Oviposition in *Cryptolaemus montrouzieri* stimulated by wax filaments of its prey

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Abstract

The oviposition responses of *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) to the soft scale *Eupulvinaria hydrangeae* (Steinweden) (Homoptera: Coccidae) and to the mealybug *Planococcus citri* (Risso) (Homoptera: Pseudococcidae) have been compared in the laboratory. The females delay oviposition and withhold mature eggs in their lateral oviducts in the absence of wax filaments produced by the prey (only present in the ovisac of *E. hydrangeae*, present in all stages of *P. citri*). Contact chemical cues perceived by females when probing the wax filaments with their mouthparts are the signals inducing the search for oviposition sites. The second step is under the control of the ovipositor by which females locate confined sites to lay eggs. This oviposition behaviour could have a considerable impact on the prey exploitation strategy of this important biocontrol agent and might help to understand its apparent ineffectiveness in situations of low prey density.

Introduction

The coccinellid *Cryptolaemus montrouzieri* Mulsant is native to Australia and has been used in many control programs against a number of mealybug species (Pseudococcidae) around the world (Bartlett, 1974; 1978). The control efficiency of *C. montrouzieri* seems restricted to high densities of its prey (Bartlett, 1978; Murray, 1978; Moore, 1988), but reasons for this have not been analysed. Oviposition can be affected by prey density. It is generally considered that reproductive numerical response in coccinellids increases with the period of time spent feeding and ovipositing in high density aphid colonies (Banks, 1956; Dixon, 1959; Hodek, 1967), and that egg production and consumption are directly correlated (Beddington et al., 1976; Gutierrez et al., 1981; Ives, 1981; Mills, 1981). Recently however, Evans & Dixon (1986) have shown that *Coccinella septempunctata* L. oviposits in response to chemical cues produced by aphids, and Hemptinne et al. (1992) explored mechanisms by which an ovipositing female of *Adalia bipunctata* (L.) seems able to assess whether an aphid colony is suitable for offspring development. As mentioned by these latter authors, it is likely that oviposition is in part triggered by certain qualitative features of aphid populations.

In this paper we describe the oviposition response of *C. montrouzieri* to the mealybug, *Planococcus citri* (Risso) and to the soft scale, *Eupulvinaria hydrangeae* (Steinweden). *E. hydrangeae* is not a recorded prey of *C. montrouzieri* but our earlier laboratory and field experiments have shown that this ladybird beetle is able to complete its life cycle on *E. hydrangeae* (Merlin, 1992; Merlin et al., 1992). Life history and morphology of the two scale species are very different. In the present context, it is important to stress that, differing from the usual mealybug prey of *C. montrouzieri* in which waxy filaments cover the bodies of most instars (Cox & Pearce, 1983), *E. hydrangeae* only produce wax filaments in abundance to construct their ovisacs...
Acer pseudoplatanus L. females were conditioned for 24 h in a glass vial with a piece of leaf bearing second or third instar E. hydrangeae larvae and one ovisac. In the second treatment, each Petri dish contained E. hydrangeae larvae as in the first treatment but no ovisac. The number of eggs laid were counted after 1 h 30 min.

The second experiment involved adult females of E. hydrangeae instead of larvae. Adults of C. montrouzieri were conditioned for 24 h in a ventilated container with nearly mature E. hydrangeae females (i.e. individuals almost ready to oviposit) on A. pseudoplatanus twigs. The oviposition of individual females was recorded in two situations (10 replicates/treatment). In the first treatment, each Petri dish contained a twig infested with nearly mature E. hydrangeae females and one ovisac. In order to have at the same time both ovisacs and scale females, ovisacs collected in nature in September were kept in the freezer (−8 °C) until nearly mature females appeared in May the following year. In the second treatment, each Petri dish contained an infested twig as in treatment 1, a pellet of cotton-wool but no ovisac. Cotton-wool pellets were added in this treatment to mimick the physical properties of wax masses. The eggs laid were counted after 2 h.

Egg retention when ovisacs of E. hydrangeae are absent. The hypothesis was that C. montrouzieri females are able to withhold mature eggs when wax masses are unavailable, which would result in a larger number of eggs laid when ovisacs are finally met. One group of C. montrouzieri adults was conditioned for 24 h in a ventilated plastic container (14 × 9 × 5.5 cm) with infested sycamore twigs (3rd instar E. hydrangeae larvae) and leaves bearing ovisacs. Another group was conditioned with infested twigs and leaves as for the first treatment but with no ovisacs. After this conditioning period, individual females from both groups (20 replicates/treatment) were each moved to a separate Petri dish (9 cm in diameter) prepared in two ways. In the first treatment, each Petri dish contained a piece of A. pseudoplatanus leaf bearing second and third instar E. hydrangeae larvae and one ovisac. In the second treatment, each Petri dish contained E. hydrangeae larvae as in the first treatment but no ovisac. The number of eggs laid were counted after 1 h 30 min.

Materials and methods

Experiments were conducted in the laboratory with female C. montrouzieri taken from stock culture. The coccinellids were reared with the mealybug P. citri on potato sprouts in containers (20 × 30 × 13 cm) maintained in an environmental chamber at 20 °C ± 1 and L16:D8 photoperiod. The strain of C. montrouzieri, a relatively cold-tolerant biotype introduced into California by B. R. Bartlett (Bartlett, 1974), was obtained from Professor K. S. Hagen (University of California at Berkeley). The beetles were between ten days and one month old and ready to lay eggs when used in the experiments. Larvae, adult females and ovisacs of the soft scale E. hydrangeae were collected on infested Acer pseudoplatanus L. on the University campus. The ovisacs were totally empty of living eggs and crawlers as they were collected sufficiently long after the egg-hatching period. They did not constitute a source of food for the ladybird beetles. The various stages of the mealybug P. citri were taken from a stock culture on potato sprouts.

Stages of E. hydrangeae stimulating oviposition. Two separate experiments were carried out. In the first experiment, we compared the egg-laying of C. montrouzieri kept with scale larvae alone with that of C. montrouzieri kept with both scale larvae and ovisac wax (10 replicates/treatment). Coccinellid adults were conditioned for 24 h in a glass vial with a piece of moist paper and without food before testing. Individual females were then moved to a separate Petri dish (9 cm in diameter) prepared in two ways. In the first treatment, each Petri dish contained a piece of A. pseudoplatanus leaf bearing second and third instar E. hydrangeae larvae and one ovisac. In the second treatment, each Petri dish contained E. hydrangeae larvae as in the first treatment but no ovisac. The number of eggs laid were counted after 1 h 30 min.

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Stages of P. citri stimulating oviposition. This experiment was planned to investigate how the different stages of P. citri (larvae, females near maturity and ovisacs) stimulate oviposition of C. montrouzieri. Coc-
cinellid females were taken directly from the stock culture (without conditioning) and placed individually in 5 cm diameter Petri dishes either with 20 nearly mature *P. citri* females (treatment 1), or with a number of larvae corresponding to a volume similar to that of 20 females (treatment 2), or with a pellet of ovisac wax roughly the size of a real ovisac (treatment 3). In each dish, a piece of cotton-wool was added to serve as a mechanical substitute of oviposition site. There were initially 20 replicates for each of these three treatments. One replicate in treatment two had to be eliminated due to mistakes in sexing the insects. The eggs laid were counted after 20 h. As the set-up used previously was somewhat artificial and did not take into account certain physical aspects of *P. citri* colonies, a new experiment was set-up to analyse oviposition of *C. montrouzieri* under more realistic conditions, using 'naturally' infested potato sprouts to compare egg-laying for 24 h in two situations. In a first treatment, potato sprouts densely infested with *P. citri* larvae were provided; in a second treatment the potato sprouts were infested with females near maturity. Each *C. montrouzieri* female was placed on a sprout stuck into a piece of plasticine and enclosed in a glass tube (2 cm in diameter, 7.5 cm long). Fourteen replicates per treatment were made.

**Kairomonal activity of the wax filaments of *P. citri***

To test the hypothesis that wax filaments contain oviposition cues, egg-laying and consumption of individual *C. montrouzieri* females were compared in three different situations: in 5 cm diameter Petri dishes with a piece of cotton-wool, we placed either 20 live *P. citri* females adults (treatment 1), or 20 *P. citri* adults females washed in acetone (three or four washings until all the wax was dissolved) (treatment 2), or 20 *P. citri* adults females killed by freezing (12 h at -8 °C) (treatment 3). Treatment 3 constitutes a control to take into account the fact that the scales are killed by washing in acetone. *C. montrouzieri* females were directly taken from the stock culture with initially 15 replicates per treatment. One replicate in the treatment 3 had to be eliminated due to mistakes in sexing the insects. The numbers of eggs laid and the numbers of scale consumed were counted after 20 h.

A further step was to confirm the presence of an acetone-soluble kairomone by trying to restore ovipositional response using an acetone solution of *P. citri* wax, obtained as in the previous experiment. About 4000 *P. citri* adult females were washed in acetone. After evaporation of the solvent, the waxy deposit remaining lost its initial filamentous structure to form a kind of dust in which pellets of cotton-wool were rolled. Each experimental Petri dish received one of these pellets. Control Petri dishes received untreated cotton-wool. No food was provided during the experiment, which involved 20 replicates. Oviposition of individual *C. montrouzieri* females was recorded after 1 h. Before testing, the beetles were conditioned for 48 h in a ventilated container with nearly mature *E. hydrangeae* females on *A. pseudoplatanus* twigs as a food supply.

**Results**

**Ovipositional response to *E. hydrangeae***

**Stages of *E. hydrangeae* stimulating oviposition.** In the presence of an ovisac, *E. hydrangeae* larvae elicited oviposition of an average (± s.e.) of 5.6 ± 1.4 eggs. All the eggs were pushed in the wax masses. In the absence of ovisacs, no eggs were laid and careful observation of the females during the experiment excluded any cannibalism.

With *E. hydrangeae* adults, an average of 9.1 ± 1.1 eggs were laid per female when ovisacs were available. No eggs were laid in the absence of any ovisac, and the cotton-wool pellets failed to mimic ovisacs and induce oviposition. Therefore, neither *E. hydrangeae* larvae nor mature females by themselves induce oviposition of *C. montrouzieri*, and ovisac wax either by itself or in combination with scales, is necessary to trigger egg-laying.

**Egg retention when ovisacs are absent.** Females conditioned without ovisacs laid significantly more eggs than those conditioned with ovisacs when transferred into Petri dish where ovisacs were available (Table 1; log transformation of variables, t test: t = 4.5, P<0.001, df = 38). We found mature eggs in lateral oviducts when dissecting the females. They were significantly more numerous in those females conditioned without ovisacs than in those conditioned with ovisacs (log transformation of variable, t test: t = 7.32, P<0.001, df = 18). Thus, females refrain from ovipositing when no ovisacs are available and withhold their eggs in their oviducts until wax masses are met.

Similarly, after a week of conditioning without ovisacs, females withheld more eggs in their oviducts than females conditioned with ovisacs (x ± s.e., n = 10: 10.8 ± 1.05 and 1.8 ± 0.5 respectively). However, the
Table 1. Effect of conditioning of *C. montrouzieri* (presence versus absence of ovisac) on the number of eggs withheld in genital tracks and on the number of eggs laid when ovisacs are provided

<table>
<thead>
<tr>
<th>No. of eggs</th>
<th>Treatment: previous conditioning of the ladybird beedes (24 h)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With ovisac + <em>E. h.</em> larvae</td>
<td>Without ovisac + <em>E. h.</em> larvae</td>
</tr>
<tr>
<td>In genital tracks</td>
<td>4.4 ± 0.7 (a)</td>
<td>18.1 ± 1.7 (b)</td>
</tr>
<tr>
<td>Laid during 2 h</td>
<td>1.2 ± 0.5 (a)</td>
<td>8.8 ± 1.5 (b)</td>
</tr>
</tbody>
</table>

In each row, means followed by different letters are significantly different (Student t-test, α = 0.001)

Table 2. Oviposition and consumption of scales by females of *C. montrouzieri* in the presence of *P. citri* adults either live, killed by acetone or killed by freezing

<table>
<thead>
<tr>
<th>Number of</th>
<th>Treatment of <em>P. citri</em> (always cotton-wool added)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>P. citri</em> alive (n = 15)</td>
</tr>
<tr>
<td>Eggs laid</td>
<td>13.4 ± 1.2 (a)</td>
</tr>
<tr>
<td>Scales consumed</td>
<td>3.1 ± 0.3 (a)</td>
</tr>
</tbody>
</table>

In each row, means followed by different letters are significantly different (Newman-Keuls test, α = 0.05)

full capacity of females to withhold eggs is probably reached after one or two days. After a long absence of oviposition sites, there is at least one mechanism for managing egg production when the oviducts are saturated with eggs, i.e. immediate cannibalism of the female on her own eggs, which were laid anywhere in the container (Merlin, 1992).

**Ovipositional response to *P. citri***

**Stages of *P. citri* stimulating oviposition.** An average (± s.e.) 7.5 ± 1 eggs per female were laid in the presence of *P. citri* larvae + cotton-wool, 9.7 ± 1.3 eggs per female were laid in the presence of *P. citri* adults + cotton-wool, and 7.3 ± 1 eggs/female were laid in the presence of ovisac wax of *P. citri* + cotton-wool. No significant difference was found in the number of eggs laid in the different treatments (one-way analysis of variance: F = 1.329, P = 0.273, df = 2, 56).

All the *P. citri* stages stimulate oviposition of *C. montrouzieri* (bear in mind that a piece of cotton-wool was provided to satisfy their ovipositor thigmotactism). The eggs were found both in the pellets of cotton-wool and inside the clusters of scales or in the masses of wax. Thus, oviposition response of *C. montrouzieri* to the prey stages of *P. citri* or *E. hydrangeae* is not the same. Since all the *P. citri* stages produce wax filaments, an obvious hypothesis is that these constitute the oviposition cue, as is probably the case with *E. hydrangeae*.

In the presence of potato sprouts infested with colonies of either larvae or adults of *P. citri*, females of *C. montrouzieri* lay significantly more eggs when placed with *P. citri* adults (10.3 ± 0.6) than when placed with larvae (2.4 ± 0.6); Student t-test; t = 9.04, P < 0.001, df = 26. This result is in apparent contradiction with that of the experiment where scales were tested in Petri dish, in which no differences were observed. Our assumption is that, compared with larvae, *P. citri* adults provide more confined sites where *C. montrouzieri* females can insert their ovipositors and lay eggs. This is supported by the fact that 30% of the eggs were found in the junctions between the glass tubes and the plasticine in the treatment involving *P. citri* larvae, whereas all the eggs were laid amongst the scales in the treatment involving *P. citri* adults.

**Kairomonal activity of the wax filaments.** Egg-laying is significantly different between treatments (log transformation of variables; one-way analysis of variance: F = 341.07, P < 0.001, df = 2, 41; Table 2). The scales washed in acetone did not stimulate oviposition. We found one hundred times more eggs with live *P. citri* than with scales washed by acetone. Therefore, it seems that acetone eliminated some of the cues involved in oviposition.

Cues are very probably associated with the wax filaments, but we can not totally exclude that other secretions, also eliminated by acetone, constitute
the stimuli. In addition, the elimination of wax filaments changed the texture of the prey bodies which could affect tactile recognition. Consumption is also significantly different between treatments (log transformation of variables; one-way analysis of variance: F = 14.34, P < 0.001, df = 2, 41). *P. citri* killed by freezing or by acetone are clearly less appreciated than live *P. citri* (Newman-Keuls test, P < 0.05). A significant difference exists also between scales killed by freezing and those killed by acetone washing, the latter being less consumed.

The following experiment was to try restoring ovipositional response using an acetone solution of *P. citri* wax, obtained as in the previous experiment. In the cotton-wool rolled in the wax deposit, we counted an average of 9.85 ± 1.30 eggs (x ± s.e., n = 20). In the control, no eggs were found in the untreated cotton-wool. Thus, oviposition is triggered by adding chemical cues to the cotton-wool. Note that egg-laying occurred without any food item in the experimental set-up.

**Discussion and conclusions**

With *E. hydrangeae*, only the ovisac stage stimulates oviposition of *C. montrouzieri*. The other stages are consumed by adults and larvae of the ladybird beetle in the laboratory (Merlin et al., 1992) but are not recognized as suitable oviposition sites by females, even when a piece of cotton-wool is added to mimic the physical properties of the ovisacs.

With *P. citri*, all stages stimulate the search for oviposition sites. Thigmotactism seems essential at this level. Colonies of *P. citri* adult females appear more suitable for oviposition than colonies of younger stages probably because they provide more confined sites where ovipositor can be inserted. For the same reason, oviposition could be directly related to host density.

The sequence of ovipositional behaviour in *C. montrouzieri* seems thus to involve two steps. In the first step, contact chemical cues seem predominant in inducing the search for an oviposition site. However, compressed *E. hydrangeae* ovisacs do not induce oviposition. This suggests that the chemical cues have to be associated with a filamentous structure. Such an association is also known in the parasitoid *Apanteles melanoscelus* (Ratzeburg) (Weseloh, 1976; 1977). Female parasites only respond to the host kairomone when it has been placed on thin strands such as cotton fibres. After a positive response in the first step, *C. montrouzieri* females extrude their ovipositors and probe the substrate. The final step seems to involve mostly physical cues perceived by the ovipositor.

Egg-laying stimulation by chemical cues in coccinellids has previously been demonstrated in *C. septempunctata*, which responds to chemical traces left by aphids previously kept in a vial (Evans & Dixon, 1986). The chemical cues perceived by *C. montrouzieri* are non-volatile because we note no significant difference between the stimulation abilities of *E. hydrangeae* ovisacs kept for about 6 months in liquid nitrogen with those kept in incubator at 60 °C (unpubl.). The nature of these chemicals is still uncertain but our results strongly suggest that they are associated with the waxy secretions or are the wax components themselves. Indeed, *E. hydrangeae* larvae and pre-mature adults do not produce wax filaments, except for a few in their stigmatic furrows. On the contrary, all stages of *P. citri* are covered with loose dermal wax. Therefore, the oviposition response of *C. montrouzieri* appears to be linked to the presence of wax filaments. Van den Meiracker et al. (1990) have shown that the wax of the mealybug *Phenacoccus manihoti* Matile-Ferrero is an arrestment stimulus for the coccinellids *Diomus* sp. and *Exochomus* sp.

When oviposition sites are absent, *C. montrouzieri* withholds its eggs in the oviducts for an estimated period of at least 24–48 h. *C. septempunctata* is also able to delay oviposition in the absence of aphids or their chemical traces, but for a few hours only (Evans & Dixon, 1986). The relatively high capacity of *C. montrouzieri* to withhold eggs allows this ladybird beetle to be particularly discriminatory in the choice of oviposition sites. Some important differences can be pointed out between aphidophagous and coccidophagous coccinellids concerning their egg-laying sites. Only the latter have a telescopic sclerotinized ovipositor which allows them to place their eggs in bark cracks or often directly under the coccids (Iperti et al., 1977; Mills, 1982; Drea & Gordon, 1990). In aphidophagous species, the eggs are deposited on the plant surface, often but not always in the proximity of an aphid colony (Banks, 1956; Dixon, 1959). This could reflect specialised reproductive strategies linked to differences in the spatial and temporal stability of the respective prey.

Oviposition behaviour could also be the cornerstone of the specificity of *C. montrouzieri*. Females of *C. montrouzieri* taken at the time of emergence produce mature eggs when they are fed on *E. hydrangeae* lar-
vae or on eggs of the moth, *Ephesia kuhniella* (Zeller) (Lepidoptera: Pyralidae), but do not oviposit owing to lack of stimuli (Merlin, 1992).

The oviposition behaviour of *C. montrouzieri*, and the chemical and the physical stimuli involved, are obviously of considerable importance in the prey exploitation strategy of this ladybird beetle. They could determine, at least partially, at what stage and, possibly, at what density of prey the offspring exerts its predatory activity. From an applied point of view, further studies on the implication of this oviposition behaviour on the population dynamics of both prey and predator could lead to an understanding of the success or failure of this important biocontrol agent, and to the development of a more rational way of using it.

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