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Oviposition-deterring pheromone in Chrysopidae (Neuroptera): Intra- and interspecific effects

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Oviposition behaviour, larval marking, spacing pheromone, egg distribution, *Chrysopa oculata*, *C. perla*, Chrysopidae

Abstract. Substrates contaminated with abdominal secretion of first instar larvae of *Chrysopa oculata* Say or *Chrysopa perla* (L.) deter females of these species from ovipositing. The intra- and interspecific responses of the females were similar; however, the smaller species, *C. oculata*, showed a stronger response overall. Although marking with oviposition-deterring pheromone is independent of encounters with conspecific larvae, the contamination of a paper substrate by crowded larvae increases a female response. The increase in response may be due either to the quantity of the pheromone secretion or the amount of marking or both.

INTRODUCTION

In various phytophagous and parasitoid insects chemicals that deter oviposition are secreted by adults (Kerkut & Gilbert, 1985) and also by larvae (Corbet, 1971). Interspecific recognition of these compounds appears to be an important phenomenon in species competing for a common food resource. Egg-associated semiochemicals may deter the oviposition of related tortricid species (Thiery & Gabel, 1993). Hosts marked by a particular parasitoid or sites that have been searched recently by a female parasitoid may also deter related species from ovipositing (Lloyd, 1942; Price, 1970).

The presence of an oviposition-deterring pheromone (ODP) in predatory insects has been described in *Chrysopa oculata* (Neuroptera: Chrysopidae) (Růžička, 1994). Females of this species are reluctant to lay eggs on substrates contaminated by a pheromonal substance secreted by their larvae from the tip of their abdomen and on substrates treated with an extract of the larval ODP. This paper describes the intra- and interspecific responses to the oviposition-deterring secretion of larvae shown by the adults of two common, geographically isolated, chrysopids, *Chrysopa oculata* Say and *C. perla* (L.).

When larvae of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) meet, they release a pheromone from their mandibular glands (Corbet, 1973). Low and high concentrations of this pheromone, respectively, stimulate and inhibit oviposition. A similar response to larval secretion has been described in *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) (Phillips & Strand, 1994). Therefore, experiments were conducted to determine whether encounters between green lacewing larvae elicit secretion of their ODP.

MATERIAL AND METHODS

Chrysopids were obtained from laboratory cultures. *C. oculata* was collected in Kentville, Nova Scotia, Canada in 1987 and *C. perla* in České Budějovice, Czech Republic in 1992. Adults were supplied

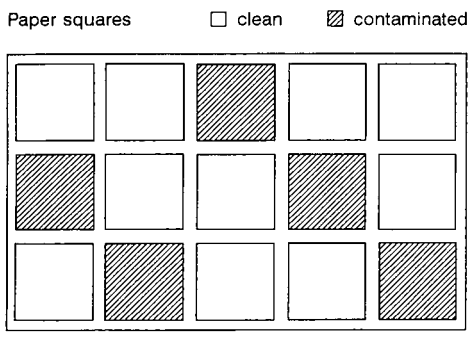

Fig. 1. The scheme of oviposition arena.

with pea aphids, *Acyrthosiphon pisum* Harris (Homoptera: Aphididae), drinking water and liquid yeast hydrolysate diet with sucrose.

The oviposition experiments were performed in a 40 × 40 × 40 cm nylon cage. Adults of both sexes were present in equal numbers and in all age categories; 100–200 females of *C. oculata* or 50–100 females of *C. perla* were present in the cage. Room temperature was 24 ± 2°C. The relative humidity was 40 ± 10%. The light source was two 40 W, white light fluorescent tubes positioned 0.4 m above and approximately 1.2 m to one side of the cage. The light regime was 18L/6D.

The oviposition arena consisted of 15 square substrates of dark blue paper (each 50 × 50 mm) arranged in a design of 3 by 5 in which 5 squares were contaminated and 10 uncontaminated (Fig. 1). The squares were attached to the underside of a 207 × 295 mm plastic sheet fixed 15 cm below and parallel with the top of the cage. One of the shorter sides was in contact with the side of the cage nearest to the light source. In order to determine whether the position of a substrate affected the number of eggs laid per square, chrysopids were offered uncontaminated substrates in all 15 positions in a blank experiment.

In the experiments of choice, contaminated squares had been exposed to unfed first instar larvae, each square in a 9 cm diameter Petri dish with a Fluon-painted rim to prevent larval escape. Substrates contaminated by 10 larvae were exposed to larvae kept together on the substrate for a period of 4 h, i.e., 40 larval hours. Substrates contaminated by a single larva were exposed to a single larva for a period of 48 h, i.e., 48 larval hours. Due to the low survival of unfed first instar larvae during the second day in a preliminary test, each single larva was replaced by a fresh first instar larva after 24 h. Females of each species were offered control squares and paper squares contaminated by either 10 first instar conspecific larvae, by 10 first instar larvae of the other species, or by a single first instar larva of *C. oculata*. In a single trial, contaminated squares of a single kind only were always present. Each trial was repeated five times.

Statistics: Although the total number of eggs laid in each of five replicates varied, it is possible to assume that the degree of avoidance of each particular type of substrate remained constant under the conditions of the experiments. Therefore, differences between numbers (%) of eggs laid per contaminated and uncontaminated square substrate unit were analysed by Student's t-test (the percentage was transformed with the arcsine transformation). Similarly, the t-test was used to analyse differences between percentages of eggs laid per contaminated squares in two different trials.

RESULTS

The number of eggs laid was dependent on the chrysopid species, the number of females present in the cage and the type of treatment with which the females had contact. In all experiments, females laid many eggs outside the oviposition arena as well as within it.

Females of both chrysopid species laid very similar numbers of eggs on clean substrates in the experimental and control positions. Average numbers of eggs ± SE per clean substrate unit in experimental and control positions were 76.32 ± 11.83 and 74.22 ± 8.79 for *C. oculata* and 27.28 ± 2.39 and 27.22 ± 2.42 for *C. perla*. The differences between the percentage of eggs laid per unit of paper substrate in the experimental and control positions were not statistically significant ($P = 0.883$ for *C. oculata* and $P = 0.916$ for *C. perla*) (Fig. 2).

The percentage of eggs laid per substrate unit by *C. oculata* was lower on contaminated than on clean substrates. This difference was statistically significant ($P < 0.0001$) in

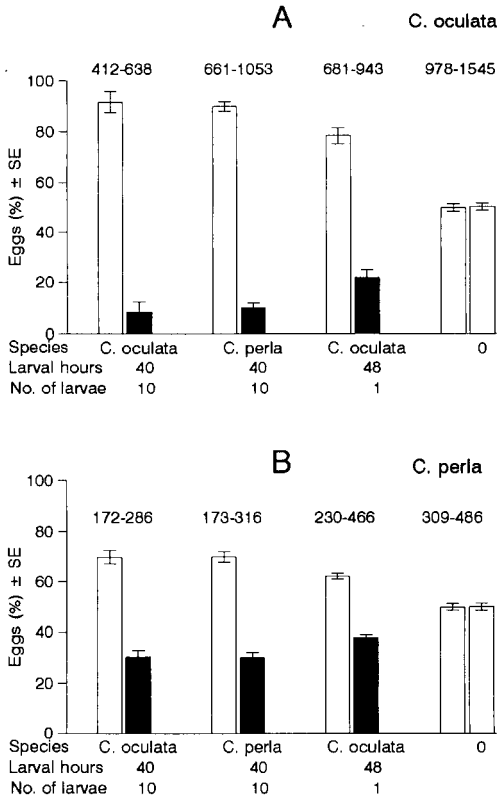

Fig. 2. Percentage of eggs laid by *Chrysopa* spp. per unit of paper substrate □ without and ■ with larval oviposition-deterring pheromone when given a choice. The range of total numbers of eggs laid on all 15 squares in each type of trial are given above the columns. Each type trial n = 5.

experiments with substrates contaminated during 40 larval hours by crowded larvae of each species, as well as in the experiment with substrates contaminated during 48 larval hours by single larvae of *C. oculata* (Fig. 2A).

Females of *C. oculata* responded similarly to substrates contaminated by 10 first instar larvae of *C. oculata* and 10 first instar larvae of *C. perla*, as the percentage of eggs laid on these two substrates did not differ statistically ($P = 0.463$). The numbers of eggs laid on substrates contaminated by single larvae of *C. oculata* were always low. Substrates contaminated by single larvae of *C. oculata* deterred females from ovipositing less than substrates exposed to 10 larvae of this species. Howewer, the difference was not statisticaly significant at level $\alpha = 0.01$ ($P = 0.032$). Crowded larvae appeared to be more mobile than single larvae but the difference was not studied in detail.

The response to substrates contaminated by larvae was generally lower in *C. perla* than in *C. oculata* (Fig. 2). The difference between the response of *C. oculata* and *C. perla* to 10 conspecific larvae was statistically significant ($P < 0.01$). Nevertheless, the differences between the percentage of eggs laid by females of *C. perla* per unit of contaminated and clean substrate were statistically significant ($P < 0.0001$) in experiments with substrates contaminated by 10 larvae of both species, as well as in the experiment with substrates contaminated by 1 larva of *C. oculata* (Fig. 2B). The response of *C. perla* to substrates contaminated by 10 larvae of its own and of the other species was again similar ($P = 0.987$). The percentage of eggs laid per substrate unit, contaminated by a single first instar larva, was slightly lower than the percentage laid per substrate unit contaminated by 10 first instar larvae of *C. oculata*. The difference was not statistically significant at level $\alpha = 0.01$ ($P = 0.026$).

DISCUSSION

Females of each species showed a similar response to sites contaminated by conspecific as well as the other species' larvae. This may indicate that the chemical markers of these species are either similar or identical. *C. perla* is slightly larger than *C. oculata*. However,

there is no evidence that the size difference affects the intensity of the response shown by the females to the pheromonal marker.

On encountering conspecifics, predators often increase their activity (Hassell et al., 1976). The crowded chrysopid larvae were more mobile and this, apparently, resulted in a certain increase of the contamination of the substrate with their ODP. However, in both species the substrates contaminated by 10 larvae of *C. oculata* (40 larval hours in total) did not deter oviposition significantly more than substrates contaminated by a single larva (48 larval hours in total). This indicates that pheromone secretion by larvae is independent of encounters with conspecifics. Thus, ovipositing chrysopids do not respond to crowds of larvae only, as reported for *A. kuehniella* (Corbet, 1971). The present author's current preliminary experiments on the persistence of chrysopid ODP indicate a very slow decrease of the repellent effect of substrates contaminated by first instar larvae.

Avoidance of deterrent semiochemicals by tortricids was more effective in isolated than crowded females (Thiery & Gabel, 1993). Both chrysopids avoided strongly contaminated substrates, despite of the high density of females in my choice experiments. It is possible to expect that single chrysopid females will avoid ODP contaminated substrates more effectively than crowded females.

Adults of *C. oculata* and *C. perla* are predatory. A search for ODPs should continue in chrysopids with non-predatory adults. Future studies should examine whether the chrysopids whose adults feed on pollen respond to the ODP of those species that have aphidophagous adults.

Egg production by satiated females of *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae) is fairly constant and independent of prey abundance (Mills, 1982). However, oviposition in this species decreases in the presence of larvae and this does not involve competition for food (Hemptinne & Dixon, 1991). Females tend to withhold eggs and leave after encountering conspecific larvae, but do not respond to conspecific eggs or pupae (Hemptinne et al., 1992). Similarly, the number of eggs laid by the coccidophagous coccinellid, *Cryptolaemus montrouzieri* Mulsant, decreased steadily as the number of conspecific larvae per Petri dish increased (Lemaitre, 1992). In the field, females of the aphidophagous syrphid, *Epistrophe nitidicollis* (Meigen) (Diptera: Syrphidae), avoid ovipositing in colonies attacked already by their larvae (Hemptinne et al., 1993). This suggests that, in addition to the two species of chrysopids studied here, ODP may also be present in these other species of aphidophagous insects.

A key factor in the evolution of these types of oviposition inhibitors is that they may reduce cannibalism. That is, they appear to enable aphidophagous insects to optimize their search for suitable patches of prey for their larvae and, apparently, serve to distribute the predators more uniformly between patches of prey. However, this could reduce substantially predator effectiveness as biocontrol agents in field crops after mass release if the released beneficials leave the target crop.

Ovipositing females of at least some aphidophagous species are able to evaluate the quality of an aphid colony, not only by its size (Hafez, 1961; Hughes, 1963) and age (Kindlmann & Dixon, 1993) relative to other colonies (Stephens & Krebs, 1986) but also, in terms of the presence or absence of conspecific larvae. In nature, the frequency of physical encounters with and the visual detection of larvae by ovipositing adults is likely to be uncommon because larvae tend to be hidden in vegetation when not feeding and

often are not active at the same times as the adults. Therefore, the oviposition-deterring chemical markers left by the larvae are more reliable indicators of their presence. Results presented here indicate that ovipositing females may also respond to the presence of larvae of other species of the same taxon. When food is scarce, larvae of many aphid predators will cannibalize larvae of related species. The ability of females to recognize the presence of potential competitors and predators that may affect the survival of their progeny is advantageous.

The inverse numerical response of some predators to prey at high prey densities (Kuchlein, 1966) indicates a tendency to avoid patches where prey are abundant. This may not be a response of predators to the high density of prey, but to the presence of ODP of the larvae of prey-competing predators.

Females of *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) avoid laying eggs on apples treated with compounds from eggs of another tortricid, *Lobesia botrana* (Denis & Schiffermüller) (Gabel & Thiery, 1994). Within each chrysopid species studied here, no difference has been found between intra- and interspecific response of females to the pheromone. The ability of females of aphid predators to respond to larval ODP of potentially competing species from other taxonomic groups should be explored.

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REFERENCES

Corbet S.A. 1971: Mandibular gland secretion of larvae of the flour moth, Anagasta kuehniella, contains an epideictic pheromone and elicits oviposition movements in a hymenopteran parasite. *Nature* **232**: 481–484.

Corbet S.A. 1973: Oviposition pheromone in larval mandibular glands of Ephestia kuehniella. *Nature* **243**: 537–538.

Gabel B. & Thiery D. 1994: Semiochemicals from Lobesia botrana (Lepidoptera: Tortricidae) eggs deter oviposition by the codling moth Cydia pomonella (Lepidoptera: Tortricidae). *Eur. J. Entomol.* **91**: 353–359.

Hafez M. 1961: Seasonal fluctuations of population density of the cabbage aphid, Brevicoryne brassicae (L.), in the Netherlands, and the role of its parasite, Aphidius (Diaeretiella) rapae (Curtis). *Tijdschr. PlZiekt.* **67**: 445–548.

Hassell M.P., Lawton J.H. & Beddington J.R. 1976: The components of arthropod predation. I. The prey death-rate. *J. Anim. Ecol.* **45**: 135–164.

Hemptinne J.-L. & Dixon A.F.G. 1991: Why ladybirds have genarally been so ineffective in biological control. In Polgár L., Chambers R.J., Dixon A.F.G. & Hodek I. (eds): *Behaviour and Impact of Aphidophaga.* SPB Academic Publishing, The Hague, pp. 149–245.

Hemptinne J.-L. Dixon A.F.G. & Coffin J. 1992: Attack strategy of ladybird beetles (Coccinellidae): factors shaping their functional response. *Oecologia* **90**: 238–245.

Hemptinne J.-L., Dixon A.F.G., Doucet J.-L. & Petersen J.-E. 1993: Optimal foraging by hoverflies (Diptera: Syrphidae) and ladybirds (Coleotera: Coccinellidse): Mechanisms. *Eur. J. Entomol.* **90**: 451–455.

Hughes R.D. 1963: Population dynamics of the cabbage aphid, Brevicoryne brassicae (L.). *J. Anim. Ecol.* **32**: 393–424.

Kerkut G.A. & Gilbert L.I. 1985: *Comprehensive Insect Physiology, Biochemistry and Pharmacology. Vol. 9. Behavior.* Pergamon Press, Oxford, 735 pp.

Kindlmann P. & Dixon A.F.G. 1993: Optimal foraging in ladybird beetles (Coleoptera: Coccinellidae) and its consequences for their use in biological control. *Eur. J. Entomol.* **90**: 443–450.

KUCHLEIN J.H. 1966: Some aspects of the prey-predator relation. In: Hodek I. (ed.) *Ecology of Aphidophagous Insects.* Academia, Prague, pp. 237–242.

LEMAITRE O. 1992: *Stimulation et Régulation de la Ponte de la Coccinelle Coccidiphage Cryptolaemus montrouzierri Mulsant.* Thesis, Université Libre de Bruxelles, 70 pp.

LLOYD D.C. 1942: Further experiments on host selection by hymenopterous parasites of the moth, Plutella maculipennis Curtis. *Rev. Can. Biol.* **1**: 633–645.

MILLS N.J. 1982: Voracity, cannibalism and coccinellid predation. *Ann. Appl. Biol.* **101**: 144–148.

PHILLIPS T.W. & STRAND M.R. 1994: Larval secretions and food odors affect orientation in female Plodia interpunctella. *Entomol. Exp. Appl.* **71**: 185–192.

PRICE P.W. 1970: Trail odors: recognition by insect parasitic on cocoons. *Science* **170**: 546–547.

RŮŽIČKA Z. 1994: Oviposition-deterring pheromone in Chrysopa oculata (Neuroptera: Chrysopidae). *Eur. J. Entomol.* **91**: 361–370.

STEPHENS D.W. & KREBS J.R. 1986: *Foraging Theory.* Princeton University Press, Princeton, New Jersey, XIV + 247 pp.

THIERY D. & GABEL B. 1993: Inter-specific avoidance of egg-associated semiochemicals in four tortricids. *Experientia* **49**: 998–1001.

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