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Effects of oviposition-deterring pheromone and allomones on *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae)

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Abstract. Larvae of *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae) secrete an oviposition-deterring pheromone (ODP). In choice tests, females of *A. aphidimyza* laid significantly fewer eggs on *Vicia faba* L. plants infested with *Aphis fabae* Scopoli (Homoptera: Aphidoidea) that were previously exposed to conspecific third-instar larvae or a water extract of their ODP.

A. aphidimyza females also laid fewer eggs on aphid-infested plants that were previously exposed to unfed first-instar larvae of *Chrysopa oculata* Say, *Chrysopa perla* (L.) or *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae), or second-instar larvae of *Coccinella septempunctata* L. (Coleoptera: Coccinellidae). However, the response to traces of *C. carnea* larvae was very weak.

INTRODUCTION

An oviposition-deterring pheromone (ODP) that deters predators from laying eggs at sites previously searched by conspecific larvae has been described in *Chrysopa oculata* Say (Růžička, 1994). Chrysopid larvae secrete and mark substrates with ODP even in the absence of conspecifics (Růžička, 1996). ODPs are also secreted by the larvae of other chrysopid species. The deterrent operates intra- and interspecifically and intergenerically. Therefore, chrysopid ODPs are also oviposition-deterring allomones (ODAs) (Růžička, 1998). The ODP of *C. oculata* is relatively stable at temperatures up to 140°C. At room temperature, contaminated substrates deter conspecific females for several weeks, and paper becomes contaminated when enclosed above glass contaminated with ODP from larvae (Růžička, 1997a).

Larvae of the aphidophagous coccinellid *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) secrete a different kind of ODP (ODA) (Růžička, 1997b). Substrates contaminated with the ODA of chrysopid larvae deterred coccinellid females from ovipositing, but that of the coccinellid had only a slight effect on chrysopid females. The presence of ODA in chrysopids and coccinellids indicates that ODAs might be secreted by other aphid predators.

Aphidoletes aphidimyza (Rondani) (Diptera: Cecidomyiidae) is a promising agent for the biological control of aphids in greenhouses (Malais & Ravensberg, 1992). A larva can kill around 50–60 adults of *Myzus persicae* (Sulzer) or *Aphis gossypii* Glover (Homoptera: Aphidoidea) during its development. *A. aphidimyza* is most effective on low aphid infestations. The inverse numerical response this species shows to high prey-densities in the field

(Hafez, 1961) is probably an effect of larval ODP. This may also account for its reluctance to oviposit in dense colonies of *A. gossypii* in greenhouses (Havelka, 1978).

In this study, the response of females of *A. aphidimyza* to aphid-infested plants previously occupied by conspecific larvae is described. In addition, the deterrent effects of plants treated with a water extract of the ODP and responses of female *A. aphidimyza* to ODAs of chrysopid and coccinellid larvae are reported.

MATERIAL AND METHODS

Experiments were carried out on adults from a laboratory culture of *A. aphidimyza*, which was established from larvae collected from colonies of *M. persicae* on *Prunus persica* in Palamos (42°N/3°E), Spain in 1996. Adults were fed 10% sucrose, honeydew of *Aphis fabae* Scopoli and water. The larvae were reared on *Acyrthosiphon pisum* Harris (Homoptera: Aphididae). Larvae of *Chrysopa oculata* and *C. perla* were from laboratory cultures, and the larvae of *Chrysoperla carnea* and *Coccinella septempunctata* were the F1 offspring of adults collected from hibernation sites in Prague and Raná near Louny, respectively, in 1996 and 1997. Experiments were done at 22 ± 1°C, 95–100% r. h. and a light regime of 18 L : 6 D.

Oviposition choice tests were performed in 40 × 40 × 40 cm nylon cages enclosed in transparent plastic sleeves. Fifty females and 50 males were placed in the cage. Drinking water and 10% sucrose solution were available during the test.

Four *Vicia faba* L. plant seedlings in the hook stage of growth were arranged in a square on the lids of four 200 ml plastic jars. The roots of these 3 cm high seedlings were inserted through small holes in the lids into the water. Each of the four plants was infested with 20 fourth-instars of *A. fabae*. The aphids were confined to the plant by enveloping it with a little glass cylinder, which was removed later. *A. fabae* was used because it is an acceptable prey for *A. aphidimyza* (Havelka & Růžička, 1984). Prior to introduction of the aphids, two of the plants, in opposite corners of the oviposition arena, were exposed to ODP contamination that is described below. The oviposition test lasted for 12 h, 6 h of which were in darkness. Each test was repeated five times.

To contaminate the test plants with ODP (or ODAs), third-instar larvae of *A. aphidimyza*, or unfed first-instar larvae of *C. oculata, C. perla* or *C. carnea* or second-instar larvae of *C. septempunctata* were placed on and left to walk over the surface of plants. Single plants were in this way contaminated in 20 ml plastic tubes for 4 h. Choice tests using these plants were carried out immediately. In the blank choice test, no plant was previously exposed to larvae or their secretions.

The treatments with ODP

A – Conspecific larvae

1 – Test plant contaminated by 30 larvae of *A. aphidimyza* for 4 h.

2 – Test plants contaminated by 30 larvae of *A. aphidimyza*, but with 1 larva left on the plant during the test.

3 – 50 larvae of *A. aphidimyza* were enclosed per plastic tube for 4 h and then the inner surface of 4 tubes and the larvae were briefly rinsed with 2 ml of distilled water. The resultant solution was filtered and then sprayed on a test plant.

The treatments with ODAs

B – Heterospecific larvae; test plants previously contaminated by

1 – 30 larvae of *C. oculata*.

2 – 30 larvae of *C. perla*.

3 – 30 larvae of *C. carnea*.

4 – 20 larvae of *C. septempunctata*.

Differences in mean numbers of eggs (%) laid on contaminated and uncontaminated substrates in choice tests were analysed by the Student's t-test.

RESULTS

In the blank choice test, the proportions of eggs laid on pairs of aphid infested plants without ODP were similar (P = 0.7978) (Fig. A4).

Plants contaminated by *A. aphidimyza* larvae (treatment A1), contaminated by larvae and having one larva (treatment A2), and sprayed with a water extract of larval traces and larvae (treatment A3), deterred conspecific females from ovipositing. The differences in the proportions of eggs laid on clean and contaminated plants were statistically significant (P < 0.0001). Similarly, the difference between numbers of eggs laid on clean plants and those sprayed with a water extract of the ODP was statistically significant (P = 0.0003).

A. aphidimyza laid fewer eggs on aphid-infested plants that were previously exposed to first-instar larvae of each of three chrysopid species (Fig. B1, 2, 3). The differences in the proportion of eggs laid on clean plants and plants contaminated by larvae were smaller than when conspecific larvae were used. The gall-midge females laid significantly fewer

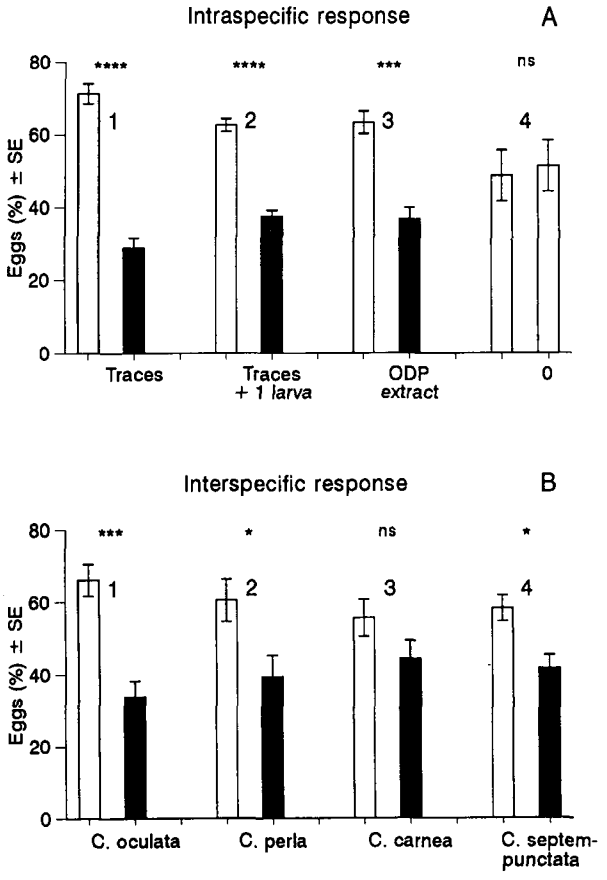

Fig. 1. Oviposition by *Aphidoletes aphidimyza* on clean (clear bars) and contaminated (black bars) plants. The latter were contaminated with ODP of third-instar conspecific larvae (A) or with ODA of unfed first-instar chrysopid or second-instar coccinellid larvae (B). Student's t-test: * 0.05 < P < 0.01, *** P < 0.001, **** P < 0.0001. Number of replicates n = 5.

eggs on plants contaminated by larvae of *C. oculata* (P = 0.0009) and *C. perla* (P = 0.0345), but not in the case of *C. carnea* (P = 0.1559).

A. aphidimyza also laid fewer eggs on plants previously exposed to second-instar larvae of *C. septempunctata*. Although the difference was relatively small, it was statistically significant (P = 0.0120) (Fig. B4).

DISCUSSION

Larvae of *A. aphidimyza* secrete an ODP that effectively deters conspecific females from ovipositing on aphid infested plants. This is the first evidence of ODP in predaceous Diptera. ODPs and ODAs have already been described in several aphidophagous species of two orders, Neuroptera and Coleoptera.

In *C. oculata* (Chrysopidae), unfed first-instar as well as third-instar larvae secrete and mark sites on which they search for prey with ODP (Růžička, 1994). Similarly, first- and second-instar larvae of *C. septempunctata* (Coccinellidae) deter conspecific females from ovipositing (Růžička, 1997b). Although only third-instar larvae of *A. aphidimyza* were used in our experiments, younger larvae of this species are also likely to secrete the ODP. It is likely that larvae of other predaceous Diptera, e.g. families Syrphidae or Chamaemyiidae, also secrete ODPs.

As in chrysopids (Růžička, 1994), the ODP in the traces left by larvae of *A. aphidimyza* was soluble in water. Plants infested with *A. fabae*, but sprayed with small amounts of the ODP water extract, deterred *A. aphidimyza* from ovipositing. This indicates that rain may reduce the deterrent effects of the ODP locally, but spread it to other parts of a plant.

Sometimes, the presence of predatory larvae did not deter conspecific females from ovipositing. While *Adalia bipunctata* (L.) avoided ovipositing in the presence of conspecific larvae in laboratory experiments (Hemptinne et al., 1991), and the syrphid *Epistrophe nitidicollis* (Meigen) laid fewer eggs in aphid colonies on bean plants already attacked by conspecific larvae in a field experiment, the presence of second-instar larva did not inhibit egg laying of conspecific females in *C. septempunctata* in the laboratory (Hemptinne et al., 1993). However, substrates previously walked upon by larvae of *C. septempunctata* strongly deterred conspecific females (Růžička, 1997b).

In our experiments here, females of *A. aphidimyza* were deterred from ovipositing on plants contaminated with traces of conspecific larvae (treatment A1), only slightly more than from those with the traces and 1 larva (treatment A2). This indicates that the physical presence of a conspecific larva is less important than the presence of larval traces. *A. aphidimyza* females oviposit mostly at night or at twilight. Therefore, the probability of visual detection of conspecific larvae by ovipositing females is relatively low. Also, the probability of physically encountering a larva is much lower than encountering larval traces on a plant surface. Whether or not the surface is contaminated with larval secretion is what is decisive in the selection of oviposition site.

Our results indicate that so-called "inverse numerical response to high prey density" (Kuchlein, 1966) reported for *A. aphidimyza* and syrphids (Hafez, 1961; Hughes, 1963) could result from the response of females to larval ODP and/or ODAs of other aphidophagous species that are highly likely to be present in dense aphid colonies of *Brevicoryne brassicae* in the field. Number of syrphid eggs declined to very low values also at high densities of psyllids (Clark, 1963). Similarly, the preference shown by syrphids for

ovipositing on *A. fabae* colonies well before they peak in abundance (Hemptinne et al., 1993) is also likely to be a consequence of the deterrent effects of ODP left by conspecific larvae and/or the larvae of other predators. The action of ODP is the likely mechanism by which females respond to the presence of larvae (Hemptinne & Dixon, 1991).

By ovipositing on aphid colonies that lack traces of conspecific and/or other aphidophagous larvae, competition for food is decreased. Females of many aphid predators lay their eggs in batches or lay several eggs in one aphid colony within a short period. Therefore, several larvae are likely to hatch there simultaneously after a few days. This significantly increases the degree of contamination of plant surface with ODP. The deterrent effect of the concentration of conspecific ODP on a plant decreases the probability that larvae of different ages will occur in an aphid colony.

While substrates walked upon by first-instar chrysopid larvae deter females of *Coccinella septempunctata* from ovipositing, deterrent effects of the traces left by first- and second-instar larvae of *C. septempunctata* on ovipositing females of *C. oculata* were negligible (Růžička, 1997b). This study shows that females of *A. aphidimyza* avoid ovipositing on plants walked upon by second-instar larvae of *C. septempunctata*, and first-instar larvae of *C. oculata* and *C. perla*. However, the ODA of *C. carnea* larvae had very little effect on ovipositing females of *A. aphidimyza*. Interestingly, the ODA of *C. carnea* had relatively little effect on ovipositing females of other chrysopids (Růžička, 1998).

As described above, ODPs (and ODAs) have now been found in larval traces of aphid predators from three insect orders: Neuroptera, Coleoptera and Diptera. Females of *A. aphidimyza*, not only respond to the ODP of conspecific larvae, but also ODAs of larvae of larger and presumably more agressive heterospecific predators that might endanger their eggs and larvae when aphid prey becomes scarce. Thus, it is advantageous for aphidophagous predators to spread their offspring more uniformly between patches of aphids. The response of females to ODPs and ODAs contributes to the optimal distribution of the progeny among prey resources. Females, by searching for ODP uncontaminated or less contaminated oviposition sites, spread their predatory larvae more evenly among aphid colonies and possibly achieve a higher efficiency of aphid control. On the other hand, a strong female response to ODP (ODAs) might favour growth of aphid colonies that survive predation but are still protected by the presence of these deterrents, which sometimes persist for very long periods. Intra- and interspecific effects of larval oviposition-deterring secretions in other predatory species, for example aphidophagous syrphids, deserve further investigation.

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