of the aphid alarm pheromone in the manipulation of *C. septempunctata* populations in the field and the response of other aphidophagous ladybird species, e.g. the two-spot ladybird, *Adalia bipunctata*, to aphid odor cues.

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