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Cornicle secretion of *Acyrthosiphon pisum* (Homoptera: Aphididae) as a contact kairomone for the parasitoid *Aphidius ervi* (Hymenoptera: Braconidae)

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Abstract. Females of the endophagous parasitoid *Aphidius ervi* Haliday (Hymenoptera: Braconidae) respond positively to the cornicle secretion of the pea aphid, *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae). The parasitoid response has been assessed in a Petri dish choice test by presenting an aphid dummy consisting of a glass bead coated with cornicle secretion along with an untreated bead, which acted as a control. Naive females responded similarly to the treated glass beads and aphids, while experienced females responded less to the treated beads than to aphids. The kairomonal activity of the cornicle secretion decreased as the wax dried. The behavioural response registered seems to be innate and not induced by associative learning.

INTRODUCTION

Host selection by insect parasitoids is regulated by a series of physical and chemical cues (Vinson, 1985, 1991). Several sources of behaviour regulating chemicals have been reported in literature for braconid aphid parasitoids. Volatiles from plants and hosts are attractive to parasitoid species (Read et al., 1970; Schuster & Starks, 1974; Powell & Zhang, 1983; Bouchard & Cloutier, 1985; Sheehan & Shelton, 1989; Wickremasinghe & van Emden, 1992; Guerrieri et al., 1993) or involved in the host recognition (Powell & Wright, 1992). Aphid honeydew stimulates host-searching in several aphid parasitoids (Bouchard & Cloutier, 1984; Gardner & Dixon, 1985; Ayal, 1987; Hågvar & Höfsvang, 1989; Budenberg, 1990; Budenberg et al., 1992) while unidentified haemolymph components can stimulate both host searching and oviposition (Srivastava & Singh, 1988). Recently, the kairomonal effect of the cornicle secretion of *Rhopalosiphum padi* (L.) on *Lysiphlebus testaceipes* (Cresson) has been reported (Grasswitz & Paine, 1992).

A comparative study (Pennacchio et al., 1994) of the host recognition and acceptance behaviour in two closely related species, *Aphidius ervi* Haliday and *Aphidius microlophii* Pennacchio & Tremblay, indicates that both cuticular and haemolymphatic kairomones, not yet identified, regulate these behavioural steps. Furthermore, it was observed that glass surfaces accidentally contaminated with the cornicle secretion of the pea aphid, *Acyrthosiphon pisum* (Harris), apparently elicited host searching behaviour in *A. ervi* females. These preliminary observations stimulated the present study, which aims to evaluate the role of the pea aphid cornicle secretion in the regulation of host recognition and acceptance behaviour of *A. ervi*.

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MATERIAL AND METHODS

The parasitoid *A. ervi* was reared in the laboratory on *A. pisum* maintained on potted broad bean plants (*Vicia faba*), in wood-framed cages closed with glass on the top and laterally with a fine mesh nylon cloth. Aphid and parasitoid cultures were maintained in two separate environmental chambers at 20 ± 1°C, 75 ± 5% R. H. and a 18 hr L : 6 hr D photoperiod.

Naive parasitoid females were obtained from aphid mummies kept singly in glass vials (8 mm × 60 mm), plugged with cotton-wool and observed twice a day. After emergence, females of the same age were grouped in adult rearing cages, consisting of cylindrical plastic boxes (50 mm × 80 mm), closed at the top with nylon cloth and bottom lined with moistened filter paper. Food was provided as a water solution of honey (50% v/v), distributed on small pieces of cotton-wool. Adult rearing cages were kept at the same environmental conditions reported above and parasitoids used between 24 hr and 48 hr after their emergence. Parasitoid females collected from the rearing cage just before performing the bioassay were considered as experienced.

To evaluate the effect of *A. pisum* cornicle secretion on *A. ervi* oviposition behaviour we designed a simple Petri dish bioassay. Two clean glass beads (2 mm in diameter) were fixed onto a Petri dish cover, 1 cm apart, with a small drop of white correction fluid, which after drying is apparently odourless and does not affect parasitoid behaviour. A choice test was then carried out by applying to the surface of one of the two beads the cornicle secretion of *A. pisum*, obtained by gently stimulating the aphids with forceps and then rapidly coating the bead with the exuding wax. Usually, about 10 aphids were required to evenly coat a test bead. The untreated bead acted as a control. The beads were covered with a circular Petri dish cover (3 cm × 1.5 cm) in which the female to be tested was released. Each parasitoid was tape-recorded for 6 minutes after release and its behaviour was subsequently analyzed with the aid of a computer and event-recording software (The Observer, Noldus Information Technology, Wageningen, The Netherlands). The behavioural events considered were the occurrence of ovipositional strikes, and the occurrence and duration of antennal examination.

Six different experiments were carried out. Naive females were tested both on fresh and dried cornicle secretion. Dried wax coated beads were used 0.5 hr and 3 hr after spreading the cornicle secretion on them. Naive females were also tested on aphids. For this experiment a single 4th instar aphid was exposed in the bioassay arena. Experienced females were tested only on fresh cornicle secretion and on aphids. All the experiments were carried out at room temperature, which fluctuated between 20 and 24°C, and within 10 hr following the onset of a photophase. Twenty-five females were used in each experiment. A χ^2 test was used to compare distributions of oviposition response registered in the different experiments, while ANOVA was used to compare transformed mean values. In most cases the log (x + 1) transformation was suitable for normalizing the distribution of the experimental data.

RESULTS

The cornicle wax of *A. pisum* strongly stimulated oviposition behaviour in *A. ervi*, which almost completely ignored the control beads but showed a high rate of response to the treated beads (Tab. 1). For naive females, in the case of cornicle wax, both fresh and air-dried for 0.5 hr, the acceptance rate of aphid dummies was not significantly different from that observed when they were in contact with aphids (Tab. 1). When the wax was allowed to dry for 3 hr its kairomonal activity clearly decreased and the acceptance rate of treated glass beads was significantly ($P \leq 0.05$) lower than that of aphids. A similar tendency was observed for the mean number of oviposition attacks per female, which decreased as the cornicle wax dried, even though the time-related differences between treatments were not significant ($P \leq 0.05$).

In comparison to naive females, experienced females, when exposed to beads coated with fresh wax, exhibited a reduced reaction, both in terms of number of individuals that reacted and of mean number of oviposition strikes, even though these differences were not

significant. However, in contrast to the results obtained with naive females, the number of individuals with experience that reacted to glass beads coated with fresh cornicle wax was significantly lower ($P \leq 0.05$) than the number of experienced parasitoids that reacted to aphids.

TABLE 1. Oviposition response of *Aphidius ervi* females, both naive and experienced, to pea aphid, *Acyrthosiphon pisum*, and to glass beads coated with fresh or dry cornicle secretion of the pea aphid. Values followed by different letters are significantly different ($P \leq 0.05$). Differences between treated and control beads were always significantly different ($P \leq 0.01$), both in terms of number of reacting individuals and number of oviposition attacks per parasitoid female.

Experimental conditions	Bead or aphid	N	N reacting	Oviposition attacks
♀ naive	treated	25	12^{ab}	$4.1^{a} \pm 3.84$
fresh wax	control	25	1	1 ± 0
♀naive dry	treated	25	12^{ab}	$3.8^{a} \pm 3.47$
wax (0.5 hr)	control	25	0	—
♀ naive	treated	25	7^{a}	$2.6^{a} \pm 2.44$
dry wax (3 hr)	control	25	0	—
♀ naive	4th instar	25	18^{b}	$9.9^{b} \pm 7.93$
aphid	pea aphid			
♀ experienced	treated	25	10^{a}	$2.7^{a} \pm 1.89$
fresh wax	control	25	0	—
♀ experienced	4th instar	25	19^{b}	$8.7^{b} \pm 5.19$
aphid	pea aphid			

There is a tendency for naive females to reduce the number of antennal examinations as the cornicle wax dries (Tab. 2). The same trend is observed in the total time spent by each naive female examining the treated bead and the mean values registered for fresh and 3 hr dry cornicle wax were significantly different ($P \leq 0.05$) (Tab. 2).

TABLE 2. Antennal examination behaviour of *Aphidius ervi* females, both naive and with experience, when in contact with glass beads coated with *Acyrthosiphon pisum* cornicle secretion and with aphids. Values followed by different letters are significantly different ($P \leq 0.05$).

Experimental conditions	N	Examination time (sec.)	Examination frequency
♀ naive, fresh wax	25	$15.46^{ab} \pm 19.27$	$4.80^{bc} \pm 5.72$
♀ naive, dry wax (0.5 hr)	25	$11.60^{bc} \pm 18.04$	$4.40^{c} \pm 5.48$
♀ naive, dry wax (3 hr)	25	$5.70^{c} \pm 8.75$	$2.0^{c} \pm 3.30$
♀ naive, aphid	25	$17.90^{a} \pm 20.77$	$12.20^{a} \pm 11.08$
♀ experienced, fresh wax	25	$22.38^{a} \pm 26.15$	$8.60^{ab} \pm 8.31$
♀ experienced, aphid	25	$16.0^{ab} \pm 17.81$	$14.80^{a} \pm 13.76$

Experience affects antennal examination behaviour. The mean number of antennal examinations was 4.8 for naive females exposed to fresh wax and up to 8.6 for females with experience (Tab. 2). Similarly, the total time spent by each female examining the treated bead was 15.46 sec for naive females and 22.38 sec for experienced females (Tab.

2). In both cases, however, these differences, even though evident, were not statistically significant. For naive females the number of antennal examinations of glass beads treated with fresh wax was significantly ($P \leq 0.05$) lower than that registered for aphids, while for experienced females these two values did not differ. The total time spent by parasitoid females examining treated glass beads and aphids was similar.

DISCUSSION AND CONCLUSIONS

Females of the endophagous parasitoid *A. ervi* are attracted by the cornicle secretion of the pea aphid, *A. pisum*. Glass beads coated with aphid cornicle wax were intensely examined by the parasitoid, which tried to oviposit in these aphid dummies. This finding is similar to the results published by Grasswitz & Paine (1992), reporting that the cornicle secretion of *R. padi* induces a similar behavioural response in the aphid parasitoid *L. testaceipes*. The reaction of *A. ervi* females to such a chemical cue seems to be innate and not influenced by learning. Associative learning might be responsible for the observed reduction, compared to aphids, in the acceptance rate of the wax coated glass beads by experienced females, which, although showing a reduced number of examinations still show an intense antennal examination of the aphid dummies. This suggests that the behavioural sequence leading to oviposition, even though stimulated, is not completed because of the absence of aphids, which are usually associated with the chemical cue under investigation. This is consistent with the general hypothesis that physical factors become important at this point in the host selection process (Vinson, 1991). A strong innate response to a chemical compound present in the cornicle secretion seems to occur in the case of *L. testaceipes*. For this parasitoid species, the kairomonal effect was specifically elicited by secretions obtained from *R. padi* and not from other aphid species (Grasswitz & Paine, 1992). This is not particularly surprising because it is well known that the chemical profile of the lipids occurring in the cornicle secretions of aphids is species specific (Callow et al., 1973; Greenway & Griffiths, 1973).

The interpretation of these experiments is difficult. Cornicle secretion is produced by *A. pisum*, when attacked by *A. ervi*, in only a negligible number of cases (Goff & Nault, 1974). Thus, the parasitoid rarely experiences the chemicals present in the cornicle wax. From an evolutionary point of view, it is hard to believe that such an unreliable cue would have been selected as host recognition kairomone. A possible explanation may be found in the composition of the cuticular lipids, which in several well documented cases have been reported to act as kairomones (Blomquist & Dillwith, 1985). It is reasonable to assume that, in the case of the pea aphid, some of these cuticular lipids are also present in the cornicle wax. In fact, unidentified hydrocarbons and triglycerids have been detected both in the cornicle secretions (Strong, 1967) and in the cuticular lipids (Stránský et al., 1973; Blomquist & Dillwith, 1985) of *A. pisum*. A comparative investigation is needed to identify the components that occur both on the cuticle and in the cornicle secretion of the pea aphid, and act as kairomones for *A. ervi* females.

The observed tendency for the kairomonal activity of the cornicle secretion to decrease as the wax dries is difficult to explain. It could be due to the low chemical stability when in contact with air or to a reduction of the availability of the bioactive compound/s in a suitable physical condition, necessary to stimulate the parasitoid's sense organs.

The strong innate response to cornicle wax recorded for *A. ervi* females suggests that behavioural manipulation in the laboratory of this species will probably be not extremely difficult. The isolation and chemical identification of the active component/s will greatly facilitate continuous in vitro rearing of *A. ervi*. For this parasitoid species we have already developed an artificial diet (Pennacchio et al., unpublished) allowing in vitro development up to mature (3rd instar) larva. The use of a chemical factor that stimulates oviposition into this artificial medium will be a further progress towards the continuous mass rearing of *A. ervi* in the absence of its natural host.

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