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Has the Attraction of Predatory Coccinellids to Cornicle Droplets Constrained Aphid Alarm Signaling Behavior?

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When attacked by a predator, aphids of many species secrete cornicle droplets, containing an alarm pheromone, that results in the dispersal of nearby conspecifics. As females are parthenogenetic, alarm signaling functions to enhance the survival of clone-mates. Enigmatically, however, aphids are physically able to, but usually do not emit alarm pheromone when initially detecting a predator, but rather signal only when captured by a predator. We hypothesized that cornicle droplets may be attractive to natural enemies and result in an increased risk of predation for the signaler, thereby selecting for prudent alarm signalers. We tested this hypothesis by investigating the olfactory cues that the multicolored Asian ladybird beetle, Harmonia axyridis *Pallas, uses to locate pea aphids,* Acyrthosiphon pisum. *In choice tests,* H. axyridis *were attracted to odors from pea aphid colonies, whether feeding or not feeding on a host plant leaf, but were not attracted to cornicle droplets containing alarm pheromone. Further, individual pea aphids emitting cornicle droplets were not located more often or in a shorter period of time by beetles than aphids not emitting cornicle droplets. Thus, the cost of emitting early alarm signals is not prohibitively high in regards to the attraction of predators such as* H. axyridis.

KEY WORDS: kairomone; searching; predator–prey interactions; pheromone; E-B-farnesene.

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INTRODUCTION

Most aphid species live as groups of genetically identical individuals during the summer months, when females are parthenogenetic (Dixon, 1998). Group-living, soft-bodied, and slow moving, aphids are vulnerable to both parasitism and predation. However, when attacked, an aphid will often secrete a small droplet of fluid from its cornicles (posterior tubes located on the end of the abdomen), containing an alarm pheromone (Dahl, 1971; Kislow and Edwards, 1972; Nault *et al.*, 1973). This pheromone causes nearby aphids to stop feeding, walk, or drop from their feeding sites to avoid predation (Nault and Phelan, 1984). The sesquiterpene E-B-farnesene (EBF) is the alarm pheromone in over 30 genera of aphids, including the pea aphid, *Acyrthosiphon pisum* (Nault and Montgomery, 1977). Other cornicle droplet compounds (fatty acids such as myristic, hexanoic, and palmitic acid) are also similar among different aphid genera (Callow *et al.*, 1973).

Particularly enigmatic is that most aphid species wait until being captured by a predator before emitting an alarm signal. Aphids detect predators before physical contact (Klingauf, 1967; E. B. Mondor and B. D. Roitberg, unpublished data), and alarm signaling is a voluntary action under nervous control (Strong, 1967). Emitting an alarm signal when a predator is first detected would be beneficial, as signaling would prevent the demise of kin of high relatedness (Maynard Smith, 1965). By emitting an alarm signal, the signaler would effectively reduce the risk of predation for nearby relatives of all ages, as aphids either disperse away from the point of alarm pheromone emission (Dahl, 1971; Kislow and Edwards, 1972) or become more alert and are less likely to be successfully attacked (Montgomery and Nault, 1978). At the same time as the colony is dispersing, the signaler itself would have a chance to escape from the predator. However, costs of alarm signaling must be taken into account as well as the benefits, and the cost of giving a signal, in many organisms, is increased risk of predatory attack (Teerling *et al.*, 1993; Allan *et al.*, 1996).

While parasitoids are not attracted to aphid alarm pheromone at levels found in aphid cornicle droplets (Du *et al.*, 1998), it is uncertain whether alarm pheromone or other components of cornicle fluid are attractive to predators (Kielty *et al.*, 1996). Obata (1986) found that adult *Harmonia axyridis* were attracted to the odor of *A. citricola*, the odor of aphid-infested leaves, and the odor of healthy leaves, but they did not test attraction to aphid cornicle droplets. Ladybirds do show antennal responses to EBF (Zhu *et al.*, 1999), but synthetic aphid alarm pheromone has not been found to alter beetle foraging paths (Nakamuta, 1991). It is possible that real EBF, or other compounds in aphid cornicle secretions in addition to EBF, may be required to attract ladybirds.

To determine whether the attraction of predatory coccinellids to cornicle fluid containing aphid alarm pheromone has constrained aphid alarm signaling behavior, we investigated the olfactory cues that the aphidophagous multicolored Asian lady beetle, *H. axyridis* Pallas, uses to locate pea aphid colonies. Our objectives in this study were to determine (1) if *H. axyridis* uses olfactory cues to locate pea aphid colonies, and (2) whether aphid cornicle secretions containing aphid alarm pheromone are attractive to *H. axyridis.*

METHODS

Insects and Plants

Pea aphids, *A. pisum*, were collected from sweet pea, *Lathyrus odouratus* 'Cuthbertson', in Burnaby, British Columbia. Aphids were reared in the laboratory on broad bean plants, *Vicia faba* 'Broad Windsor', potted in standard garden soil. Bean plants used for colony maintenance and for the experiments were grown at 22–28°C, 50 ± 25% rh, and 16:8 L:D. Aphids were maintained at 21 ± 2°C, 50 ± 20% rh, and 16:8 L:D.

H. axyridis were collected as adults from several different locations in Burnaby, British Columbia. They were maintained in the laboratory on pea aphids, banana, and honey-water at 21 ± 2°C, 50 ± 20% rh, and 16:8 L:D. Adults used for the experiments were not more than two generations from wild-collected stock. All ladybird beetles used for the experiments were placed in individual Petri dishes and were deprived of food for at least 12 hr to facilitate searching behavior.

Experimental Protocol

In Experiment 1, we placed four model trees (Obata, 1986) constructed out of three 15-cm lengths of bamboo cane inside a Plexiglas cage measuring 32 × 32 × 32 cm. On the top of each "tree" was a fine gauze bag (6 cm in diameter) containing one of four experimental treatments: empty bag, 100 apterous aphids (3rd instar to adult), an excised bean leaf pair, or 100 apterous aphids (3rd instar to adult) actively feeding on a bean leaf pair. A Petri dish containing a single ladybird was placed between all four "trees" and the beetle was allowed to search for 30 min. At the end of the 30 min, the ladybird was removed, the cage wiped out, and the "trees" randomly rearranged. The experiment was conducted in a room at 23–24°C, 32–48% rh, and 16:8 L:D, with lighting provided from above by two 40-W fluorescent bulbs.

Experiment 2 was similar to Experiment 1, the only difference being that we had two model "trees" in the Plexiglas cage. One "tree" held a gauze bag with a piece of filter paper, the other containing a piece of filter paper with cornicle secretions from five apterous aphids (3rd instar to adult). Each ladybird was introduced into the Plexiglas cage and allowed to search for 30 min. The experiment was conducted under the same conditions as Experiment 1.

In Experiment 3 a ladybird was introduced into a 15-cm diameter Petri dish, approximately 1 cm from the edge of the dish. On the opposite side of the dish, 1 cm from the edge, a mature adult aphid was adhered with Stikem. In half the trials an aphid was induced to emit a cornicle droplet (by rubbing gently with a fine paintbrush). In the other trials the aphid was not induced to secrete cornicle fluid. A ladybird beetle was allowed to search for 15 min or until it discovered the aphid. The experiment was conducted in a room at 21–22°C, 60–69% rh, and 16:8 L:D, with lighting provided from above by two 40-W fluorescent bulbs.

Statistical Analyses

In Experiment 1, the number of visits to each gauze bag by each lady beetle was recorded. The total number of visits to each bag were combined for all beetles and analyzed by G^2 likelihood ratio test. The overall analysis was subdivided and individual G^2 contrasts between the control bag (containing nothing) and the three treatments were performed. The total time that each lady beetle spent searching each bag type was summed. Time spent on bags was compared with a one-way Kruskal–Wallis test. A Tukey-type multiple comparison procedure was used to determine which groups were significantly different from the control bag.

For Experiment 2, the number of visits to and time spent on each gauze bag by each beetle were recorded. The total numbers of visits to filter paper versus filter paper and cornicle droplets were combined for all beetles and analyzed by G^2 likelihood ratio test. The times that ladybirds spent searching each bag were compared with a Mann–Whitney U test.

The numbers of ladybird beetles finding versus not finding an aphid, when it was alarm signaling versus not alarm signaling, were entered into a 2 × 2 contingency table and analyzed with a G^2 likelihood ratio test. A two-tailed, nonpaired t-test compared the time taken for predators to find the aphid when emitting versus not emitting cornicle droplets.

RESULTS

When allowed to search the four trees, ladybird beetles did not visit the gauze bags randomly ($G^2 = 8.44$, $df = 3$, $P = 0.0377$). Subdividing the

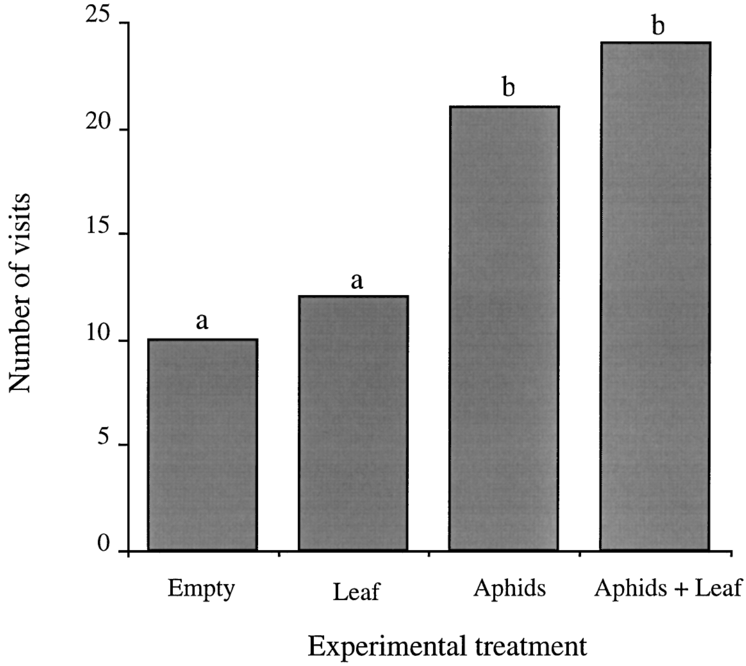

Fig. 1. Number of visits *H. axyridis* made to gauze bags containing different experimental treatments. Columns with different letters than the control "empty" gauze bag are significantly different from the control at $P < 0.05$.

analysis, beetles did not visit gauze bags containing a bean leaf pair more often than empty gauze bags ($G^2 = 0.18$, $df = 1$, $P = 0.6696$). However, beetles did visit gauze bags containing aphids ($G^2 = 3.99$, $df = 1$, $P = 0.0458$), and aphids feeding on a bean leaf pair ($G^2 = 5.94$, $df = 1$, $P = 0.0148$), more often than empty gauze bags (Fig. 1). Ladybirds did not spend equal amounts of time on the four different treatments ($G^2 = 9.07$, $df = 3$, $P = 0.0284$). Beetles spent more time on bags containing aphids versus empty gauze bags ($q = 2.982$, $P < 0.05$). However, there was no difference in the time spent on empty bags versus bags containing a bean leaf pair ($q = 1.395$, $P > 0.05$), nor between empty bags versus bags containing aphids feeding on a bean leaf pair ($q = 1.217$, $P > 0.05$) (Fig. 2).

Ladybirds did not show a preference for visiting gauze bags containing filter paper versus filter paper with cornicle droplets ($G^2 = 1.1468$, $df = 1$, $P = 0.2842$). Beetles did not spend different amounts of time on bags containing filter paper versus filter paper with cornicle droplets ($S = 99$, $z = -0.73987$, $P = 0.4594$). In Petri dish searching bioassays, ladybirds did not

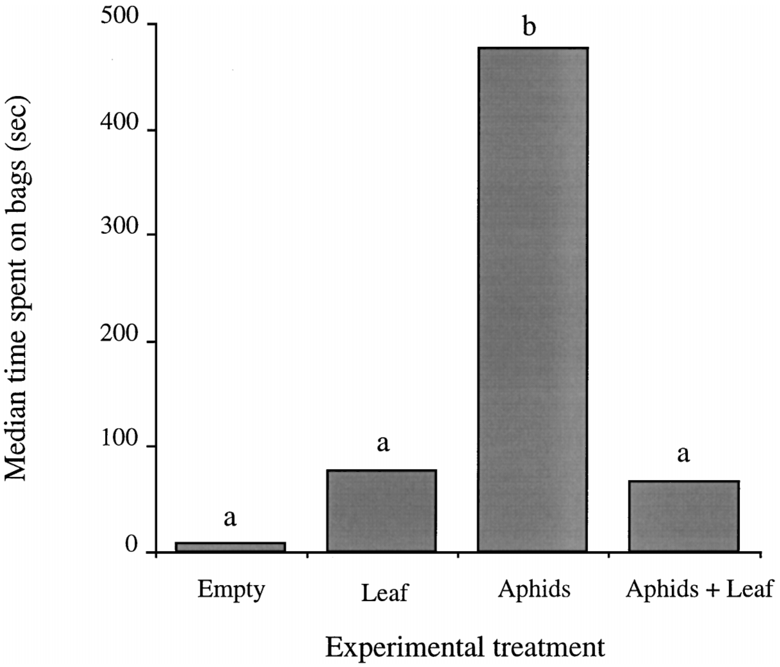

Fig. 2. Time spent searching by *H. axyridis* on gauze bags containing different experimental treatments. Columns with different letters than the control "empty" gauze bag are significantly different from the control at $P < 0.05$.

find aphids more often when the aphid was emitting cornicle droplets versus not emitting cornicle droplets ($G^2 = 0.862$, $df = 1$, $P = 0.3533$). Among aphids that were found by predators, aphids emitting cornicle droplets were not discovered in a shorter amount of time than aphids not emitting cornicle droplets ($t = 0.184$, $df = 13$, $P = 0.8588$).

DISCUSSION

Natural selection acts on herbivores to be inconspicuous so that they avoid detection by natural enemies (Vet and Dicke, 1992). Aphid colonies may give off cues of their presence through unavoidable cues (e.g., cuticle components, honeydew production, feeding damage, etc.) and voluntarily emitted cues (e.g., alarm pheromone emission). If voluntarily emitted cues are attractive to predators, signals should be emitted so as to benefit the individual and the clone. Thus, we hypothesized that aphids do not emit alarm signals before being captured by a predator, as emitting a cornicle droplet may enable the predator to more easily locate and attack the signaler. However, we found that *H. axyridis* is attracted to a kairomone(s) from pea aphids, but is not attracted to cornicle droplets containing alarm pheromone.

H. axyridis is attracted to aphid odors whether the aphids are on or off-plant. Ladybirds were not attracted to a host plant that had never been fed on by aphids. Similar results were obtained by Obata (1986) for the *H. axyridis, Aphis citricola*, annual fleabane system. However, one difference is that Obata (1986) also found undamaged host plant leaves to be attractive to *H. axyridis.* Thus, it appears that the attractive aphid odor is not species-specific, but, rather, is a compound(s) common to at least two different genera of aphids. Other aphid predators such as syrphids (Shonouda *et al.*, 1998) are also attracted to aphid volatiles. Further, nonaphidophagous coccinellids such as the mite predator *Stethorus punctum* (Colburn and Asquith, 1970) and the scale predator *Chilocorus nigritus* (Hattingh and Samways, 1995) are also attracted to host kairomones when foraging.

Ladybirds spent more time searching areas that have aphid odor present compared to areas not having aphid odor. It is puzzling, though, that beetles spent more time, as compared to empty gauze bags, on bags containing aphids, but not on bags containing aphids feeding on a leaf. Perhaps leaf odors or feeding stimuli are not required to extend predator search times. It is also possible that the removal of the aphids from the bean leaves generated additional kairomones not present in the other treatment. It could also be that the aphids, not able to feed, probed the gauze bag and were actually contacted by the lady beetle. Contacts with aphid body fluid (Nakamuta, 1985) and honeydew (Carter and Dixon, 1984) both increase search times, although this has not been documented for contact with whole, undamaged aphids. Further experimentation is required to document the exact cues leading to more thorough searching of a habitat.

H. axyridis was not attracted to cornicle droplets containing the aphid alarm pheromone EBF. We believe that we conducted a strong test of the hypothesis, as we collected and tested five cornicle secretions at once, as opposed to a single cornicle secretion. We also found that aphids are not more susceptible to capture by a predator if they emit a cornicle secretion than if they do not emit cornicle fluid. In this experiment, the signaling aphids were unable to move, unlike under natural conditions, and thus it should have been relatively easy to find if cornicle droplets were attractive to the predators.

It is important to note that the compound EBF is not only found in aphid cornicle droplets (Kislow and Edwards, 1972), but is also given off by aphid-infested plants (Du *et al.*, 1998). However, the concentration of pheromone emitted in cornicle droplets is much lower than that emitted by the plants (E. B. Mondor and D. S. Baird, unpublished data; Du *et al.*, 1998). Thus, antennal responses (Zhu *et al.*, 1999) indicate that it may be beneficial for ladybirds to respond to EBF at relatively high concentrations. For example, aphid EBF is only secreted when an aphid is already under attack. It would not be beneficial for a predator to locate areas where alarm pheromone is present, because the emitter may have been eaten and other aphids will be dispersing from the area. Second, aphid EBF is not secreted regularly, but is only emitted when a predator attacks an aphid. Thus, aphid alarm pheromone would be an inefficient host-finding cue for ladybirds. Conversely, EBF in large concentrations, such as is given off by damaged plants, would be an efficient host-finding cue, as the strong signal would indicate a plant heavily infested with aphids.

Irrespective of the reasons EBF is not attractive to ladybirds at the concentrations found in aphid cornicle droplets, it is clear that the attraction of predatory coccinellids to this fluid has not constrained aphid alarm signaling behavior. Thus, it remains enigmatic why pea aphids wait until being captured by a predator before emitting alarm signals.

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REFERENCES

Allan, R. A., Elgar, M. A., and Capon, R. J. (1996). Exploitation of an ant chemical alarm signal by the zodariid spider *Habronestes bradleyi* Walckenaer. *Proc. R. Soc. Lond. B* **263:** 69–73.

Callow, R. K., Greenway, A. R., and Griffiths, D. C. (1973). Chemistry of the secretion from the cornicles of various species of aphids. *J. Insect Physiol.* **19:** 737–748.

Carter, M. C., and Dixon, A. F. G. (1984). Honeydew: An arrestant stimulus for coccinellids. *Ecol. Entomol.* **9:** 383–387.

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

Dahl, M. L. (1971). Uber einen schreckstoff bei aphiden. *Dtsch. Entomol. Z.* **18:** 121–128.

Dixon, A. F. G. (1998). *Aphid Ecology: An Optimization Approach*, 2nd ed., Chapman and Hall, London.

Du, Y., Poppy, G. M., Powell, W., Pickett, J. A., Wadhams, L. J., and Woodcock, C. M. (1998). Identification of semiochemicals released during aphid feeding that attract parasitoid *Aphidius ervi. J. Chem. Ecol.* **24:** 1355–1368.

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

Kielty, J. P., Allen-Williams, L. J., Underwood, N., and Eastwood, E. A. (1996). Behavioral responses of three species of ground beetle (Coleoptera: Carabidae) to olfactory cues associated with prey and habitat. *J. Insect Behav.* **9:** 237–251.

Kislow, C. J., and Edwards, L. J. (1972). Repellent odor in aphids. *Nature* **235:** 108–109.

Klingauf, F. (1967). Abwehr- und Meideraktionen von Blattlausen (Aphididae) bei Bedrohung durch Raubern und Parasiten. *Z. Angew. Ent.* **60:** 269–317.

Maynard Smith, J. (1965). The evolution of alarm calls. *Am. Nat.* **99:** 59–63.

Montgomery, M. E., and Nault, L. R. (1978). Effects of age and wing polymorphism on the sensitivity of *Myzus persicae* to alarm pheromone. *Ann. Entomol. Soc. Am.* **71:** 788–790.

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

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

Nault, L. R., and Montgomery, M. E. (1977). Aphid pheromones. In: Harris, K. F., and Maramorosch, K. (eds.), *Aphids as Virus Vectors*, Academic Press, New York.

Nault, L. R., and Phelan, P. L. (1984). Alarm pheromones and sociality in pre-social insects. In: Bell, W. J., and Carde, R. T. (eds.), *Chemical Ecology of Insects*, Sinauer Associates, Sunderland, Massachusetts.

Nault, L. R., Edwards, L. J., and Styer, W. E. (1973). Aphid alarm pheromones: Secretion and reception. *Environ. Entomol.* **2:** 101–105.

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

Shonouda, M. L., Bombosch, S., Shalaby, A. M., and Osman, S. I. (1998). Biological and chemical characterization of a kairomone excreted by the bean aphids, *Aphis fabae* Scop. (Hom., Aphididae) and its effect on the predator *Metasyrphus corollae* Fabr. II. Behavioral response of the predator *M. corollae* to the aphid kairomone. *J. Appl. Entomol.* **122:** 25–28.

Strong, F. E. (1967). Observations on aphid cornicle secretions. *Ann. Entomol. Soc. Am.* **60:** 668–673.

Teerling, C. R., Gillespie, D. R., and Borden, J. H. (1993). Utilization of western flower thrips alarm pheromone as a prey-finding kairomone by predators. *Can. Entomol.* **125:** 431–437.

Vet, L. E. M., and Dicke, M. (1992). Ecology of infochemical use by natural enemies in a tritrophic context. *Annu. Rev. Entomol.* **37:** 141–72.

Zhu, J. W., Cosse, A. A., Obrycki, J. J., Boo, K. S., and Baker, T. C. (1999). Olfactory reactions of the twelve-spotted lady beetle, *Coleomegilla maculata* and the green lacewing, *Chrysoperla carnea* to semiochemicals released from their prey and host plant: Electroantennogram and behavioral responses. *J. Chem. Ecol.* **25:** 1163–1177.