Olfactory Response of the Lady Beetle *Hippodamia convergens* (Coleoptera: Coccinellidae) to Prey Related Odors, Including a Scanning Electron Microscopy Study of the Antennal Sensilla

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ABSTRACT The olfactory ability of the convergent lady beetle, *Hippodamia convergens* Guérin-Méneville, was investigated using an 8-arm airflow olfactometer. Lady beetles tested were normal or had their antennae, antennal tips, or maxillary palps amputated. Normal beetles and those with their maxillary palps removed were highly attracted by the odor of radish leaves infested with green peach aphids, *Myzus persicae* (Sulzer). Beetles with their antennae or antennal tips removed were not attracted. In addition, the normal lady beetles were significantly attracted to clean radish leaves. These results indicate that *H. convergens* can perceive olfactory stimuli released by their prey and their prey's host plant (or a combination of the two) and that the beetles' olfactory receptors are located principally on the tips of their antennae. Also as part of this study, the antennal sensilla of male and female convergent lady beetles were examined using scanning electron microscopy. The sensilla were counted, the majority of which were located on the terminal segment, and 4 morphological classes were identified: chetiform, Böhm, basiconic, and trichoid. Chetiform sensilla were observed on all 11 antennal segments, while the Böhm sensilla were located only on the first two segments. The basiconic and trichoid sensilla were located exclusively on the terminal two segments. The most abundant sensilla on the terminal segment, trichoid sensilla, were suggested to function in olfaction.

KEY WORDS *Hippodamia convergens*, *Myzus persicae*, olfactometer, olfaction, scanning electron microscopy, sensilla

THE POSSIBILITY OF using lady beetles, or coccinellids, as biological control agents to suppress agricultural pests such as aphids, mites, or coccids has intrigued scientists for years. Researchers have therefore attempted to gain an understanding of the mechanisms of prey search and detection of these organisms to more effectively use them in pest management.

Before 1980, scientists generally accepted the view that coccinellids find their prey by coincidence or random encounter (Putman 1955, Banks 1957, Dixon 1959, Kehat 1968, Kesten 1969, Murdie 1971). It was believed that "neither optic nor olfactory orientation operates in prey searching behavior" (Hodek and Honek 1996) and that prey were not perceived until actual physical contact was made (Dixon 1959, Storch 1976, Ferran and Dixon 1993). This idea was typified by Murdie's (1971) inference, in modeling coccinellid search behavior, that lady beetles were no more than "blundering idiots."

Since 1980, researchers have investigated more seriously the entire mechanism by which coccinellids locate their prey, including the role of vision and olfaction in prey detection. Several of these authors have suggested that lady beetles do respond to visual (Allen et al. 1970, Stubbs 1980, Nakamuta 1984, Khalil et al. 1985, Obata 1986, Collett 1988, Heidari and Copland 1992, Hattingh and Samways 1995, Udayagiri et al. 1997) and olfactory (Bhatkar 1982, Obata 1986, Hattingh and Samways 1995) stimuli either directly or indirectly associated with their prey.

Of the post-1980 reports, olfactometer studies (Garcia and Ribeiro 1983, Liu and Şengonca 1994, Şengonca and Liu 1994) have provided some of the most convincing evidence that lady beetles perceive prey-related odors. Other post-1980 investigators have characterized the antennal, maxillary palp, and labial palp sensilla of a few coccinellid species using electron microscopy and concluded that many of the sensilla are likely olfactory in nature (Yan et al. 1982, 1987; Barbier et al. 1989; Jourdan et al. 1995).

The studies conducted during the past 20 yr have dramatically increased our knowledge of coccinellid sensory reception; however, even after years of intense research, "the question, whether coccinellids can find their prey by visual and olfactory cues, cannot yet be answered unambiguously" (Hodek and Honek 1996).

The convergent lady beetle, *Hippodamia convergens* Guérin-Méneville, is one of the most abundant and widely distributed native coccinellids in North America (Rankin and Rankin 1980, Gordon 1985). However, virtually no information exists on its prey finding mechanisms and abilities.

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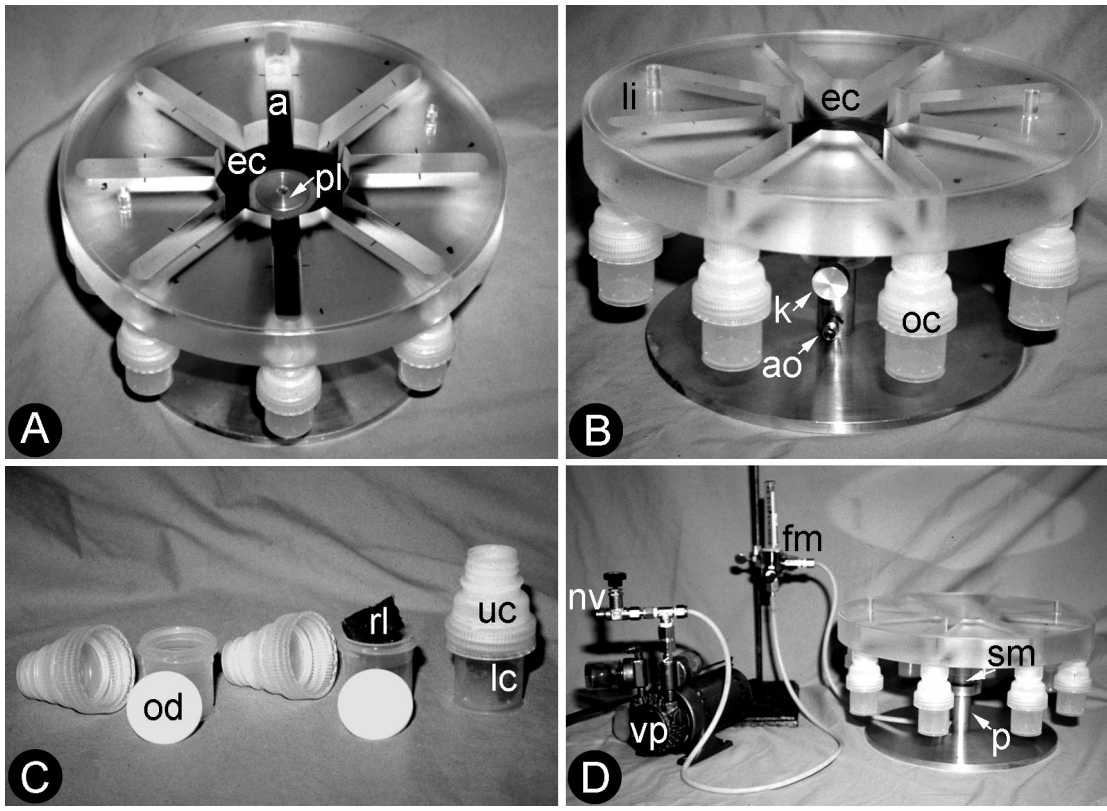

Fig. 1. Details of 8-arm airflow olfactometer. (A) Superior view (a, arm; ec, exposure chamber; pl, plate on piston). (B) Side view (ao, air outlet; k, knob on piston; li, lid; oc, odor chamber). (C) Odor chamber (lc, lower compartment; od, opaque disk; uc, upper compartment; rl, radish leaf). (D) Vacuum pump with olfactometer (fm, flow meter; nv, needle valve; p, piston; sm, sample chamber; vp, vacuum pump).

We conducted this study to investigate the olfactory ability of the convergent lady beetle, using an 8-arm airflow olfactometer, and to determine the general location of the beetles' olfactory receptors. An additional objective of the study was to describe the antennal sensilla using scanning electron microscopy (SEM).

Materials and Methods

***Hippodamia convergens* Storage and Handling.** Field-collected *H. convergens* lady beetles were purchased from A-1 Unique Insect Control (Citrus Heights, CA). They were maintained in a growth chamber with a 16-h photoperiod (lights on at 0600 hours and off at 2200 hours) and temperature set at 25°C (Nakamuta 1991); white fluorescent lights were used as the source of illumination. The lady beetles were kept in 473-ml (1-pint) unwaxed paper cups with 9-cm petri dish halves as lids. Holes poked in the sides of the cups permitted ventilation. Wood excelsior placed in the bottom of the cups provided a substrate for the beetles to crawl on.

Lady beetles were fed to satiation with green peach aphids, *Myzus persicae* (Sulzer), every morning. At feeding time, aphid-infested radish leaves were placed in the cups with the beetles. The aphids were reared on 'Scarlet White Tip' radishes (Charles H. Lilly, Portland, OR) growing in the laboratory under continuous illumination from wide spectrum fluorescent lights.

Olfactometer Design. An 8-arm airflow "population" olfactometer, based on the design of Liu and Şengonca (1994), was constructed (Fig. 1 A and B). The olfactometer consisted of a central exposure chamber (10.2 cm diameter, 2.7 cm deep) with 8 arms (2 cm wide, 2.7 cm deep, 10.1 cm long) extending radially every 45° from the exposure chamber (Fig. 1A). The arms and exposure chamber were milled into a clear, solid, acrylic disk (33.5 cm in diameter, 3.5 cm thick) and were covered by a clear acrylic lid (32 cm diameter, 1.0 cm (⅜ in) thick). The arms were sequentially numbered clockwise from 1 to 8. At the end of each arm, a 1.3-cm (½-in) hole exited down into an odor chamber that could contain an odor source (Fig. 1B). A sample chamber (4.0 cm diameter, 5.0 cm deep) extended perpendicularly from the center of the exposure chamber's floor (Fig. 1D). Within, and extending below the sample chamber was an aluminum piston (Fig. 1D) that could be driven upward so that it was level with the floor of the exposure chamber. Lady beetles were temporarily contained in the sample chamber until the beginning of each experi-

ment, at which time we slid the piston upward to release the beetles into the exposure chamber.

Piston Design. The piston was 2.5 cm (1 in) in diameter with a 4.0-cm plate (Fig. 1A) on top that slid snugly within the sample chamber. A removable aluminum knob could be screwed horizontally into the side of the piston either 3.3 cm or 8.3 cm below the top of the piston (Fig. 1B). When in place, the sample chamber rested on the shaft of the knob, preventing the chamber from sliding down the piston. In the 3.3-cm position, the piston plate sat in the bottom of the sample chamber, but in the 8.3-cm position (Fig. 1A) it was flush with the floor of the exposure chamber.

At the bottom of the sample chamber, an O-ring created an air tight seal between the piston and the sample chamber. The bottom of the piston screwed into an aluminum base plate (25.4 cm diameter, 0.8 cm (5/16 in) thick) that served as a stand. A 6.4-mm (1/4-in) hole passed through the center of the piston (Fig. 1A) and exited laterally (Fig. 1B) 12.5 cm below the top of the piston. Air was pumped out of the olfactometer through this hole using a vacuum pump (Fig. 1D). The top of the hole (Fig. 1A) was covered with wire mesh.

Odor Chamber Design. Each semiopaque odor chamber was made by cutting a Nalgene 60-ml high density polyethylene bottle (Part # 2002–0002, Nalge Company, Rochester, NY) in two, 3.5 cm below its rim, and gluing the cut edge of the upper half to the lid of a Nalgene 30-ml polypropylene bottle (Part # 2118–0001) (Fig. 1 B and C). A hole, 3.2 cm (1¼ in) in diameter, was cut through the lid. The surfaces to be glued were roughed with sandpaper and then glued using 5-min epoxy (Devcon, Danvers, MA). The lower half of the 60-ml bottle was discarded, and the 30-ml bottle was retained to be the lower half of the odor chamber. Eight 1.6-mm (1/16-in) holes were drilled every 45° around the sides of the 30-ml bottles, 2 mm above the bottom. The holes allowed air to be drawn into the odor chamber from the outside.

Eight opaque white acrylic disks (3.8 cm diameter, 0.3 cm (⅛ in) thick) were cut to sit on the rims of the 30-ml bottles, allowing the lids to screw down snugly on top of them (Fig. 1C). When in place the disks divided the odor chamber into 2 compartments; odor sources were placed in the lower compartment (Fig. 1C). Around the edge of each disk, 16 holes (each 0.3 mm [1/32 in] diameter) were drilled (every 22.5°) at a 30° angle radially inward. The holes in the disks allowed passage for air drawn through the chamber; however, they were small enough to prevent aphids placed in the lower compartment from crawling into the upper compartment and into the arms and exposure chamber. Because the holes were drilled at an angle, lady beetles could make no visual contact with the odor source.

Lids of the 60-ml bottles had 1.3-cm (½-in) holes drilled through them and were glued below the arms. The odor chambers were attached to the arms by screwing the 60-ml bottles into their lids.

Airflow and Vapor Tests. Air was drawn through the olfactometer by a General Electric (model 5KHM40NG1A, 1/15 hp) vacuum pump. Rubber tubing connected the pump to the olfactometer via an oxygen flow meter (Timeter Instrument, model TLO-8, St. Louis, MO) (Fig. 1D).

An airflow olfactometer is functional only if the plumes of air from the different arms remain separate from each other (Vet et al. 1983) in the periphery of the exposure chamber. Small irregularities in the olfactometer, obstructions, or even an improperly adjusted airflow can produce air turbulence (Rowlands 1985) in the exposure chamber and cause mixing of air from different odor chambers. This could potentially mislead the lady beetles and yield invalid results. By pumping water vapor through the system, the airflow in the olfactometer was visually observed and adjusted to eliminate mixing.

Water vapor was produced by placing chunks of dry ice (solid CO_2) in several beakers of warm water placed between the odor chambers all the way around the olfactometer. The vapor was contained in the vicinity of the odor chambers by enclosing the beakers and the entire lower portion of the olfactometer in a plastic bag. The opening of the bag was held in place, around the olfactometer, by a large rubber band that encircled the acrylic disk containing the exposure chamber and arms. Once the bag was in place, the pump was turned on and the vapor was drawn through the odor chambers and arms into the exposure chamber. The airflow was adjusted with an oxygen needle valve (Fig. 1D) to 4.8 liter/m, at which rate the air plumes appeared to be separate and distinct with virtually no mixing until the plumes converged in the center of the exposure chamber.

Olfactometer Experimental Protocol. Before each run, the olfactometer was disassembled and thoroughly washed with warm soapy water to remove any residues left by either the lady beetles or the odor sources. The apparatus was then dried using an unfiltered jet of compressed air. To create an airtight seal with the lid, Vaseline 100% Pure Petroleum Jelly (Cheeseborough-Pond's, Greenwich, CT) was applied to the top of the dry, reassembled olfactometer in a ring just beyond the distal ends of the arms and in streaks between the arms. To deter the beetles from climbing up onto the lid, a thin band of the jelly was applied to the upper portion of the walls of the exposure chamber and arms (Rowlands and Chapin 1978). It was also applied to the sides of the sample chamber to prevent the beetles from crawling into the exposure chamber before the beginning of the experiment. After petroleum jelly was applied, an odor source was placed in the lower compartment of one odor chamber (designated the active chamber), which was then attached to a randomly selected arm. This arm was referred to as the active arm. The seven empty chambers, designated nonactive chambers, were attached to the other arms, designated nonactive arms.

Experiments were conducted between 0930 and 1730 hours (the lady beetles being most active during this period) in another growth chamber, separate from where the lady beetles were maintained, with

Table 1. Specific treatments used in the olfactometer experiments, and the median ± SE number of entrances into the arms for each treatment

Treatment	Lady beetle status	Active chamber odor source[a]	Odor chamber type	Median ± SE
1	Normal	Nothing[b]	Active chamber	15.8 ± 2.4
2			Nonactive chambers	14.1 ± 1.7
3	Normal	Aphid-infested radish leaf	Active chamber	36.4 ± 5.7
4			Nonactive chambers	16.9 ± 2.1
5	Normal	Clean radish leaf	Active chamber	24.7 ± 4.0
6			Nonactive chambers	20.1 ± 2.6
7	Antennae removed	Aphid-infested radish leaf	Active chamber	10.9 ± 1.7
8			Nonactive chambers	11.1 ± 1.4
9	Maxillary palps removed	Aphid-infested radish leaf	Active chamber	31.6 ± 5.0
10			Nonactive chambers	16.9 ± 2.1
11	Antennae tips removed	Aphid-infested radish leaf	Active chamber	11.4 ± 1.8
12			Nonactive chambers	12.3 ± 1.6

Median = Antilogarithm of the mean of the log values.
[a] The specified odor source refers only to the active chamber; by definition, all of the nonactive chambers are empty.
[b] The designation "Nothing" refers to an empty active chamber, used as a control.

temperature set at 25°C. In this chamber, the olfactometer was always oriented in the same direction (e.g., with arm 1 pointing south). After the olfactometer was properly oriented, the vacuum pump was attached to the air outlet and turned on (Fig. 1D). Six active *H. convergens* lady beetles deprived of food for at least 24 h (but not >36 h) were then placed in the sample chamber (beetles were used only once and then discarded). After the lid was in place on the olfactometer, the piston was driven up to release the beetles in the center of the exposure chamber. The beetles could then move freely about the exposure chamber, sample air from the various arms, and enter any of them.

Every run lasted 45 min. During this time, the number of entrances into each arm was recorded. An entrance was defined as the arrival of a beetle at the opening to an odor chamber after traveling all the way from the exposure chamber. A second entrance into the same arm by the same beetle was recorded only if the beetle exited to the exposure chamber and then returned to the odor chamber entrance.

Treatments. Our olfactometer experiments consisted of 12 treatments (Table 1). The treatments were defined by a combination of lady beetle status, odor source, and odor chamber type. *Lady beetle status* referred to the state of the beetles—whether they were normal or had appendages amputated. Lady beetles without their antennae or maxillary palps removed were designated as *normal. Antennae-removed* lady beetles had both antennae amputated at the second or third segment. The beetles with antennal tips amputated had the 11th or the 10th and 11th segments excised from both antennae. Lady beetles with maxillary palps removed had each palp amputated at the second or third segment. Active chamber odor sources consisted of nothing (a control) or a clean or heavily aphid-infested small (≈15-cm^2) radish leaf placed in the active chamber. Clean radish leaves were defined as leaves that never had aphids on them. Chamber type referred to whether the odor chambers were active or nonactive; the nonactive chambers were always empty. Thus, for every run of the olfactometer, 2 treatments were simultaneously tested, because the beetles were exposed to air plumes from both the active chamber and the seven nonactive chambers. For each pair of these active and nonactive treatments, associated with a given lady beetle status and active chamber odor source, 16 runs were conducted. Contrasts involving two or more of the treatments (Table 2) were used to answer specific research questions.

To determine the location of the lady beetle olfactory receptors (i.e., whether on the antennae or maxillary palps), olfactometer runs were conducted with beetles that had their respective appendages excised. Aphid-infested and clean radish leaves were used in some of the treatments to determine whether the lady beetles were attracted to the odor of the combination of aphids and radish leaves or to the odor of radish leaves alone.

Entrance data for each treatment were collected from the 16 corresponding runs. Treatments 2, 4, and 6 were assumed to be essentially identical and were pooled together in some of the contrasts. This assumption was validated by a nonsignificant multiple degree of freedom contrast, which compared the median number of entrances into the nonactive arms among these treatments ($F = 2.21$; df = 2, 659; $P = 0.11$). When pooled together, entrance data were collected from a total of 48 runs.

Randomization. For every run conducted with each combination of lady beetle status and odor source, the active arm was randomly selected. Each arm was used twice as the active arm for every such combination. For the first two treatments, although no scent source was placed in any of the odor chambers, as a control one arm was still designated as the active arm in every run.

Antennae and Maxillary Palp Amputation Procedures. Lady beetles about to have their antennae or maxillary palps amputated were first anesthetized with carbon dioxide. Anesthetized beetles were picked up with a vacuum tweezer and mounted under a dissecting microscope, ventral side up. The antennae or maxillary palps were removed by grasping the tip of either with watchmaker forceps and cutting at the base with iri-

Table 2. Contrasts involving two or more of the treatments, used to answer specific research questions

Contrasts	Description	Estimated difference (log scale)	Asymptotic			95% CI for ratio of median number of entrances	
			Standard error	Z statistic	*P*-value	Lower limit	Upper limit
3 vs 2, 4, 6	Aphid main effect	0.7704	0.1380	5.58	0.0001	1.6486	2.8316
2, 4, 6 vs 1	Empty active vs nonactive arms	0.0679	0.1349	0.50	0.6152	0.8215	1.3942
3 vs 1	Aphid vs empty active arms	0.8382	0.2104	3.98	0.0001	1.5309	3.4924
5 vs 2, 4, 6	Radish vs nonactive arms	0.3856	0.1414	2.73	0.0066	1.1145	1.9403
3 vs 5	Aphid vs Radish	0.3847	0.2171	1.77	0.0768	0.9601	2.2483
3, 8 vs 2, 4, 6, 7	Antennae × aphid interaction	0.2678	0.0850	3.15	0.0017	1.1065	1.5440
3, 10 vs 2, 4, 6, 9	Palps × aphid interaction	0.0725	0.0874	0.83	0.4072	0.9059	1.2760
3, 12 vs 2, 4, 6, 11	Tips × aphid interaction	0.3124	0.0850	3.68	0.0003	1.1570	1.6145
7, 12 vs 8, 11	(Tips vs antennae) × aphid	0.0335	0.0758	0.44	0.6588	0.8913	1.1997
12 vs 11	Aphid effect for tip beetles	0.0835	0.1072	0.78	0.4364	0.8811	1.3412
8 vs 7	Aphid effect for antennae beetles	0.0165	0.1072	0.15	0.8776	0.8240	1.2544
9 vs 10	Aphid effect for palp beetles	0.6254	0.1072	5.83	0.0001	1.5149	2.3061

Aphid, an aphid-infested radish leaf in the active chamber. Radish, a clean radish leaf in the active chamber. Antennae, beetles with their antennae amputated. Palp, beetles with their maxillary palps amputated. Tips, beetles with their antennal tips amputated.
Median = Antilogarithm of the mean of the log values.

dectomy scissors. The tips of the antennae were also amputated with iridectomy scissors, but without the use of forceps. After the amputation procedures, the beetles were fed and returned to the growth chamber for at least 36 h before use.

Statistical Analysis of Olfactometer Data. A natural logarithmic transformation of the number of entrances into each arm was performed to equalize the variances. The entrance data were analyzed using a mixed model analysis of variance (ANOVA) (PROC MIXED, SAS Institute 1997) based on an unbalanced split plot design, with runs as whole units and odor chambers as subunits. Terms were included in the model for the various arms because of an observed preference of the beetles for certain arms regardless of the content of the odor chamber. Contrasts (Table 2) were used to determine the effect of the presence of aphid-infested leaves, clean radish leaves, absence of odor source, antennae amputation, antennal tip amputation, and maxillary palp amputation on the number of entrances into the active arm. Asymptotic *P* values and confidence intervals were calculated using the normal distribution. All confidence intervals were based on a 95% level of confidence.

Scanning Electron Microscopy. Male and female lady beetles were fed to satiation on green peach aphids until the female beetles oviposited. Eggs were removed to a 473-ml (1-pint) paper cup. Upon hatching, the larvae were fed green peach aphids until they pupated. Newly emerged adults were collected, frozen in liquid nitrogen, and freeze-dried.

Under a dissecting microscope, antennae of the freeze-dried beetles were carefully dissected from the heads. The scape (first segment) of each antenna was glued to the tip of an insect pin and placed in a mount that allowed the antenna to rotate 360° about its longitudinal axis. Mounted antennae were gold coated and examined with a SEM at a 20-kV accelerating potential. Digital images of every segment were obtained at 1,000× for segments 2–10, and at 1,500× for the scape and terminal (11th) segments. Each antenna was then rotated by nearly 90° and another set of images was obtained. This procedure continued until the antenna had been imaged from all sides. Prints of the images of the terminal segment for each different view were taped together into a montage. Using the prints, sensilla on each segment were numbered.

The sensilla of three male and three female antennae were counted. However, the first segment of all but two of the antennae were sufficiently damaged by the mounting process that accurate counts could not be obtained. Therefore, the sensilla on the first segment of four additional antennae were counted. Because of the unbalanced nature of the data set, a mixed model analysis of variance (ANOVA), which allowed heterogeneous variances among the segments, was used to obtain means and standard errors.

In addition to counting the sensilla, different morphological classes of sensilla were identified. High magnification images of these sensilla were obtained.

Results

Olfactometer Studies. The observation that the lady beetles preferred some arms over others, regardless of the contents of the odor chambers, was found to be statistically significant ($F = 3.53$; df = 7, 659; $P = 0.001$). Because this was taken into account in the analysis, the results are unaffected by these preferences.

Hippodamia convergens Response to Odor Source. The lady beetles showed no significant preference ($Z = 0.5, P = 0.62$) (SAS Institute 1997) for the empty active arm over the nonactive arms (see Table 1 for median number of entrances for each treatment; see Table 2 for specific contrasts and statistics). However, they were significantly more attracted to the active arm containing an aphid-infested radish leaf than to the nonactive arms ($Z = 5.58, P < 0.0001$) (SAS Institute 1997); the number of entrances into the active arm increased by 65–183% over the nonactive arms. The lady beetles also visited the active arm containing

a clean radish leaf 11–94% more frequently than the nonactive arms ($Z = 2.73$, $P = 0.007$) (SAS Institute 1997). Although the median number of visits to the active arm containing a clean radish leaf was less than the number of visits to the active arm containing an aphid-infested leaf (Table 1), the difference was not significant ($Z = 1.77$, $P = 0.08$) (SAS Institute 1997).

Amputation Experiments. The beetles' preference for or their ability to detect the aphid-infested radish leaves was significantly decreased when their antennae were removed as opposed to when they were not *(*$Z = 3.15$, $P = 0.002$) (SAS Institute 1997). Likewise, the beetles with their antennal tips amputated demonstrated a significantly decreased ability to detect the aphid-infested radish leaves as compared with the normal beetles ($Z = 3.68$, $P = 0.0003$) (SAS Institute 1997). With regard to their response to the odor of an aphid-infested radish leaf, the behavior of the beetles with their antennal tips removed was no different than the behavior of the antennae-removed beetles ($Z = 0.44$, $P = 0.66$) (SAS Institute 1997). However, the ability of the lady beetles, with their maxillary palps removed, to detect the aphid-infested radish leaves was not significantly different from the normal beetles ($Z = 0.83$; $P = 0.41$) (SAS Institute 1997). Similar to the normal beetles, the number of entrances into the active arm by the maxillary palp-removed beetles was 51–131% greater than the number of entrances into the nonactive arms.

Electron Microscopy: Antennal Sensilla. *H. convergens* antennae were composed of 11 segments, including a scape, a pedicel, and a 9-segment flagellum (Fig. 2 A and B). They were somewhat clavate and were ≈1.2 mm in length. Four major morphological types of sensilla were identified—trichoid, basiconic, chetiform, and Böhm—based on the classification scheme of Jourdan et al. (1995). Excluding Böhm sensilla, the average number of sensilla on the male antennae was 560, and female antennae averaged 571 (Table 3). The terminal (11th) segment of the male beetles had significantly more sensilla than the corresponding segment in females ($Z = 2.68$, $P = 0.01$) (SAS Institute 1997); the number of sensilla on all other segments did not differ significantly between the sexes (Table 3).

Most of the sensilla were located on the terminal segment (Fig. 2 A–C). This segment not only had the highest concentration, but also the greatest diversity of sensilla. Trichoid sensilla, the most abundant sensilla type on the terminal segment, were densely clustered on the distal end of this segment (Fig. 2C). A few of these sensilla were also located in a small cluster medially on the distal end of segment 10, but were found on no other segments. The trichoid sensilla were small, somewhat blunt needle-like projections. They had no articulatory socket and were smooth, tapering gradually to a point (Fig. 2D).

Also on the 10th and 11th segments, three subtypes of basiconic sensilla (Fig. 2C) were identified —types 1, 2, and 3—based on the classification scheme of Jourdan et al. (1995). All three subtypes were relatively short peg- or needle-like projections. On the terminal segment they were located among the trichoid sensilla in a relatively small region of the distal, ventral surface. A few were also intermixed in the small cluster of trichoid sensilla on the 10th segment. Type 1 were smooth and needle-like in appearance (Fig. 2E). Type 2 were broader than type 1 and had grooves at the distal end that converged at the tip (Fig. 2F). Type 3 were smooth and cone- or peg-shaped (Fig. 3A).

Chetiform sensilla were mostly long, somewhat needle-like projections (Figs. 2C, 3 B–D). They were divided into three subtypes—types 1, 2, and 3—based on the criteria of Jourdan et al. (1995). All three subtypes were set in articulatory sockets. Type 1 sensilla were abundant and were found on all segments (Fig. 3B) and were the only sensilla found on segments 3–9. They exhibited a large variation in length. Some of the type 1 sensilla, such as those on the pedicel, were the longest sensilla found on the antennae, whereas others were relatively short. These sensilla projected forward at an acute angle from the surface of the antennae. They were relatively slender with shallow longitudinal grooves running along the entire length of the sensilla. Type 2 chetiform sensilla (Figs. 2C, 3C) occurred only on the terminal segment and were not very abundant. They were the shortest of the three subtypes and were very slender. Type 3 sensilla (Fig. 3D) occurred mainly on segments 9, 10, and 11; however, they were occasionally found on other segments. They were stout, somewhat curved with deep, spiraling grooves running the entire length of the sensilla, and were not very abundant.

The Böhm sensilla were relatively small and were located only on the proximal ends of the scape and pedicel (Fig. 3E). They were smooth, needle-like projections, set in a socket. These sensilla were not counted because many were severely damaged, covered by debris, or otherwise obscured by the dissection and mounting process.

Discussion

Our study suggests that *H. convergens* lady beetles are attracted by the odor of aphid infested radish leaves. These results coincide with those of Liu and Şengonca (1994) and Şengonca and Liu (1994) who used *Coccinella septempunctata* L. lady beetles in a nearly identical olfactometer. Liu and Şengonca (1994) reported that nearly 10 times more beetles entered a single aphid-containing odor chamber than entered any of the seven empty odor chambers. Şengonca and Liu (1994) reported that significantly more beetles entered aphid-containing odor chambers than odor chambers containing either nonprey insects (*Epilachna varivestis* Mulsant) or nothing at all.

The experimental protocol of our study was somewhat different from that used by Şengonca and Liu (1994) and Liu and Şengonca (1994). These authors released 15–28 lady beetles into the exposure chamber and recorded the number of beetles that were in the odor chambers at the end of each run. Based on our preliminary observations, it was determined to record entrances into the arms rather than into the odor

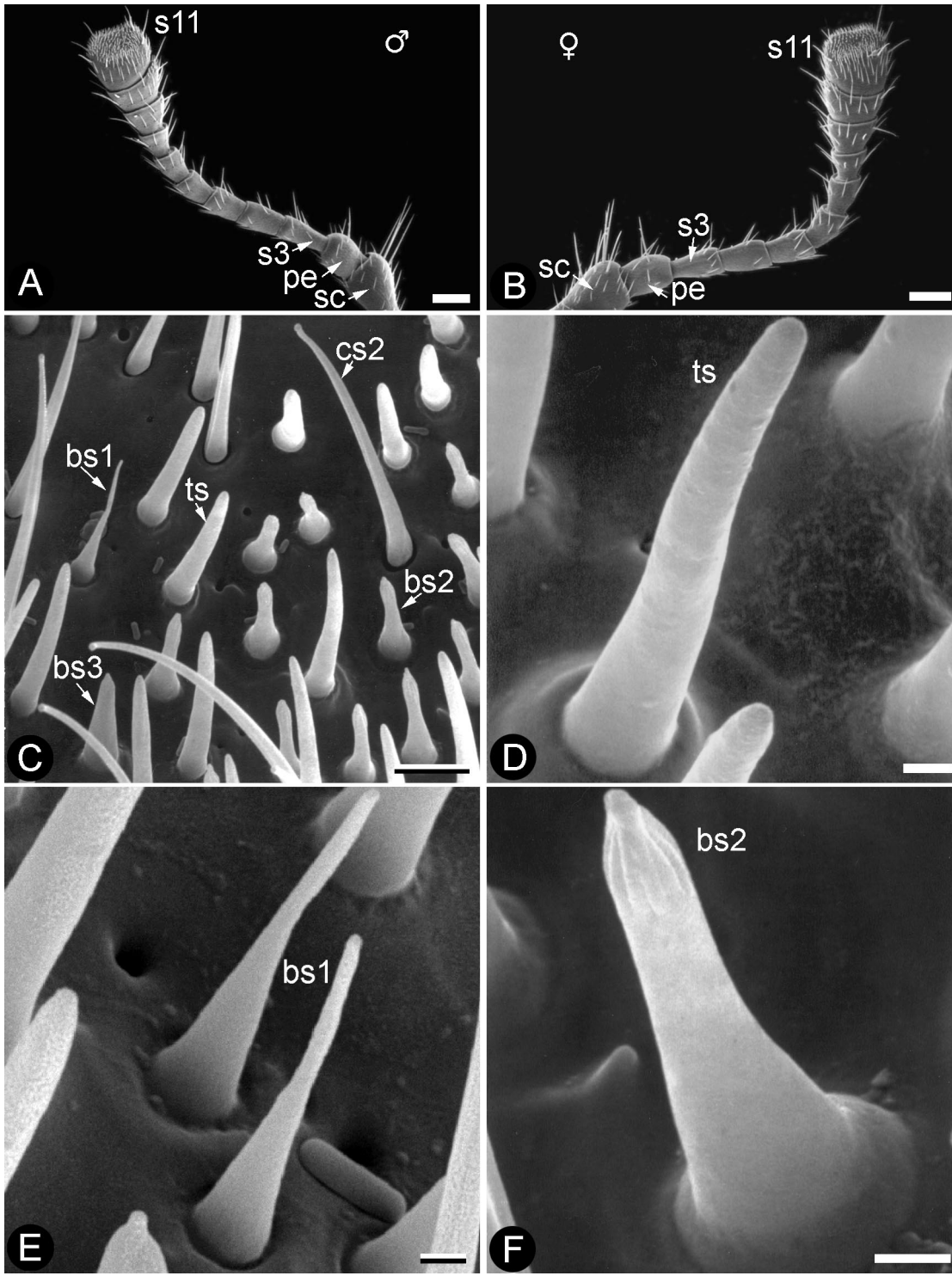

Fig. 2. Electron micrographs of *H. convergens* antennal sensilla. (A) Ventral side of a male antenna; bar = 100 μm (sc, scape; pe, pedicel; s3, third segment, s11, terminal segment). (B) Ventral side of a female antenna; bar = 100 μm. (C) Ventral tip of 11th segment; bar = 5 μm (ts, trichoid sensillum; bs1, type 1 basiconic sensillum; bs2, type 2 basiconic sensillum; bs3, type 3 basiconic sensillum; cs2, type 2 chetiform sensillum). (D) High magnification view of a trichoid sensillum; bar = 1 μm. (E) High magnification of type 1 basiconic sensillum (bs1); bar = 1 μm. (F) High magnification of type 2 basiconic sensillum (bs2); bar = 1 μm.

Table 3. Number of sensilla (mean ± SE) on male and female antennal segments

Segment	Male	Female
1	58.7 ± 1.9	60.4 ± 1.4
2	27.3 ± 1.9	28.0 ± 1.9
3	16.7 ± 1.6	18.3 ± 1.6
4	10.7 ± 1.2	10.7 ± 1.2
5	12.7 ± 0.9	14.3 ± 0.9
6	10.3 ± 1.7	12.7 ± 1.7
7	10.7 ± 0.7	10.0 ± 0.7
8	10.0 ± 0.6	9.0 ± 0.6
9	20.3 ± 1.1	18.7 ± 1.1
10	41.7 ± 3.2	42.3 ± 3.2
11	352.3 ± 4.4*	335.7 ± 4.4
Total per antenna	571.3 ± 6.8	560.0 ± 6.6

*, The number of sensilla in males is significantly greater than in females ($P < 0.05$). Means calculated from three male and three female antennae, respectively. Böhm sensilla excluded.

chambers, because frequently the beetles appeared to fall accidentally into the odor chambers (as opposed to purposefully crawling into them). They were also observed to crawl out of the odor chambers after entering into them. Entrances into the arms, however, appeared to be rarely if ever accidental occurrences. Because we observed entrances into the arms rather than counting the number of beetles in the odor chambers at the end of each run, we released only six beetles at a time into the olfactometer because of the difficulty of continually observing the active beetles.

The olfactometer built by Liu and Şengonca (1994) used 50-ml, noncompartmentalized bottles as the odor chambers. Dual compartment odor chambers were used in our study to eliminate any physical contact between the beetles and the odor sources. Physical contact could cause the beetles to alter their behavior (Rowlands and Chapin 1978, Nakamuta 1985a, b) and aggregate in the active arm (Nakamuta 1982) even if they had entered that arm by random chance alone. The use of opaque disks, in addition to preventing physical contact with the odor source, prevented the beetles from making visual contact with the odor source.

Other research using olfactometers has also reported olfactory responses in coccinellids. Colburn and Asquith (1970) observed in a simple 4-arm olfactometer that spider mite destroyers, *Stethorus punctum* (LeConte), were preferentially attracted to their prey, European red mites, *Panonychus ulmi* (Koch), on apple leaves over apple leaves alone. Garcia and Ribeiro (1983) made a similar observation for two other coccinellid species, *C. septempunctata* and *Adonia variegata* (Goetze), which exhibited significant preferences for aphid-infested leaves over clean leaves.

Similarly, Obata (1986) reported that *Harmonia axyridis* (Pallas) were significantly more attracted by the odors of aphid-infested leaves contained in opaque gauze bags than by the odors of clean leaves in gauze bags. Hedin and Phillips (1991) isolated various volatile chemicals from the honeysuckle aphid *Hyadaphis tataricae*, which they suggested might attract coccinellids from a distance. Ben Saad and Bishop (1976) reported that *H. convergens*, along with other lady beetle species, were attracted by the odor of an artificial honeydew applied to potato plants. The authors suggested that an olfactory response was involved in the attraction because the beetles were not permitted to make physical contact with the stimulus. The results of our study suggest that such a response might be expected and that it could be olfactory in nature, in agreement with Ben Saad and Bishop's (1976) assertion.

Although many of the studies mentioned provide strong evidence for olfaction in lady beetles, some relatively recent publications (along with many pre-1980 studies) still contend that coccinellids do not use olfaction in prey detection. For example, da Silva et al. (1992) concluded that adult *Curinus coeruleus* Mulant did not perceive their prey until physical contact took place, thus excluding any olfactory attraction. Similarly, Nakamuta (1984) stated that *C. septempunctata* did not perceive their prey by olfaction because they were unable to recognize their prey in the dark, except by contact. Nakamuta (1991) also showed that at least one aphid-associated scent, an aphid alarm pheromone, (*E*)-β-farnesene, had no effect on the behavior of the lady beetle *C. septempunctata.*

In addition to an attraction to the odor of aphid infested radish leaves, our results also indicate that *H. convergens* lady beetles are attracted by the odor of radish leaves alone. Similarly, Kesten (1969) found that the lady beetle *Anatis ocellata* L. was strongly attracted to aromatic substances released into the air by pine needles. In this way, the odor indirectly attracted the beetles to their primary prey, the pine aphid. Obata (1986) made a similar observation with the lady beetle *H. axyridis;* however, the author's data are somewhat unclear. In one experiment, Obata (1986) observed that *H. axyridis* showed no significant preference for gauze bags containing clean leaves as compared with empty bags, yet in another experiment a significant preference was observed. Our study reports on entomophagous coccinellids responding to odors associated with their prey's host plant.

Because our olfactometer results indicated that *H. convergens* could perceive olfactory stimuli, we wanted to identify the location of the olfactory receptors. When we amputated the maxillary palps, the beetles appeared to act no differently than the normal beetles. However, when their antennae were amputated, they appeared to loose their ability to perceive prey associated odors. We reasoned that the major olfactory receptors were likely on the terminal antennal segment because it had the highest number of sensilla of all the segments. Hence, we amputated the terminal segment and again found that the beetles lost their ability to perceive olfactory stimuli. From these experiments we suggest that the majority, if not all, of the olfactory receptors are located on the terminal antennal segments in *H. convergens.*

The SEM portion of our study revealed Böhm, trichoid, basiconic (with three subtypes), and chetiform (with 3 subtypes) sensilla on *H. convergens* antennae.

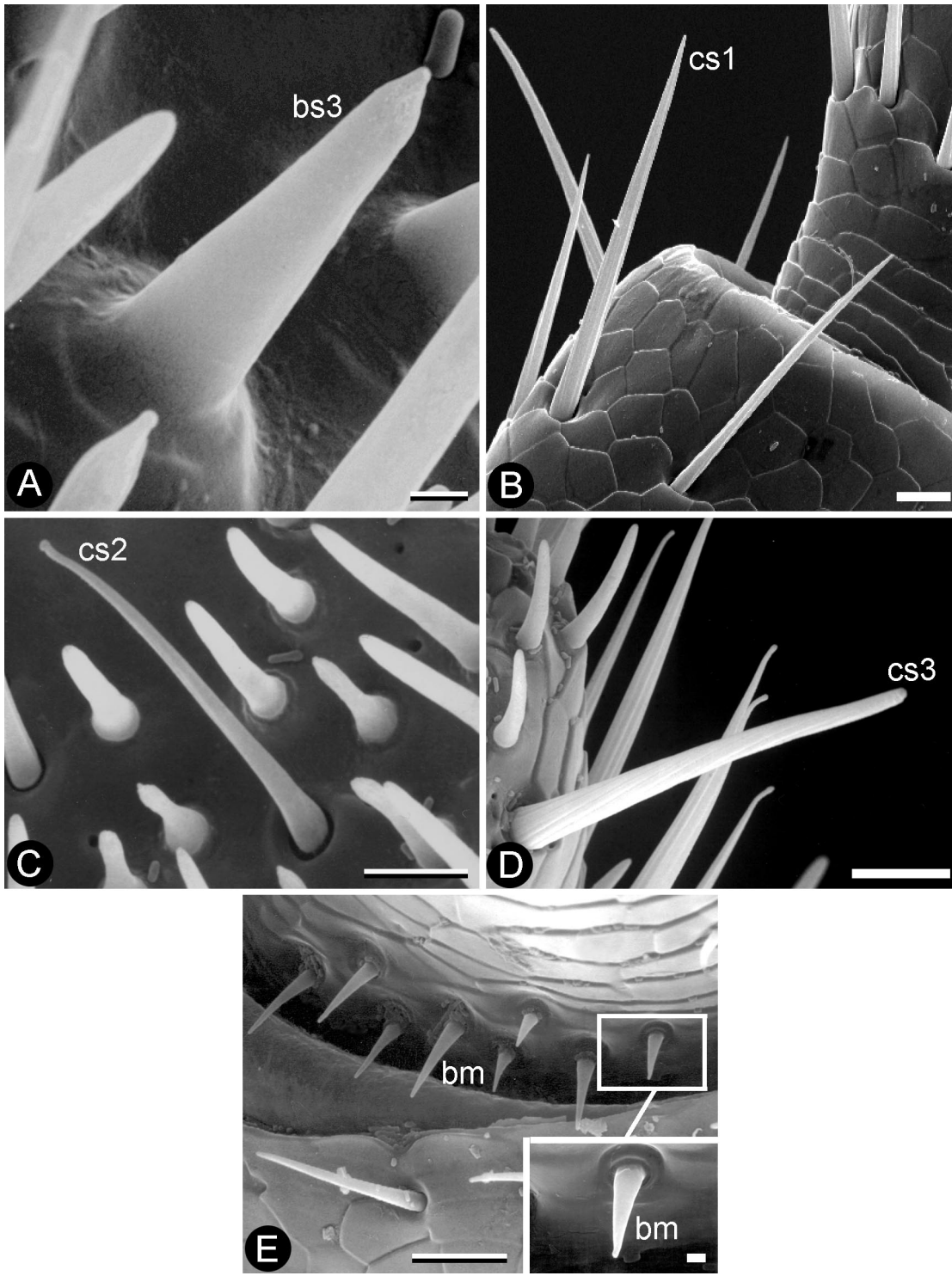

Fig. 3. Scanning electron micrographs of *H. convergens* antennal sensilla. (A) High magnification of type 3 basiconic sensillum (bs3); bar = 1 μm. (B) Chetiform sensillum, type 1 (cs1); bar = 10 μm. (C) Type 2 chetiform sensillum (cs2); bar = 5 μm. (D) Chetiform sensillum, type 3 (cs3) on 10th segment; bar = 10 μm. (E) Böhm sensilla (bm) located on the pedicel at the junction between the pedicel and scape; bar = 10 μm, insert bar = 1 μm.

Jourdan et al. (1995), using SEM and TEM identified Böhm, trichoid, coeloconic, basiconic (three subtypes), and chetiform (six subtypes) sensilla on the antennae of *Semiadalia undecimnotata* Schneid. All of the sensilla on *H. convergens* antennae appeared to be nearly identical to those described by Jourdan et al. (1995). However, Jourdan et al. (1995) observed three subtypes of chetiform sensilla that were unique to the sexes—these sensilla were not found on *H. convergens* antennae. The authors suggested that these sensilla, most of which were unique to the males, might function in sex pheromone detection. In *H. convergens*, such sexual dimorphism was not observed. However, the additional sensilla observed on the 11th segment of male antennae may enhance their ability to detect sex pheromones that could be released by the females. Although the existence of sex pheromones in coccinellids is not well established, Barbier et al. (1992) reported that at least one coccinellid species, *S. undecimnotata*, may release such pheromones.

Jourdan et al. (1995) suggested that the trichoid, type 2 chetiform, and types 1 and 2 basiconic sensilla located on the antennae of *S. undecimnotata* could be olfactory in nature. In addition, the authors inferred that the trichoid sensilla, located only on the terminal two antennal segments, seemed to be mainly responsible for long-range chemo- (or olfactory) reception. This is consistent with our results, as our beetles no longer responded to odors when their trichoid sensilla, which were located only on the terminal 2 segments, were removed. Jourdan et al. (1995) also speculated that the trichoid sensilla could even be involved in olfactory detection of plants.

In conclusion, the results of our study indicate that adult *H. convergens* lady beetles can perceive olfactory stimuli associated with their aphid prey and their prey's host plant. The beetles' olfactory receptors appear to be located principally on the terminal antennal segment and not on the maxillary palps. Trichoid sensilla, which are densely packed on the terminal segment, may perform the olfactory function.

The significance of olfaction and the role it plays in the entire mechanism used by lady beetles to successfully locate and consume their prey is still poorly understood. In addition, identification of the chemical compound(s) that elicit an olfactory response in coccinellids has received little attention. Clearly, additional research needs to be performed before the mechanisms of prey finding in coccinellids can be understood. A greater understanding of these mechanisms will surely lead to more effective use of coccinellids in integrated pest management.

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References Cited

Allen, D. C., F. B. Knight, and J. L. Foltz. 1970. Invertebrate predators of the jack-pine budworm, *Choristoneura pinus,* in Michigan. Ann. Entomol. Soc. Am. 63: 59–64.

Banks, C. J. 1957. The behaviour of individual coccinellid larvae on plants. Br. J. Anim. Behav. 5: 12–24.

Barbier, R., A. Ferran, J. Le Lannic, and A. Le Strat. 1989. Ultrastructure et fonction des organes sensoriels des palpes maxillaires de la coccinelle *Semiadalia undecimnotata* Schn. (Coleoptera: Coccinellidae). Bull. Soc. Zool. Fr. 114: 119–128.

Barbier R., A. Ferran, J. Le Lannic, and M.-R. Allo. 1992. Morphology and ultrastructure of integumentary glands of *Semiadalia undecimnotata* Schn. (Coleoptera: Coccinellidae). Int. J. Insect Morphol. Embryol. 21: 223–234.

Ben Saad, A. A., and G. W. Bishop. 1976. Attraction of insects to potato plants through use of artificial honeydews and aphid juice. Entomophaga 21: 49–57.

Bhatkar, A. P. 1982. Orientation and defense of ladybeetles (Coleoptera, Coccinellidae), following ant trail in search of aphids. Folia Entomol. Mex. 53: 75–85.

Colburn, R., and D. Asquith. 1970. A cage used to study the finding of a host by the ladybird beetle, *Stethorus punctum.* J. Econ. Entomol. 63: 1376–1377.

Collett, T. S. 1988. How ladybirds approach nearby stalks: a study of visual selectivity and attention. J. Comp. Physiol. A 163: 355–363.

da Silva, P. G., K. S. Hagen, and A. P. Gutierrez. 1992. Funct. response of *Curinus coeruleus* (Col.: Coccinellidae) to *Heteropsylla cubana* (Hom.: Psyllidae) on artificial and natural substrates. Entomophaga 37: 555–564.

Dixon, A.F.G. 1959. An experimental study of the searching behaviour of the predatory coccinellid beetle *Adalia decempunctata* (L.). J. Anim. Ecol. 28: 259–281.

Ferran, A., and A.F.G. Dixon. 1993. Foraging behaviour of ladybird larvae (Coleoptera: Coccinellidae). *In* R. J. Chambers, A.F.G. Dixon, I. Hodek, and J.-M. Rabasse [eds.], Behavioural ecology of aphidophagous insects. Eur. J. Entomol. 90: 383–402.

Garcia, V., and J. A. Ribeiro. 1983. A olfactometria como método de selecçao de Coccinelídeos afidífagos. Arquipel. Cienc. Nat. 4: 31–41.

Gordon, R. D. 1985. The Coccinellidae (Coleoptera) of America north of Mexico. J. N.Y. Entomol. Soc. 93: 1–912.

Hattingh, V., and M. J. Samways. 1995. Visual and olfactory location of biotypes, prey patches, and individual prey by the ladybeetle *Chilocorus nigritus.* Entomol. Exp. Appl. 75: 87–98.

Hedin, P. A., and V. A. Phillips. 1991. Volatile constituents from honeysuckle aphids, *Hyadaphis tataricae,* and the honeysuckle, *Lonicera* Spp.: search for assembling pheromones. J. Agric. Food Chem. 39: 1304–1306.

Heidari, M., and M.J.W. Copland. 1992. Host finding by *Cryptolaemus montrouzieri* (Col., Coccinellidae) a predator of mealybugs (Hom., Pseudococcidae). Entomophaga 37: 621–625.

Hodek, I., and A. Honek. 1996. Ecology of Coccinellidae. Kluwer Academic, Boston.

Jourdan, H., R. Barbier, J. Bernard, and A. Ferran. 1995. Antennal sensilla and sexual dimorphism of the adult ladybird beetle *Semiadalia undecimnotata* Schn. (Coleoptera: Coccinellidae). Int. J. Insect Morphol. Embryol. 24: 307–322.

Kehat, M. 1968. The feeding behaviour of *Pharoscymnus numidicus* (Coccinellidae), predator of the date palm scale *Parlatoria blanchardi.* Entomol. Exp. Appl. 11: 30–42.

Kesten, U. 1969. Zur Morphologie und Biologie von *Anatis ocellata* (L.) (Coleoptera, Coccinellidae). Z. Angew. Entomol. 63: 412–455.

Khalil, S. K., M. A. Shah, and U. K. Baloch. 1985. Optical orientation in predatory coccinellids. Pakistan J. Agric. Res. 6: 40–44.

Liu, B., and Ç. Şengonca. 1994. Development of 8-armed airflow olfactometers for measuring olfactory responses of insect predators. Anz. Schadlingsk. Pfl. Umweltsch. 67: 30–34.

Murdie, G. 1971. Simulation on the effects of predator/parasite models on prey/host spatial distribution, pp. 215–233. *In* G. P. Patil, E. C. Peilou and W. E. Waters [eds.], Statistical ecology 1. Pennsylvania State University Press, Harrisburg.

Nakamuta, K. 1982. Switchover in searching behavior of *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) caused by prey consumption. Appl. Entomol. Zool. 17: 501–506.

Nakamuta, K. 1984. Visual orientation of a ladybeetle, *Coccinella septempunctata* L., (Coleoptera: Coccinellidae), toward its prey. Appl. Entomol. Zool. 19: 82–86.

Nakamuta, K. 1985a. Area-concentrated search in adult *Coccinella septempunctata* L. (Coleoptera: Coccinellidae): releasing stimuli and decision of giving-up time. Jpn. J. Appl. Entomol. Zool. 29: 55–60.

Nakamuta, K. 1985b. Mechanism of the switchover from extensive to area-concentrated search behaviour of the ladybird beetle, *Coccinella septempunctata bruckii.* J. Insect Physiol. 31: 849–856.

Nakamuta, K. 1991. Aphid alarm pheromone component, (E)- 36 -farnesene, and local search by a predatory lady beetle, *Coccinella septempunctata* bruckii Mulsant (Coleoptera: Coccinellidae). Appl. Entomol. Zool. 26: 1–7.

Obata, S. 1986. Mechanisms of prey finding in the aphidophagous ladybird beetle, *Harmonia axyridis* (Coleoptera: Coccinellidae). Entomophaga 31: 303–311.

Putman, W. L. 1955. Bionomics of *Stethorus punctillum* Weise (Coleoptera: Coccinellidae) in Ontario. Can. Entomol. 87: 9–33.

Rankin, M. A., and S. Rankin. 1980. Some factors affecting presumed migratory flight activity of the convergent ladybeetle, *Hippodamia convergens* (Coccinellidae: Coleoptera). Biol. Bull. 158: 356–369.

Rowlands, M.L.J. 1985. A fusiform olfactometer chamber. Entomol. Mon. Mag. 121: 163–165.

Rowlands, M.L.J., and J. W. Chapin. 1978. Prey searching behavior in adults of *Hippodamia convergens* (Coleoptera: Coccinellidae). J. Ga. Entomol. Soc. 13: 309–315.

SAS Institute. 1997. SAS/STAT Software: changes and enhancements through release 6.12. SAS Institute, Cary, NC

Şengonca, Ç., and B. Liu. 1994. Responses of the different instar predator, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), to the kairomones produced by the prey and non-prey insects as well as the predator itself. Z. Pflkrankh. Pflschutz. 101: 173–177.

Storch, R. H. 1976. Prey detection by fourth stage *Coccinella transversoguttata* larvae (Coleoptera: Coccinellidae). Anim. Behav. 24: 690–693.

Stubbs, M. 1980. Another look at prey detection by coccinellids. Ecol. Entomol. 5: 179–182.

Udayagiri, S., C. E. Mason, and J. D. Pesek, Jr. 1997. Coleomegilla maculata, Coccinella septempunctata (Coleoptera: Coccinellidae), *Chrysoperla carnea* (Neuroptera: Chrysopidae), and *Macrocentrus grandii* (Hymenoptera: Braconidae) trapped on colored sticky traps in corn habitats. Environ. Entomol. 26: 983–988.

Vet, L.E.M., J. C. Van Lenteren, M. Heymans, and E. Meelis. 1983. An airflow olfactometer for measuring olfactory responses of hymenopterous parasitoids and other small insects. Physiol. Entomol. 8: 97–106.

Yan, F. S., J. Quin, and X. F. Xiang. 1982. The fine structure of the chemoreceptors on the labial palps of *Coccinella septempunctata.* Acta Entomol. Sin. 25: 135–140.

Yan, F. S., J. Quin, and X. F. Xiang. 1987. The chemoreceptors on the maxillary palps of the adult lady bird beetle *Coccinella septempunctata.* Acta Entomol. Sin. 30: 146–151.

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