

Orientation of *Hippodamia convergens* (Coleoptera: Coccinellidae) Larvae to Volatile Chemicals Associated with *Myzus nicotianae* (Homoptera: Aphidiidae)

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ABSTRACT Larval coccinellids are thought to exhibit random search behavior but recent work has suggested that closer analysis of search paths could reveal more olfaction-driven behavior than previously realized. To test this idea, the use of volatile chemical cues by larvae of the coccinellid *Hippodamia convergens* Guérin-Ménéville in searching for prey tobacco aphids, *Myzus nicotianae* Blackman, were studied. Larvae (second, third, and fourth instars) were exposed to three volatile sources; aphids alone, aphids on tobacco leaves and tobacco leaves previously exposed to aphids. Larvae were placed in an arena and their search path traced onto a grid then divided into seven different components relating approach angles and velocities at various time intervals. In general, older larvae were more efficient at searching than were younger ones. Older larvae had lower approach angles and higher approach velocities associated with treatments than did younger larvae. Different search behavior components responded differently to the various treatments but in most cases significant effects attributable to olfactory cues were obtained. The results support the potential use of olfactory cues by these larvae and suggest that a more extensive set of analyses and treatments should be conducted.

KEY WORDS *Hippodamia convergens*, *Myzus nicotianae*, behavior, prey searching, biological control, olfactory cues

ADULT LADY BEETLES use various chemical cues to locate their aphid prey. Nakamuta (1991) found that adult *Coccinella septempunctata bruckii* Mulsant oriented toward point sources of the aphid alarm pheromone, (E)- β -farnasene. Aphid honeydew is known to increase oviposition in some coccinellids (Carter and Dixon 1984). In a possibly related case, Evans and Dixon (1986) found that *Hippodamia convergens* Guérin-Ménéville females oviposited more in glass vials that had previously contained aphid prey than in clean vials. Obata (1986) found that *Harmonia axyridis* Pallas adults used chemical cues to locate sites where aphids were likely to exist and concluded that olfaction-directed search was more "advantageous" than random searching.

In contrast, searching behavior of coccinellid larvae has been thought to be nearly random (Kehat 1968), at least in artificial arenas (Banks 1957). On leaves, these larvae have been reported to follow leaf surface irregularities such as leaf veins or ribs (Banks 1957, Dixon 1959) until they physically encounter prey (Fleschner 1950) and they are nevertheless believed to be unable to detect prey before actual contact (Hodek 1973, Hunter 1978, Liu and Stansly 1999). However, Stubbs (1980) found that larvae in an arena with crushed prey would attach their anal organ to the

substrate and "cast about with the front part of the body," often contacting prey in the process. She also found that prey location time was significantly reduced compared with uncrushed prey further suggesting that chemical cues may be important in prey location. Her experiments involved very short-range prey detection, though, and she concluded that the ability to detect chemical cues from prey was limited to a mean distance of 0.7 cm.

Most of these studies have emphasized prey capture rates as an indicator of searching behavior rather than the behavior itself (Storch 1976). In a recent review, however, Dicke (1999) noted that this approach may underestimate the role of olfaction in searching behavior and that, as a result, the use of chemical cues in predator searching behavior may be more common than previously believed. He further noted that a better approach might be to emphasize the analysis of the search path itself rather than prey capture rates. Because such studies have not been performed on the larvae of many common coccinellid species such as *H. convergens*, we investigated the role of chemical cues in the searching behavior of this predator by analyzing various components of the search path. This article reports the results of those analyses for different age classes of *H. convergens* larvae exposed to three different odor sources.

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Materials and Methods

Tobacco aphid (*Myzus nicotianae* Blackman) colonies were established on greenhouse tobacco plants, *Nicotiana tabacum* (L.) ('KY-14'). Plants were irrigated as needed with fertilized water (0.02% 20:20:20, N:P:K) and provided with a high pressure sodium vapor light source (16:8 [L:D] h). The coccinellid population consisted of *Hippodamia convergens*, initially obtained from Gardens Alive! (Lawrenceburg, IN) and maintained on aphid-infested plants in a greenhouse. Coccinellid larvae used in experiments were obtained from egg masses; leaf tissue bearing egg masses were excised, placed in a petri dish with moist cotton and incubated 48 h under a photoperiod of 16:8 (L:D) h and 25°C. Neonate larvae were then isolated and provisioned with a leaf disk infested with >100 aphids at all times until they reached the desired development stage (second, third, or fourth instar, based on molt counts). Because neonate larvae perform little prey searching before their first molt, only second, third and fourth instars were evaluated.

The attractiveness to volatile sources was tested in a response chamber constructed from a clear plastic crisper (26 by 18.5 by 9.5 cm). A glass inlet tube (3 mm i.d.) was installed through each opposing end of the arena and connected via clear plastic tubing to a flowmeter (model 62A, Gilmont, Atlanta, GA) set to 0.2 liter/min at each tube. The charcoal-filtered air source was an aquarium fish pump. Treatments and controls were placed directly in front of the inlet tubes so that air entering the arena flowed over the source and toward the center of the arena. Finally, graph paper was photocopied onto a mylar transparency which was affixed to the arena bottom so that the larval search paths could be recorded directly onto the mylar.

Larvae were starved for 12 h, and were then confined under an inverted petri dish top at the center of the arena for 5 min to acclimate. The petri dish enclosing the acclimated larva was then removed. Thereafter, every 30 s for second instars and 15 s for later instars, the location of the larva was recorded on the transparency using a felt tip pen. Each trial lasted for a maximum of 5 min or until a larva touched the arena side, whichever occurred first. Each individual larva was tested twice, once with the treatment on each end of the arena. The average of these two trials represented a single replication.

The first odor source tested was 'aphids alone'; 100 adult aphids were placed into a cheesecloth bag while the control (on the opposite end of the arena) was an empty bag. Fifty replications were performed. The second odor source tested was 'tobacco leaves with aphids'; two leaf disks (1.5 cm diameter) were removed from the uppermost leaf of an aphid-infested plant and placed at one end of the arena. Fifty aphids were placed on each disk. The control consisted of two leaf disks taken from plants never infested with aphids. The third odor source tested was 'leaves previously exposed to aphids'. This treatment was the same as the previous one except that all aphids were removed from the leaf disks. The control consisted of leaves

from plants never exposed to aphids. Both of the latter two treatments were replicated 25 times.

A general measure of attraction was scored by dividing the arena into thirds. Any larva which crossed into the treatment section was recorded having oriented toward the treatment while any larva that crossed into the control section was recorded as not oriented toward the treatment. Larvae that remained in the middle section after 5 min were recorded as "indeterminate" and discarded. These responses were analyzed using a chi-square (chi-square test, Snedecor and Cochran 1989).

In addition to a simple scoring system, the search path of each larva was analyzed through six of its components measured at each time interval. These were as follows: (1) distance to treatment—the distance between a larva's position observed at each time interval and the treatment's fixed location (mm); (2) heading-treatment angle—the angle between a larval course heading and the direct course to the treatment (radians); (3) course heading change—the angular change in a larva's course heading between time intervals (radians); (4) accumulative length—the total distance traveled by each larva from the beginning of the trial through each time interval (mm); (5) larval velocity—the net distance traveled between time intervals divided by the time interval (mm/s); and (6) net deflection—the distance between the larval location and the arena mid-line (mm), positive values denoted a net deflection to the right while negative values denoted a net deflection to the left.

Data from each component were analyzed two ways, by larva and by time interval. Analyses by larva were conducted by calculating a mean value of each component for each larva (i.e., across time intervals) and degrees of freedom were based on the number of larvae tested. Analyses by time interval were conducted by calculating a mean value for each component at each time interval (i.e., across larvae) and degrees of freedom were based on the number of larvae remaining in each time interval. Each component was analyzed using an analysis of variance with Tukey's honest significant difference (HSD) test for mean separation (SAS Institute 1998).

Results and Discussion

More than half of the larvae migrated toward the treatment end of the arena in all treatments that involved tobacco leaves with tobacco aphids (Table 1). Second instars exhibited a net migration toward this treatment but not toward the others while fourth instars oriented toward all treatments. Third instars were intermediate, orienting only toward those treatments with aphids. However, with one exception (second versus fourth instars tested with aphids alone), there were no significant differences between the instars on any treatment, nor were there any significant differences within an instar between treatments (Table 1). In other words, although there were clear age-dependent trends, aphids and/or aphid products or effects resulted in a generally equal attraction of all

Table 1. Mean proportion of trials in which larvae crossed into the treatment section of the arena

Larval instar	Aphids alone	Tobacco leaves with tobacco aphids	Tobacco leaves without tobacco aphids
Second	0.55bA	0.67*aA	0.53aA
Third	0.68*abA	0.88*aA	0.56aA
Fourth	0.80*aA	0.90*aA	0.83*aA

Proportions followed by an asterisk indicate significantly more than half of the larvae crossed into the treatment section of the arena than crossed into the control section ($P < 0.05$, chi-square). Proportions followed by the same lower case letter in the same column are not significantly different, rows having the same upper case letter are not significantly different ($P < 0.05$, chi-square).

larval instars and these odor sources were equally attractive within a given instar.

Distance to treatment generally declined with increasing larval age in all treatments (Table 2). The distance from the center of the arena to the center of the treatment was 125 mm so that, by the end of the average trial, second instars had barely approached any of the treatments while later instars showed a somewhat greater tendency to approach the treatment. In general, the 'aphids alone' treatment was less attractive than either leaf treatment when results were averaged over time intervals, but no trends were found when results were averaged over individual larvae. Analysis by individual larva retained the last distance achieved of those larvae that contacted the side of the arena and were thereby terminated whereas analysis by time interval excluded these larvae from those time intervals subsequent to disqualification.

Although the fourth instars tended to approach the treatment more than other instars, they did so less directly. For all treatments and in both analysis methods, there was a clear tendency for the heading-treatment angle to be greater with older larvae (Table 3). Because the angles in Table 3 are in radians, any angle >1.57 ($\pi/2$) represents a mean angle $>90^\circ$ or a net trajectory away from the treatment. Collectively, Tables 2 and 3 suggest that younger larvae tended to

Table 2. Mean \pm SEM distance to treatment (mm) observed when *H. convergens* larvae were tested with three different odor sources

Larval instar	Tobacco aphid alone	Tobacco leaves with tobacco aphid	Tobacco leaves without tobacco aphid
Across time intervals			
Second	114.9 \pm 1.4aB	128.6 \pm 3.5aA	115.5 \pm 1.1aB
Third	118.0 \pm 2.2aA	103.3 \pm 0.8abB	105.1 \pm 9.2aB
Fourth	105.5 \pm 2.0bA	87.9 \pm 21.2bB	66.1 \pm 6.1bB
By individual larvae			
Second	116.1 \pm 1.2aB	121.4 \pm 2.1aA	111.9 \pm 1.7aA
Third	110.1 \pm 1.7abA	114.0 \pm 2.9abA	109.1 \pm 2.6aA
Fourth	104.7 \pm 1.2bA	94.2 \pm 3.3bA	88.5 \pm 4.1bB

For this table, distances were averaged two different ways, across time intervals and by larvae (see text). Means followed by the same lower case letter in the same column and analysis category are not significantly different, rows having the same upper case letter are not significantly different ($P < 0.05$; Tukey's HSD).

Table 3. Mean \pm SEM heading-treatment angle (radians) observed when *H. convergens* larvae were tested with three different odor sources

Larval instar	Tobacco aphid alone	Tobacco leaves with tobacco aphid	Tobacco leaves without tobacco aphid
Across time intervals			
Second	0.3 \pm 0.3bB	0.4 \pm 0.0bA	0.2 \pm 0.0bC
Third	0.5 \pm 0.1abA	0.5 \pm 0.1bA	0.3 \pm 0.1bB
Fourth	1.1 \pm 0.1aA	2.0 \pm 0.2aA	2.0 \pm 0.3aA
By individual larvae			
Second	0.4 \pm 0.0bB	0.6 \pm 0.11bA	0.2 \pm 0.4bB
Third	1.3 \pm 0.1aA	1.9 \pm 0.6aA	1.9 \pm 0.3aA
Fourth	1.1 \pm 0.1aB	2.0 \pm 0.2aA	2.0 \pm 0.3aA

For this table, angles were averaged two different ways, across time intervals and by larvae (see text). Means followed by the same lower case letter in the same column and analysis category are not significantly different, rows having the same upper case letter are not significantly different ($P < 0.05$; Tukey's HSD).

orient their body more toward the treatment (lower heading-treatment angle) but did not actually move toward it as much as fourth instars. Fourth instars, however, spent much time oriented away from the treatment (Table 3) without moving. When they did move, they tended to move more toward the treatment (Table 2).

The course heading change results suggest a relatively frequent turning rate (Table 4). All means, regardless of how they were calculated, were mostly near $\pi/2$ or 90° . There are several significant differences among the various comparisons in Table 4 but no clear pattern emerges. This lack of a pattern with respect to instar or treatment suggests that these larvae are not altering their course headings in direct response to either of these variables.

With respect to distance traveled, larval age interacted with treatment (Table 5). Younger larvae traveled further in the presence of treatments with aphids than in the presence of leaves without prey but, with fourth instars, the opposite was observed. Further, these different responses were fairly dramatic with

Table 4. Mean \pm SEM course heading change (radians) observed when *H. convergens* larvae were tested with three different odor sources

Larval instar	Tobacco aphid alone	Tobacco leaves with tobacco aphid	Tobacco leaves without tobacco aphid
Across time intervals			
Second	1.70 \pm 0.05abA	1.59 \pm 0.08aA	1.51 \pm 0.07bA
Third	1.32 \pm 0.13bB	1.69 \pm 0.11aA	1.85 \pm 0.09aA
Fourth	1.50 \pm 0.07abB	1.88 \pm 0.12aA	1.52 \pm 0.19bB
By individual larvae			
Second	1.63 \pm 0.04bA	1.42 \pm 0.04bB	1.52 \pm 0.05aAB
Third	2.71 \pm 0.11aA	1.70 \pm 0.09aB	1.71 \pm 0.21aB
Fourth	1.74 \pm 0.14bA	1.76 \pm 0.12aA	1.78 \pm 0.28aA

For this table, angles were averaged two different ways, across time intervals and by larvae (see text). Means followed by the same lower case letter in the same column and analysis category are not significantly different, rows having the same upper case letter are not significantly different ($P < 0.05$; Tukey's HSD).

Table 5. Mean \pm SEM accumulated length (mm) observed when *H. convergens* larvae were tested with three different odor sources

Larval instar	Tobacco aphid alone	Tobacco leaves with tobacco aphid	Tobacco leaves without tobacco aphid
Second	106.5 \pm 12.1bA	134.8 \pm 14.1bA	10.9 \pm 1.1bB
Third	154.8 \pm 8.5aA	177.8 \pm 10.6aA	21.2 \pm 5.3bB
Fourth	67.9 \pm 4.8cB	35.3 \pm 3.7cB	126.7 \pm 12.0aA

By definition, accumulated length occurred only between time intervals so these results were analyzed only across time intervals. Means followed by the same lower case letter in the same column and analysis category are not significantly different, rows having the same upper case letter are not significantly different ($P < 0.05$; Tukey's HSD).

second and third instars responding to tobacco leaves lacking aphids with only 10–15% of the searching distance observed for larvae responding to the two treatments with aphids. Tobacco leaves lacking aphids induced a two- to four-fold increase in search path length of fourth instars.

Searching velocity generally declined over time regardless of treatment (Fig. 1). After about a minute, larval search speed became roughly constant for the remainder of the trial. As one would expect, second instars were generally slower than older larvae and this difference was significant when tested with tobacco aphids alone ($F = 26.8$; $df = 1, 135$; $P < 0.05$), tobacco aphids on leaves ($F = 10.0$; $df = 1, 74$; $P < 0.05$) or tobacco leaves previously exposed to aphids ($F = 52.2$; $df = 1, 64$; $P < 0.05$).

Larvae in the presence of aphids alone had a much stronger tendency to bias their search to the left (Table 6). Large positive deflections observed for second instars exposed to tobacco leaves with aphids reflects the tendency of these larvae to leave the arena to the right. The relatively low deflections obtained with fourth instars on this same treatment reflects a nearly equal tendency to leave the arena on both sides.

Overall, the behavioral responses to these treatments were age-dependent. Second instars lifted their forelegs, prothorax and head when the air pumps started but this behavior occurred only in the presence of aphids. Kaddou (1960) also noticed such behavior during prey search by the larvae of *Hippodamia quinquesignata* (Kirby). In that case, second instars searched very slowly, had a low search path length per unit time and made numerous and frequent turns. In contrast, fourth instars exhibited fewer and shallower turns during search. In the work reported here, the shallower turns and higher velocity resulted in 50% of the fourth instars locating prey in the first 30 s of the trial compared with only 10% of the second and third instars.

Generally, tobacco leaves with aphids triggered more searching by second instars than did either aphids alone or leaves previously exposed to aphids. Thus, while these predators are known to orient toward aphid alarm pheromone (Nakamura 1991) as well as honeydew (Carter and Dixon 1984), the blend

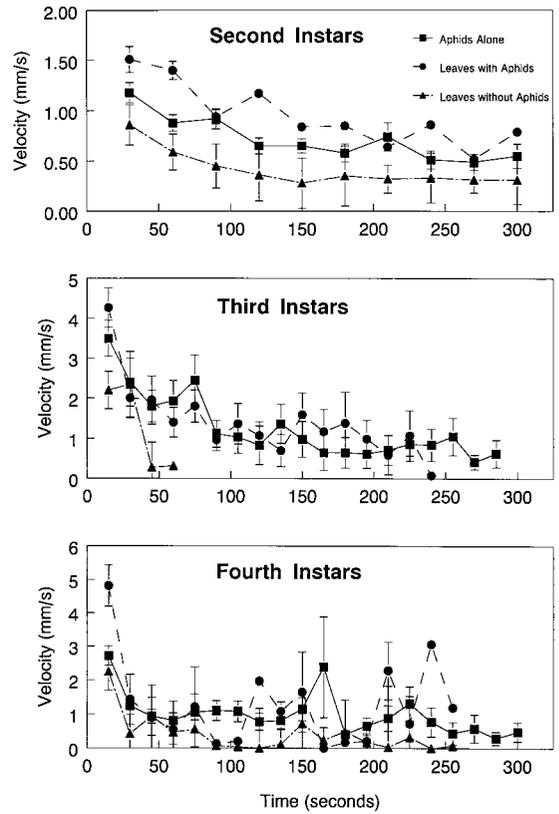


Fig. 1. Searching speed exhibited by larval *Hippodamia convergens* confined to an arena with air streams over aphids alone (in a gauze bag) versus gauze alone, aphids on tobacco leaves versus tobacco leaves with aphids removed, and leaves with aphids removed versus leaves never exposed to aphids. Three sizes of larvae were tested: second, third, and fourth instars (C).

of these along with other volatile components produces a stronger action than either alone.

These experiments strongly suggest that larvae of *H. convergens* alter their search path in response to olfactory cues that are prey-related. All instars were significantly attracted in a general sense (Table 1). When analyzed by individual larva, fourth instars had reduced inter-interval distances to leaves with live aphids or aphid residues showing a nonrandom bias either toward the treatment or away from the corresponding control (Table 2). The right versus left deflection of search paths was clearly influenced by treatment (Table 6). This deflection is probably not a methodological artifact because each larva was tested twice, once with the treatment at one end of the arena and again with the treatment at the other end. Had there been a methodological fault (e.g., odor flow, lighting, temperature gradient), no net effect would have been observed.

To be sure, the responses were subtle and deviation from randomness would not be detectable without examining different components of the searching behavior itself as suggested by Dicke (1999). However,

Table 6. Mean \pm SEM deflection from the arena centerline (mm) observed when *H. convergens* larvae were tested with three different odor sources

Larval instar	Tobacco aphid alone	Tobacco leaves with tobacco aphid	Tobacco leaves without tobacco aphid
Across time intervals			
Second	-9.4 \pm 2.0bB	48.9 \pm 13.3aA	-5.9 \pm 2.2aB
Third	-20.1 \pm 2.0cB	-15.2 \pm 2.7cB	9.8 \pm 2.8aA
Fourth	-3.6 \pm 1.7aA	1.0 \pm 1.0bA	11.8 \pm 20.3aA
By individual larvae			
Second	-9.0 \pm 4.1aA	80.5 \pm 22.1aB	-3.4 \pm 2.9aA
Third	-21.0 \pm 4.1bB	-7.7 \pm 6.9bA	7.4 \pm 5.3aA
Fourth	-4.7 \pm 2.9aA	0.6 \pm 5.9bB	-5.7 \pm 7.0aA

Negative numbers denote a deflection to the left while positive numbers denote a deflection to the right. For this table, deflection distances were averaged two different ways, across time intervals and by larvae (see text). Means followed by the same lower case letter in the same column and analysis category are not significantly different, rows having the same upper case letter are not significantly different ($P < 0.05$; Tukey's HSD).

the deviation from randomness as detected here suggests that a more sophisticated analysis complemented with a more extensive set of treatments is needed to better separate the various tritrophic components in this system. This work is important because, although it has little immediate practical application, it identifies a subject that must be more fully explored if we are to better understand the searching behavior of these economically important larvae.

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