Role of Visual and Olfactory Cues from Agricultural Hedgerows in the Orientation Behavior of Multicolored Asian Lady Beetle (Coleoptera: Coccinellidae)

Author(s): C. A. Bahlai, J. A. Welsman, E. C. Macleod, A. W. Schaafsma, R. H. Hallett, and M. K. Sears

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Role of Visual and Olfactory Cues from Agricultural Hedgerows in the Orientation Behavior of Multicolored Asian Lady Beetle (Coleoptera: Coccinellidae)

C. A. Bahlai,1 J. A. Welsman,1 E. C. MacLeod,1 A. W. Schaafsma,2 R. H. Hallett,1,3 and M. K. Sears1

ABSTRACT  Harmonia axyridis Pallas is an introduced lady beetle common in eastern North American agroecosystems. Two-choice behavioral bioassays were performed to determine whether visual and olfactory stimuli from prey and host habitats could elicit taxis in wild-collected H. axyridis adults and whether beetles exhibit a preference among stimuli. Soybean aphid (Aphis glycines Matsumura) spends much of the year in agricultural hedgerows residing on buckthorn (Rhamnus cathartica L.), and H. axyridis is frequently observed feeding on aphids in this habitat. Olfactory bioassays were performed in a Y-tube olfactometer and tested the response of beetles to the odor of buckthorn leaves, apple leaves (Malus domestica Borkh.), and buckthorn leaves both naturally and artificially infested with A. glycines. No differences were observed between the numbers of beetles moving toward the odor of buckthorn artificially infested with A. glycines and uninfested buckthorn, but more beetles preferred naturally infested buckthorn over uninfested buckthorn. Visual bioassays were performed in an acrylic tube arena, and tested beetle response to silhouettes and to apple and buckthorn leaves. Beetles were significantly more likely to choose silhouettes over blank space in visual trials. Significantly more beetles moved toward buckthorn leaves than blank space, but beetles did not discern between apple and buckthorn until olfactory cues were also included. This study lays the foundation for future work examining the response of H. axyridis to visual and olfactory cues in Ontario agroecosystems, which could help enhance effectiveness of H. axyridis as a biological control and mitigate its impacts as a pest species.

KEY WORDS  Harmonia axyridis, preference assay, soybean aphid, Aphis glycines, buckthorn
Because of the widespread use of lady beetles as biological control, the foraging behavior of many species of lady beetles has been studied, specifically with respect to searching behaviors involved with prey location (Dixon 1959. Nakamuta 1983, 1984a, b, Nakamuta and Saito 1985, Obata 1985, Hattingh and Samways 1995, Mondor and Warren 2000, Schaller and Nentwig 2000). Other natural enemies of aphids such as parasitic wasps rely on aphid host plant cues to locate their prey (Du et al. 1998), and recently, the ladybeetle Coccinella septempunctata L. has been shown to respond to plant volatiles induced by the feeding of soybean aphids (Zhu and Park 2005). Volatiles collected from the headspace surrounding aggregated females proved attractive to males of H. axyridis, and aphid alarm pheromones attracted both males and females (Verheggen et al. 2007). However, the role of volatiles has been studied less in predatory coccinellid–prey interactions than in parasitoid–host interactions (Nakamuta and Saito 1985). Foraging behaviors in coccinellids previously were thought to be based primarily on random-walk patterns (Hodek 1973), but a number of studies suggest the importance of tactile, gustatory, visual, and olfactory cues (each of which may be specific to a prey item, host plant, or habitat) in modulating nonrandom search patterns (Stubbs 1980, Nakamuta 1983, 1984a, b, Nakamuta and Saito 1985, Obata 1985, Hattingh and Samways 1995, Obata 1997, Schaller and Nentwig 2000). The random-walk pattern hypothesis for foraging in coccinellids assumes that prey detection only occurs with physical contact (Banks 1954, Kehat 1968). Under the random-walk foraging model, intensive and extensive random walk search enhances foraging success with aggregated prey. After encountering a prey item, a beetle will increase turning frequency and decrease walking speed to increase the likelihood of encountering a nearby prey item (i.e., intensive searching) (Hodek 1973). If no prey items are encountered after a period of intensive search, the extensive search pattern, marked by an increase in speed of movement and decreased turning, is resumed (Carter and Dixon 1982). Several studies have since reported the use of additional cues associated with prey and prey host plants in the enhancement of foraging success in coccinellids; adults or larvae move toward visual and olfactory cues associated with prey, prey hosts, feeding damage on prey hosts, and/or prey waste products (Stubbs 1980, Nakamuta 1983, 1984a, b, Nakamuta and Saito 1985, Obata 1985, Hattingh and Samways 1995, Obata 1997, Schaller and Nentwig 2000). Conditioning based on previous experience may also play an important role in the foraging of H. axyridis (Mondor and Warren 2000).

This study examines the roles of visual and olfactory cues from selected components of a hedgerow ecosystem in the foraging of H. axyridis. Specific objectives were to determine whether simulated odors and visual cues derived from R. cathartica, M. domestica Borkh. (domestic apple), and A. glycines could elicit taxis toward the source from adult H. axyridis, and if so, to determine whether H. axyridis exhibit preferences for certain of these stimuli. The results of this study should provide information regarding the cues used in foraging of H. axyridis, which may provide insight into the mechanisms behind the pest behavior of this species.

Materials and Methods

Experimental System. Response of H. axyridis to olfactory and visual cues originating from R. cathartica, M. domestica Borkh. (domestic apple), and A. glycines was tested using two-choice behavioral bio-assays in two different arena types. M. domestica was selected as the experimental control because of its prevalence in agricultural hedgerows, its superficial resemblance to R. cathartica in leaf shape, and its lack of host association with A. glycines in Ontario (OMAFRA 2006).

Insect and Plant Material. In spring and summer 2006, adult H. axyridis were collected from the wild in the vicinity of Ridgetown, Ontario, Canada (42.35° N, 81.84° W). Beetles were maintained in captivity in mesh cages for 24–72 h before experimentation at a photoperiod of 16 L:8 D and 21 ± 2°C and were provided with moistened paper toweling and sliced spoiled grapes ad libitum. A random allotment of beetles was removed from the cages, placed in plastic containers, and starved for 24 h before their use in experiments. Branch segments of M. domestica and R. cathartica were collected from agricultural hedgerows and ornamental plantations at Guelph, Ontario, Canada (43.59° N, 80.27° W). New plant material was collected before each repetition.

Specimens of A. glycines for artificial infestation of R. cathartica were obtained from a laboratory culture maintained at the University of Guelph Ridgetown Campus, Ridgetown, Ontario, Canada. Foliage of R. cathartica with natural infestation of A. glycines was obtained in autumn 2006 from several ornamental shrubs growing on the University of Guelph Campus, Guelph, Ontario, Canada. Identification of insect specimens used in experiments was accomplished by comparison to published keys (Voegtlin et al. 2004).

Olfactometry Assays. A glass Y-tube olfactometer (Fig. 1) was used to test adult H. axyridis preferences for olfactory cues derived from R. cathartica, M. domestica, and A. glycines in isolation from other relevant cues. The following treatment pairs were examined: (1) R. cathartica foliage versus blank air, (2) R. cathartica foliage versus M. domestica foliage, (3) R. cathartica foliage alone versus R. cathartica foliage artificially infested with A. glycines, and (4) R. cathartica foliage versus R. cathartica foliage naturally infested with A. glycines (leaves for both treatments were taken from the same plants). Four repetitions, each consisting of 10 trials with single beetles, were performed in randomized order for each of the first three treatment pairs using new plant material and new aphids for each repetition; these trials were performed in May and June 2006. The final treatment was performed in four sequential repetitions of 10 beetles, with new plant and aphid material for each repetition,
in October 2006, because naturally aphid-infested *R. cathartica* were unavailable earlier in the season.

Treatments consisted of 5 g of foliage from *R. cathartica* or *M. domestica* that had been removed from branches and placed loosely in the bottom of one of the two bell jars. Foliage was examined visually to exclude leaves with insects and insect damage and rinsed in distilled water and allowed to air dry before use. For leaves artificially infested with aphids, rinsed foliage of *R. cathartica* was infested with *A. glycines* by placing two to three laboratory-cultured aphids on each leaf in the 5-g foliage sample 30 min before use in experiments. Artificially infested foliage was used to examine the response of *H. axyridis* to foliage with aphids that had not had a chance to induce volatiles because of feeding damage or honeydew. Foliage of *R. cathartica* infested naturally with *A. glycines* was obtained by gathering leaves with two to three aphids present on each from shrubs with autumn aphid populations. Leaves with aphids were left unwashed to preserve any volatiles originating from honeydew or feeding damage.

All experiments took place in a windowless laboratory. Insect and plant material were housed in a glass bell jar and connected to the olfactometer by Teflon semiflexible tubing. All joints were sealed with Teflon tape. Components of the olfactometer system subsequent to the charcoal filter, including juncture seals, were manufactured from materials that emit no organic volatile compounds (e.g., glass and Teflon). Most components were obtained from Analytical Research Systems (Gainesville, FL).

The olfactometer was housed in a frame made of double-sided mesh to provide diffuse light. Light was provided by two banks of Sylvania 60-W fluorescent bulbs mounted at equal distances and heights above the olfactometer. Bell jars were placed outside of the mesh frame and behind black walls to remove the influence of visual stimuli on insect behavior. Air was passed through an activated charcoal filter and humidified to at least 70% RH by passing the air stream through a column of distilled water. Air stream temperature was maintained at 23 ± 1°C throughout the experiment. The relative humidity and temperature of air exiting the olfactometer was measured using a hygrometer (Mannix, New York, NY) before the beginning of each repetition. Air flow rate was regulated by flow meters (Key Instruments, Trevose, PA) and maintained at 1 liter/min entering each arm of the olfactometer; air flow was equalized before the beginning of each trial involving a new insect.

Treated air streams were assigned randomly to an arm of the Y-tube based on a coin toss at the beginning of each trial. Before introducing each insect to the arena, the Y-tube arena was washed with denatured ethanol and air-dried with compressed, purified air passed through a charcoal filter.

Individuals were placed in the arena through the entry port at the base of its main arm, and their movements were observed. Choice was recorded when the subject insect moved past a “choice line” located 5 cm past the junction of the two arms, toward one of the olfactory sources. Beetles not moving past the choice line within 10 min were recorded as nonresponders and excluded from analysis.

**Shape Preference Assays.** Vertical and horizontal silhouettes were used to examine beetle preference for visual stimuli in an I-tube arena. The following treatment pairs were examined: horizontal bars versus blank space, vertical bars versus blank space, and horizontal bars versus vertical bars. An open-ended clear acrylic tube 120 cm long by 12 cm diameter (Sterling Creations, Guelph, Ontario, Canada) was placed in a 50 by 50 by 130-cm white cotton tent in a darkened room (Fig. 2). Any air flow occurring in the arena was passive, and the tent functioned to minimize any out-
side air currents from natural air movement in the room. The white tent was suspended on a frame constructed of white 1-in PVC tubing and placed on a 70 by 140-cm table. Each end of the tent was illuminated from the outside with an identical 150-W incandescent spotlight attached to the table at the center of its width. Treatment pairs were placed at opposite ends of the tube, inside the tent, and attached to the frame of the enclosure. The horizontal bar treatment was made using five 5-cm-wide by 50-cm-long strips of black foam-core board affixed to the frame of the tent horizontally at 5-cm intervals. The vertical bar treatment was made using bars identical to the horizontal treatment, attached vertically to the tent frame, with the same white space present between bars. Before each repetition, the arena was rinsed with denatured ethanol and air dried. Before the introduction of each subject insect, a coin toss determined whether the tube was to be rotated 180° to randomize the end of the tube pointing at each treatment in the treatment pair. From June through September 2006, assays of six repetitions, each consisting of 10 individual adults of *H. axyridis* for each treatment pair, were performed in randomized order. Subject insects were placed individually in the I-tube arena by placing them on a plastic lid from a vial and raising them through a 1-cm-diameter hole drilled at the midpoint of its length in the bottom of the tube. Choice was recorded when a subject insect passed a “choice line” located 20 cm from the treatment stimulus. Beetles not crossing the choice line within 5 min were recorded as nonresponders and excluded from analysis.

**Statistical Analyses.** All analyses were conducted in SAS 9.1.3 (SAS Institute, Cary, NC) using PROC FREQ. Choice distributions for each treatment pair in all assays were subjected to a χ² goodness-of-fit analysis to determine whether the choice response deviated significantly from random (1:1 ratio of choice for each treatment in the pair). A significance level of α = 0.05 was used for all analyses, and only statistically significant differences are reported.

### Results

Several treatment-pair comparisons yielded significant, nonrandom results in each assay type (Table 1). From 65.0 to 98.3% of beetles made a choice depending on the treatment pair.

**Olfactometry Assays.** *Harmonia axyridis* moved toward the odor of *R. cathartica* leaves in absence of other stimuli (Table 1) but preferred the odor of *M. domestica* foliage over that of *R. cathartica* foliage. *H. axyridis* was not able to discern between odors of *R. cathartica* artificially infested with aphids and *R. cathartica* without aphids, but a strong preference was observed for the odor of *R. cathartica* foliage naturally infested with *A. glycines* over clean *R. cathartica* foliage.

**Shape Preference Assays.** *Harmonia axyridis* moved toward both vertical and horizontal bars in the absence of other visual stimuli (Table 1). Beetles did not discriminate between horizontal and vertical bars.

**Foliage Preference Assays.** Foliage of *R. cathartica* was more visually attractive to *H. axyridis* than the control blank space (Table 1). *H. axyridis* preferred *R. cathartica* foliage with olfactory stimuli than the visual stimuli alone. *H. axyridis* exhibited no preference be-

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**Fig. 2.** Schematic of I-tube arena used for assays examining the behavior of *H. axyridis* in response to olfactory and visual cues originating from a hedgerow ecosystem. The assembly consisted of a 120-cm-long by 12-cm-diameter, open-ended, clear acrylic tube (1) placed in a 50 by 50 by 130-cm white fabric tent (2) in a darkened room. Each end of the tent was illuminated with an identical spotlight (3). Treatment pairs (T1, T2) were placed at opposite ends of the tube. Adult beetles were placed individually in the arena through a 1-cm-diameter hole drilled at the center of its length (4).
tween visual stimuli of *R. cathartica* and *M. domestica* when olfactory stimuli were absent, but a strong preference for *R. cathartica* was observed when both visual and olfactory stimuli were present.

**Discussion**

Volatiles associated with undamaged *R. cathartica* foliage elicited a response in adult *H. axyridis*, but beetles were preferentially attracted to *R. cathartica* with actively feeding natural populations of *A. glycines*. No such preference was observed for *R. cathartica* foliage infested artificially with *A. glycines*, suggesting that volatiles associated with prey feeding damage on a host and prey waste products may play a more important role in prey-finding of *H. axyridis* than volatiles associated with prey items alone. This treatment likely did not provide sufficient time for aphids to produce a significant amount of honeydew or other olfactory cues associated with feeding damage. Olfactory cues from prey items or their byproducts (such as honeydew) assure a beetle of the presence (or recent presence) of prey, yet a number of previous studies involving direct aphid scent cues alone do not support this finding (Nakamura 1991, Schaller and Nentwig 2000).

When damaged by herbivores, plants may release volatile semiochemicals that are used by natural enemies seeking herbivorous prey (Schaller and Nentwig 2000). This chemical signaling by the plant may provide an adaptive advantage to both the plant and natural enemy of an herbivore attacking the plant by shortening search times required to find and kill pest insects (Hattingh and Samways 1995). Volatiles associated with *R. cathartica* were able to elicit responses in *H. axyridis* even in the absence of prey items in our study. Olfactory cues from plants undamaged by herbivores could be employed in foraging for a number of reasons. Diets of many predaceous coccinellids consist mainly of aphids and other soft bodied arthropods, but often include small amounts of pollen and other plant tissues (Schaller and Nentwig 2000). Plant olfactory cues could thus play an important role in coccinellid foraging behavior because, in addition to seeking prey item habitats and hosts, the lady beetle may also be seeking out plant food, oviposition sites, or mates (Schaller and Nentwig 2000). Olfactory cues from an herbivore host plant may play a role in sustaining a natural predator population to keep herbivorous insect populations in check (Dicke and Sabelis 1988).

Visual cues play important roles in host-finding, but studies are conflicting as to the scale at which they act and as to the visual acuity of lady beetles. This study suggests that adult *H. axyridis* rely on long range visual cues to locate host habitats: *H. axyridis* will move toward both silhouetted shapes and leaves of prey-host plants, even when scent cues are eliminated. Similarly, Hattingh and Samways (1995) found that *C. nigrithus* adults were attracted to simulated horizons and were attracted preferentially to horizons that simulated treelines over flat horizons.

When presented in isolation in the Y-tube assays, *H. axyridis* preferred the odor of *M. domestica* foliage over that of *R. cathartica* foliage, but in the I-tube assays, when presented with both types of foliage with odor, a strong preference for *R. cathartica* was observed. This apparent contradiction may have been caused by a number of factors. It is possible that beetles behave differently in the presence of visual stimuli, but it is more likely that other factors could have affected this result. Assays performed in the Y-tube arena were conducted in May and June 2006 using leaves collected from local populations of *M. domestica* and *R. cathartica*, whereas the I-tube assays involving foliage were conducted in July and August 2006 using leaves from plants in the same area. It is thus possible that leaf age plays an important role in its

### Table 1. Observed preferences of wild-collected adult *H. axyridis* for various stimuli derived from a hedgerow ecosystem in two different arena types

<table>
<thead>
<tr>
<th>Arena type</th>
<th>Comparison</th>
<th>N</th>
<th>% Response</th>
<th>% Choice</th>
<th>χ²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td></td>
<td></td>
<td>T1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Olfactometry assays</td>
<td>Y tube <em>R. cathartica</em> odor</td>
<td>30</td>
<td>75.0</td>
<td>70.0</td>
<td>4.5</td>
<td>0.029</td>
</tr>
<tr>
<td></td>
<td>Y tube <em>R. cathartica</em> odor</td>
<td>31</td>
<td>77.5</td>
<td>32.3</td>
<td>67.7</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>Y tube <em>R. cathartica</em> odor</td>
<td>32</td>
<td>80.0</td>
<td>53.1</td>
<td>46.9</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Y tube <em>R. cathartica</em> odor</td>
<td>35</td>
<td>95.0</td>
<td>31.6</td>
<td>68.4</td>
<td>5.2</td>
</tr>
<tr>
<td>Shape preference assays</td>
<td>I tube horizontal bars</td>
<td>39</td>
<td>65.0</td>
<td>66.7</td>
<td>33.3</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>I tube vertical bars</td>
<td>43</td>
<td>71.7</td>
<td>65.1</td>
<td>34.9</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>I tube horizontal bars</td>
<td>55</td>
<td>91.7</td>
<td>43.6</td>
<td>56.4</td>
<td>0.89</td>
</tr>
<tr>
<td>Foliage preference assays</td>
<td>I tube <em>R. cathartica</em> without odor</td>
<td>46</td>
<td>76.7</td>
<td>69.6</td>
<td>30.4</td>
<td>7.0</td>
</tr>
<tr>
<td></td>
<td>I tube <em>R. cathartica</em> without odor</td>
<td>43</td>
<td>71.7</td>
<td>32.6</td>
<td>67.4</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td>I tube <em>R. cathartica</em> without odor</td>
<td>54</td>
<td>90.0</td>
<td>53.7</td>
<td>46.3</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>I tube <em>R. cathartica</em> without odor</td>
<td>59</td>
<td>98.3</td>
<td>71.2</td>
<td>28.8</td>
<td>10.6</td>
</tr>
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</table>

A Y-tube olfactometer was used to test adult preference for olfactory cues derived from *R. cathartica* and *A. glycines*. An I-tube flight chamber was used to test adult preference for silhouetted shapes and to plant material with and without odor stimuli. Choice distributions were subjected to χ² goodness-of-fit analysis to determine whether the response was random at α = 0.05. Number of *H. axyridis* adults, percent choosing each treatment, Yates-corrected χ² statistics, and P values are given.

*a* Significant, nonrandom responses.
attractiveness to *H. axyridis*, and beetles prefer younger *M. domestica* leaves but older *R. cathartica* leaves. Future work should specifically examine the effect of leaf age on attractiveness to *H. axyridis*.

Obata (1997) asserted that *H. axyridis* uses both olfactory and visual cues to detect prey “from a distance”; however, different cues for foraging may vary in importance at different scales. At a more distant spatial scale, visual cues seem to be most important in finding suitable prey habitats, as was observed in this study with *H. axyridis*, and for *C. nigrinus* preferentially moving toward simulated treelines (Hattingh and Samways 1995). Within a prey habitat, cues associated with finding prey host plants are largely visual (Hattingh and Samways 1995), but some plants are known to be attractive to *C. septempunctata* (Schaller and Nentwig 2000).

*Harmonia axyridis* has status as a pest in Ontario. In early autumn, *H. axyridis* has been observed to aggregate on grape clusters as they ripen, and these beetles may be harvested with ripened grapes (Ker and Carter 2004). When processed with the grapes, chemicals produced by *H. axyridis* can adversely affect the quality of wines and juices (Pickering et al. 2004). Ecological mechanisms responsible for aggregation of *H. axyridis* on grapes in autumn are unknown, and explanations proposed to date are speculative: no large populations of aphids are known to occur in Ontario vineyards in late autumn (N. Carter, personal communication). Aggregations of *H. axyridis* in vineyards are often associated with damage or overripening of the fruit (Ker and Carter 2004, Pervez and Omkar 2006).

A better understanding of cues used by *H. axyridis* in foraging may explain why beetles aggregate on grape vines. We observed *H. axyridis* in agricultural hedgerows in autumn, foraging on *R. cathartica* and feeding on *A. glycines*. Beetles are more numerous in hedgerows in years when very few beetles are present in vineyards (Bahlaio 2007). When aphids are not numerous in hedgerows, beetles may use similar cues to find their way to vineyards. Fermentation products associated with ripe or damaged grapes may be similar to fermentation products of honeydew and thus be attractive to *H. axyridis*. Future work should examine the response of *H. axyridis* to specific volatiles associated with aphids and grape varieties, as well as chemical analyses of these volatiles. Studies should also incorporate satiated and starved beetles to compare responses to stimuli in that context; a beetle that is satiated on aphids may have different feeding preferences and foraging behaviors than a beetle that was starved.

Acknowledgments

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