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Chemical cues produced by conspecific larvae deter oviposition by the coccidophagous ladybird beetle, *Cryptolaemus montrouzieri*

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Abstract

Experimental evidence shows that oviposition in *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) is deterred by the presence of conspecific larvae. A similar deterrent effect is also recorded when females are tested in an experimental set up that previously housed conspecific larvae. It is shown that an oviposition-deterring pheromone is associated with the abundant wax filaments produced by the larvae of *C. montrouzieri*.

Introduction

Cryptolaemus montrouzieri is a coccinellid of Australian origin extensively used to control populations of some pseudococcid pests (Bartlett, 1978; Moore, 1988). In addition to these usual prey, it has also been observed preying on *Pulvinaria* spp. (Coccidae) and *Eriococcus* spp. (Eriococcidae) colonies (Puttarudriah *et al.*, 1952; Bartlett, 1978; Panis, 1981). While some control programs were very successful, others failed for different causes such as poor *C. montrouzieri* adaptation to climate and inadequate prey density (Bartlett, 1978; Moore, 1988), although experimental evidence to support this latter hypothesis is lacking. As oviposition success could play an important role in the population dynamics of *C. montrouzieri*, especially when prey are scarce, experiments have been undertaken to study the cues involved in the oviposition behaviour of this species (Merlin *et al.*, 1996). It was found that chemical cues perceived by females when they probe the wax filaments produced by *Planococcus citri* (Risso) (a well known pseudococcid prey) and the wax of the ovisacs of *Eupulvinaria hydrangeae* (Steinweden) (an unrecorded coccid prey) stimulate oviposition in *C. montrouzieri*. The females withhold eggs in their oviducts in the absence of these cues. In addition, physical cues lead the females to select confined places to lay eggs. Abundance of adequate oviposition sites was shown to depend on the stage and density of the scale colonies.

On the other hand, the predator population in rearing boxes was observed to reach rapidly (in about three weeks) a stable size in spite of the abundance of food and the protracted (three or four months) egg-laying period of the females. This suggested that some mechanism is regulating oviposition.

Regulation is possibly exerted by conspecific larvae. First, it was studied if their presence affects oviposition. Then, the source of the cues was investigated. *C. montrouzieri* larvae produce abundant wax filaments (Pope, 1979) and their resemblance to their mealybug prey is remarkable. Considering the stimulating role of wax produced by scale insect prey in the oviposition of *C. montrouzieri* (Merlin *et al.*, 1996), the abundant wax filaments produced by the larvae of this coccinellid could possibly also be involved in regulating oviposition. Finally, the effect of the oviposition-deterring cue on egg spatial distribution was investigated.

Materials and methods

Origin of *C. montrouzieri*, stock culture conditions and physiological state of the beetles used in the experiments have been described in Merlin *et al.* (1996).

Three experiments involved *E. hydrangeae*. Fresh ovisacs collected on *Acer pseudoplatanus* L. (University campus) were used. They were fixed on the bottom of the experimental arena with a drop of hot paraffin-wax. *C. montrouzieri* always oviposit in ovisacs, where they push their eggs into the mass of wax (Merlin *et al.*, 1996). In these three experiments, *C. montrouzieri* females were tested individually after a conditioning period of 24 h with *E. hydrangeae* larvae or adults as food. Under these conditions, females do not oviposit and withhold their eggs in their oviducts (Merlin *et al.*, 1996). Two experiments involved *P. citri*, taken from a stock culture maintained on potato sprouts. The ladybird beetles used in these experiments were taken directly from the stock culture before testing.

All experiments were conducted in an environmental room where the stock culture of *C. montrouzieri* was maintained.

Effect of the presence of conspecific larvae on oviposition. In the first experiment, individual females of *C. montrouzieri* were placed in 5 cm diameter Petri dishes containing two ovisacs of *E. hydrangeae* and either 0, 1, 2 or 4 larvae of *C. montrouzieri*. There were 15 replicates of each treatment. The numbers of eggs laid by the ladybird beetles were recorded after 2 h.

In the second experiment, before testing females of *C. montrouzieri*, four *E. hydrangeae* ovisacs were placed in a 5 cm diameter Petri dish with either four *C. montrouzieri* larvae, or four larvae of another coccinellid species, *Rhyzobius forestieri* (Mulsant) (Coleoptera: Coccinellidae), or nothing as a control. There were 20 replicates of these 3 treatments. Larvae of *R. forestieri* were used as a control because they do not produce cottony wax but forage in the ovisacs in the same way as *C. montrouzieri* larvae. In this way, a possible effect of the foraging activity is controlled for. In order to avoid cannibalism and disturbance, the larvae were removed before testing the females. The ladybird beetle larvae were kept feeding on the ovisacs for 24 h before being removed; individual females of *C. montrouzieri* were then placed in the Petri dishes. The numbers of eggs laid were counted after 2 h.

Source of the oviposition-deterring cue. Oviposition of individual females of *C. montrouzieri* was compared in two situations. In the first treatment, 20 *P. citri* adults and one pellet of cotton-wool were placed in 5 cm Petri dishes. In the second one, *P. citri* and cotton-wool were similarly placed in dishes but wax filaments from *C. montrouzieri* larvae (collected by shaking a number of last instar larvae in a Petri dish) were spread over the scales, the cotton-wool and the bottom of the dishes. Since the wax of 150 larvae was collected on a piece of paper and spread as equally as possible amongst the 15 replicates, an amount of wax approximatively equivalent to 10 larvae was spread per Petri dish. There were initially 15 replicates of these two treatments but one replicate in treatment 1 had to be eliminated due to mistakes in sexing the insects. The eggs laid were counted after 24 h.

In another experiment, about 4000 *P. citri* adults were washed in acetone in order to dissolve their wax threads. The same washing was applied to wax threads of *C. montrouzieri* collected as in the previous experiment. After evaporation of the acetone in a glass Petri dish, a waxy dust remained on the bottom. Egg-laying of individual *C. montrouzieri* females in two situations was then compared. In the first treatment, one pellet of cotton-wool rolled in the waxy deposit of *P. citri* was placed in each Petri dish (5 cm diameter). In the second one, one pellet of cotton-wool rolled in the waxy deposit of *P. citri* and then rolled in that of *C. montrouzieri* was placed in each Petri dish (5 cm diameter). These latter pellets received an amount of *C. montrouzieri* wax approximatively equivalent to three larvae. There were 15 replicates. No coccids were provided to the ladybird beetles during the experiment. The numbers of eggs laid were counted after 2 h.

Effect of the oviposition-deterring cue on egg distribution. A consequence of an oviposition-deterring pheromone marking a cluster of prey would be to affect the spatial egg distribution of *C. montrouzieri*. This hypothesis was tested in an experimental set-up involving 3 *E. hydrangeae* ovisacs as oviposition sites (Fig. 1). Just before testing, females of *C. montrouzieri* were individually placed in a piece of straw (3 cm long) and kept inside by a plug of cotton-wool. Ovisac 1 was placed in front of the opening of the straw so that the ladybird beetle had to encounter it when it left the straw. Ovisac 2 was placed just behind and against the first one. Ovisac 3 was placed 4 cm away from the second one. Two treatments were compared. In the first one, ovisac 1 was spread with wax threads

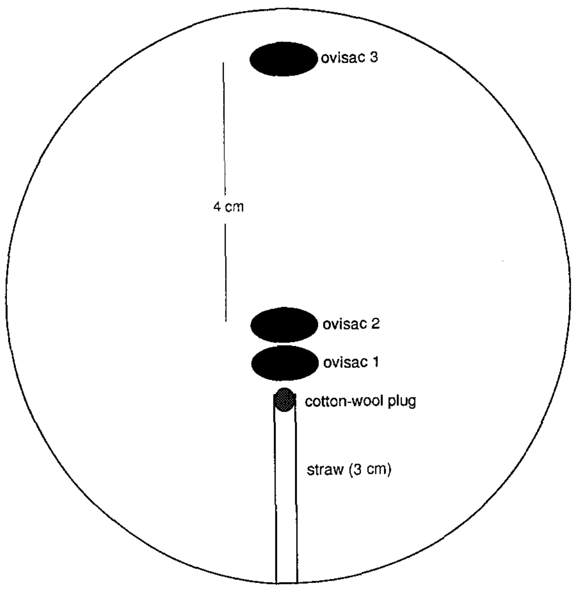

Fig. 1. Experimental set-up installed in 9 cm diameter Petri dishes to test changes in *C. montrouzieri* egg distribution induced by the oviposition-deterring cue.

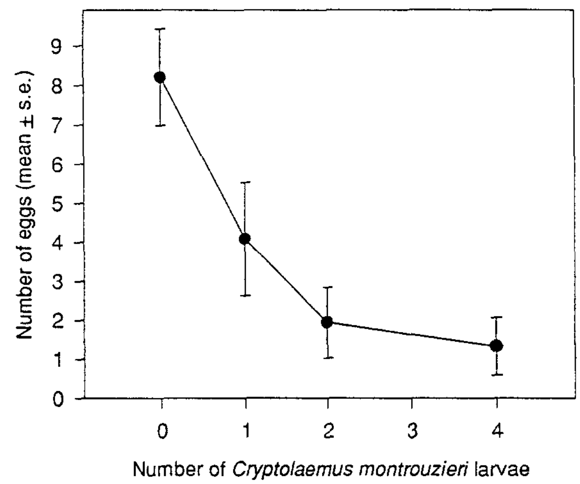

Fig. 2. Oviposition response of individual females of *C. montrouzieri* to an increasing number of larvae of their own species present in the experimental containers (n = 15).

of *C. montrouzieri* larvae collected as in the previous experiments. The two other ovisacs were untreated. In the second treatment (control), nothing was added to the ovisacs. The plug on the straw was removed when the experiment started and the females went out freely. The eggs laid were counted after 2 h. There were 14 replicates.

Results and discussion

Effect of the presence of conspecific larvae on oviposition. The number of *C. montrouzieri* eggs decreases with an increasing number of conspecific larvae in the experimental arena (Fig. 2). Larvae enter the ovisacs to feed on *E. hydrangeae* eggs. These results could be explained either by a regulation mechanism mediated by some cues perceived by females when they encounter conspecific larvae or traces of their activity, or by larval cannibalism on the coccinellid eggs laid in the ovisacs, or by the physical disturbance of ovipositing females by the larvae.

Another experiment was therefore carried out to test the first hypothesis and more particularly the possible role of traces left by the larvae. The mean numbers (± s.e.) of eggs laid were 3.13 ± 1.05 in the presence of 4 *C. montrouzieri* larvae, 10.60 ± 1.58 in the presence of 4 *R. forestieri* larvae and 10.85 ± 1.28 with no coccinellid larva. These results suggest that traces left by *C. montrouzieri* larvae induce a significant decrease in egg-laying, whilst the presence of *R. forestieri* larvae does not influence oviposition (Newman-Keuls multiple-range test, $\alpha = 0.05$).

Source of the oviposition-deterring cue. It is reasonable to assume that *C. montrouzieri* larvae lose some wax filaments when they forage in the ovisacs, and these could be the oviposition-deterring cue perceived by the females. Indeed, the presence of *C. montrouzieri* wax threads significantly inhibits egg-laying [mean numbers (± s.e.) of eggs laid on cotton-wool alone: 6.93 ± 1.47; on cotton-wool + wax threads: 2.07 ± 1.12; one-way analysis of variance: $F = 7.00$, $P = 0.013$, $df = 1, 27$].

In a previous paper (Merlin *et al.*, 1996), it was shown that when *P. citri* are washed in acetone, the waxy deposit remaining in the box after evaporation of the solvent stimulates the oviposition of *C. montrouzieri*. Here, it was tested whether, conversely, oviposition might be deterred by adding a similar waxy deposit from *C. montrouzieri* larvae to that from *P. citri*. The results obtained from this experiment confirm that the egg-laying of *C. montrouzieri* females is significantly reduced when the dust of *C. montrouzieri* wax is added to the stimulating dust of *P. citri* wax [mean numbers (± s.e.) of eggs laid on cotton-wool + waxy deposit from *P. citri*: 3.40 ± 0.73; on cotton-wool + waxy deposit from *P. citri* + waxy deposit from *C. montrouzieri*: 1.07 ± 0.44; one-way

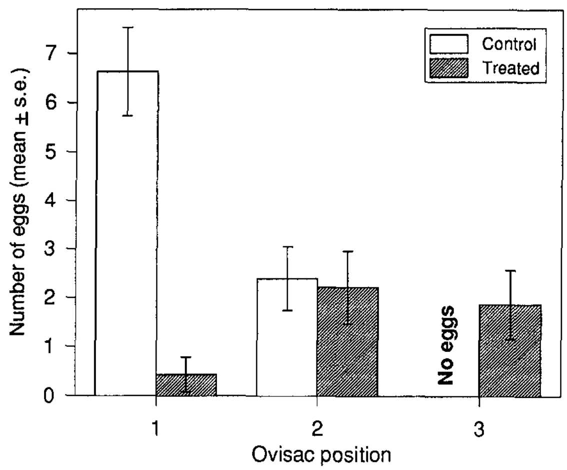

Fig. 3. Change in the egg distribution of females of *C. montrouzieri* when one of the oviposition sites (ovisac 1, see Fig. 1) is inhibited by wax of conspecific larvae (n = 14). On the x-axis, 'Ovisac position' refers to the three oviposition sites in Fig. 1.

analysis of variance on log-transformed variables: F = 7.254, P = 0.012, df = 1, 28].

Effect of the oviposition-deterring cue on egg distribution. The presence of *C. montrouzieri* larval wax on the first ovisac found by the female leaving the straw notably changes the spatial distribution of eggs (Fig. 3). Almost no eggs were laid in ovisac 1 treated with the coccinellid wax, whereas the corresponding ovisac in the control collected most of the eggs. Conversely, eggs were found in ovisac 3 in the experimental treatment whereas no eggs were observed in the corresponding ovisac in the control.

In conclusion, the results demonstrate that oviposition of *C. montrouzieri* females is deterred by the presence of conspecific larvae in the experimental arena. Hemptinne *et al.* (1992) reached the same conclusion for *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae). Furthermore, it is shown that an oviposition-deterring pheromone is associated with wax filaments produced by larvae of *C. montrouzieri*. Females, therefore, would be deterred from laying eggs on a colony of mealybug where larvae are, or have been preying. It seems that the extent of this inhibition is modulated by the density of conspecific larvae (see the first experiment). The last experiment suggests that the rate of encounter with conspecific larvae or traces of their activity modifies the dispersal behaviour of females and egg distribution. This would clearly limit intraspecific competition.

Avoiding local overexploitation of resources in *C. montrouzieri* could be the more advantageous as the larvae appear inefficient dispersers. Indeed, it was observed both in the field (Merlin *et al.*, 1992) and in the laboratory (Merlin, 1992; A. Drumont, pers. comm.) that *C. montrouzieri* larvae are poorly adapted for crawling on the leaf and twig surfaces and very easily fall from the plant. Therefore, a batch of *P. citri* on a plant would constitute a spatially limited food resource. Conversely, they show an outstanding ability to forage in sticky masses constituted by a mixture of wax, ovisacs, scales and honeydew, as produced by dense infestations of *P. citri* in rearings on potato sprouts.

Thus, the quality of egg-laying sites is at least partially assessed by ovipositing females, on the one hand through chemical and physical stimuli produced by the prey (Merlin *et al.*, 1996) and on the other hand through an oviposition-deterring pheromone from conspecific larvae already preying.

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