



Research Article

Ovipositional responses of *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) to the presence of prey, *Maconellicoccus hirsutus* (Green)

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ABSTRACT: Studies to understand the influence of prey-specific cues on the clutch size of predatory coccinellid, *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) were carried out under laboratory conditions to determine factors which act as positive ovipositional cues to the female. Correlation analysis between the clutch size and the oviposition time indicated that the clutch size is influenced by prey specific cues like prey eggs and honeydew. The regression analysis also explained the maximum variability in the clutch size in the presence of prey specific cues viz., prey eggs along with honeydew ($y = 0.2725x + 3.5593$; $R^2 = 0.8742$; 0.94^{**}); prey eggs along with exuviae and honeydew ($y = 0.2865x + 3.2844$; $R^2 = 0.8384$; $r = 0.92^{**}$) and prey eggs alone ($y = 0.257x + 3.7467$; $R^2 = 0.7805$; $r = 0.88^{**}$). The study indicated that prey specific cues like prey eggs and honeydew serve as positive ovipositional cues for gravid *C. montrouzieri*.

KEY WORDS: Predator, coccinellid, *Cryptolaemus montrouzieri*, clutch size, prey specific cues

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INTRODUCTION

Generally visual and chemical cues provide insect predators with the information on the abundance and quality of prey in a particular patch thereby, encouraging the females to oviposit. Usually, clutch size (number of eggs laid per day per female) varies among and within coccinellid species (Stewart *et al.*, 1991), depending on the factors such as body size (Dixon and Guo, 1993), age (Smith, 1966) and food abundance (Dixon and Guo, 1993; Agarwala and Bardhanroy, 1999). In the laboratory, the clutch size in *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) was significantly affected by the time interval in oviposition (Osawa, 2003). Further, the oviposition deterrent effects of conspecific and heterospecific larvae have been reported under field conditions in several coccinellid predators viz. *Cheilomenes sexmaculata* F. and *Scymnus pyrocheilus* Mulsant (Jha *et al.*, 1991; Bind and Bind, 1993; Sugiura and Takada, 1998; Agarwala and Bardhanroy, 1999; Ulrichs *et al.*, 2001; Omkar, 2004). Although there has been considerable interest in the reproductive tactics of predatory insects (Dixon and Guo, 1993), studies on influence of prey-specific cues on oviposition are limited. This is also true in the case of *Cryptolaemus montrouzieri* Mulsant, a

potential predator of mealybugs and a recommended biocontrol agent as well. However, its predation efficacy is not sustained over time and predation in *C. montrouzieri* tended to be random or chance encounters, and visual/olfactory cues seemed to have less role in enhancing predation (Verghese *et al.*, 2001; Kamala Jayanthi *et al.*, 2010, 2012). One of the reasons may be the non-availability of prey which fluctuates temporally and this seems to affect the predatory viability, necessitating inundative releases costing huge efforts. Usually, *C. montrouzieri* is considered a successful predator under high prey densities (Fand *et al.*, 2010). Therefore, prey specific cues may have a role in influencing the oviposition decision of the mother. Here, we make an attempt to study whether the prey specific cue is a prerequisite to obtain satisfactory clutch size in *C. montrouzieri*.

MATERIALS AND METHODS

The present study was carried out at the Indian Institute of Horticultural Research, Bangalore (12°58'N; 77°35'E), India. Laboratory cultures of *C. montrouzieri* reared on *Maconellicoccus hirsutus* (Green) (Pseudococcidae: Heteroptera) (pink hibiscus mealybug) with *Cucurbita moschata* (pumpkin) fruit as laboratory host (Kairo *et al.*, 1997) were used for the study. The pupae

of *C. montrouzieri* of uniform age were randomly selected from the established laboratory culture and kept in a Petri dish till the adults emerged. After adult emergence, the beetles were fed with second instar nymphs of *M. hirsutus* daily for 7 days. The adult beetles were then sexed and the post-mated female *C. montrouzieri* were selected and placed individually on a filter paper in a 90 mm diameter Petri dish (approximately 64 cm²) lined with Whatman filter paper (90 mm diameter).

The prey (*M. hirsutus*) specific cues included for the study were prey eggs (E), adult mealybugs (A), white mealy covering or exuviae (M) of adult mealybugs and honeydew (H) excreted by the adult mealybugs. In case of prey eggs, uniform sizes of intact egg masses of *M. hirsutus* were selected for the study. The exuviae of the adult females was collected from the mealybug rearing cages using camel hair brush. These were rolled into small fluffy balls (5 mm diameter) and used for the study. The honeydew, a semi solid sugary material excreted by the adult mealybugs was collected on to the small bits (1 x 1 cm) of Whatman filter paper No. 1 from the mealybug infested pumpkins using a camel hair brush.

These prey cues *viz.*, adult mealybugs, eggs, exuviae and honeydew were deployed in different Petri plates in the following combinations. Each gravid female was provided with i., adult prey alone (10 uniform size of female mealybugs without egg sac) (treatment A) , ii. with

prey eggs alone (10 egg sacs for each Petri plate) (treatment E), iii. with a combination of prey eggs (10 egg sacs for each Petri plate) and exuviae (treatment E+M), iv. with prey eggs (10 egg sacs for each Petri plate) and honeydew (treatment E+H) and v. with a combination of prey eggs, mealy covering and honeydew (treatment E+M+H). Observations were made on the number of eggs laid each day by *C. montrouzieri* for 10 days continuously and each treatment had ten beetles. All the cues used in the study were changed afresh daily. The data were subjected to ANOVA, correlation, linear and nonlinear analyses (Little and Hills, 1978).

RESULTS AND DISCUSSION

Cryptolaemus montrouzieri gravid females laid significantly ($P < 0.001$, F ratio: 13.42, df: 45) more eggs in the presence of mealybug (prey) eggs alone (5.16 ± 0.28 /adult/day) compared to mealybug adults alone (4.09 ± 0.19 /adult/day) as well as combination of prey eggs and mealy covering (3.00 ± 0.15 /adult/day) (Fig. 1). No significance differences ($P > 0.05$) were detected in the number of eggs laid by *C. montrouzieri* females when exposed to prey specific cues *viz.*, prey eggs alone, prey eggs in combination with mealy covering/ honeydew (4.86 ± 0.30 /adult/day) and prey eggs along with honeydew (5.06 ± 0.28 /adult/day) (Fig. 1). However, the lowest eggs ($P < 0.001$) were laid when exposed to prey eggs in combination with exuviae or mealy covering (Fig. 1). It

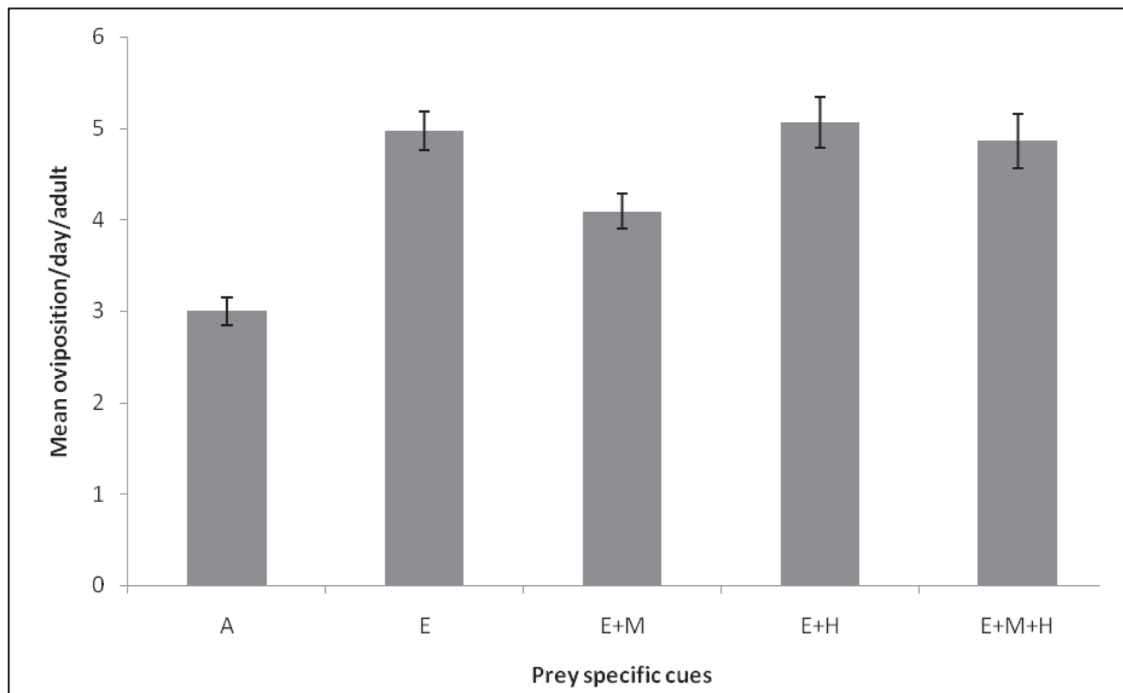


Fig. 1. Differences in clutch size of gravid *C. montrouzieri* in different treatments; treatments followed by same letter are not significantly different from each other (A: adult mealybugs alone; E: mealybug eggs alone; E+M: mealybug eggs and exuviae; E+H: mealybug eggs and honeydew; E+M+H: mealybug eggs with exuviae and honeydew)

was observed that the presence of exuviae hindered the female oviposition in spite of the presence of the mealybug eggs. Further, when prey eggs were combined with exuviae and honeydew produced by the mealybugs, the number of eggs laid by *C. montrouzieri* increased significantly (4.86/day) ($P < 0.05$) over prey eggs in combination with mealy covering (Fig. 1). Overall, the clutch size per day varied among treatments and ranged from 3.93 – 6.80 (prey eggs alone), 2.13 – 3.47 (adult mealybugs alone), 3.33 – 4.93 (prey eggs with exuviae), 3.86 – 6.12 (prey eggs with honeydew) and 3.67 – 6.40 (prey eggs along with exuviae and honeydew). The correlation analysis showed a highly significant ($P < 0.01$) positive correlation between clutch size and oviposition period (number of days) for treatments *viz.*, prey eggs alone ($r = 0.88^{**}$), prey eggs with honeydew ($r = 0.94^{**}$) and prey eggs with mealy covering and honeydew ($r = 0.92^{**}$). However, there was no significant correlation between the prey eggs with mealy covering ($r = 0.22$) and adult mealybugs alone ($r = -0.02$) as days progressed during experimentation.

The maximum variability in the egg number was explained by simple linear regression models for the treatments: prey eggs alone, prey eggs with honeydew and prey eggs along with mealy covering and honeydew. The regression analysis showed that the highest significant (F ratio: 55.62, $P < 0.0001$) variability of 87% in the clutch size of female *C. montrouzieri* was explained by simple linear model, $y = 0.2725x + 3.5593$; $R^2 = 0.8742$ when exposed to a combination of prey eggs with honeydew followed by 84%, $y = 0.2865x + 3.2844$; $R^2 = 0.8384$

(F ratio: 41.50, $P = 0.0002$) in the treatment combination of prey eggs along with mealy covering and honeydew and 78% ($y = 0.257x + 3.7467$; $R^2 = 0.7805$; F ratio: 28.45, $P < 0.001$) in treatment with prey eggs alone. Further, the treatments *viz.*, adult mealybugs alone ($y = -0.002x + 3.01133$, $R^2 = 0.0002$; F ratio: 0.002, $P = 0.97$) and prey eggs in combination with exuviae did not exhibit significant R^2 through linear equation ($y = 0.0446x + 3.8453$, $R^2 = 0.0496$; F ratio: 0.42, $P = 0.54$). However, here the polynomial order (2) explained the variability up to 74% ($y = 0.0658x^2 - 0.6789x + 5.2922$, $R^2 = 0.7419$) (Fig. 2). The variability in clutch size was explained sufficiently by the presence of honeydew, prey eggs and mealy covering. The former two had a positive effect while the mealy covering /exuviae seemed to inhibit the egg number laid per day.

In the present study, the prey specific cues like prey eggs and honeydew excreted by prey are the signs of active prey abundance which increased the clutch size in the predatory coccinellid, *C. montrouzieri*. Previous studies reported that the oviposition in ladybirds is determined by the availability of prey (Dixon, 1959; Evans and Dixon, 1986) without specific mention of cues. In the present study the prey eggs and the honeydew that are the indicators of the fresh infestation of prey influenced the clutch size in the *C. montrouzieri* more positively than the exuviae which is the left over product of old infestation or a sign of declining prey populations. Presence of adult mealybugs alone did not exhibit any influence on the clutch size proving the earlier studies that *C. montrouzieri* prefers to oviposit on the mealybug egg sacs (Raupp

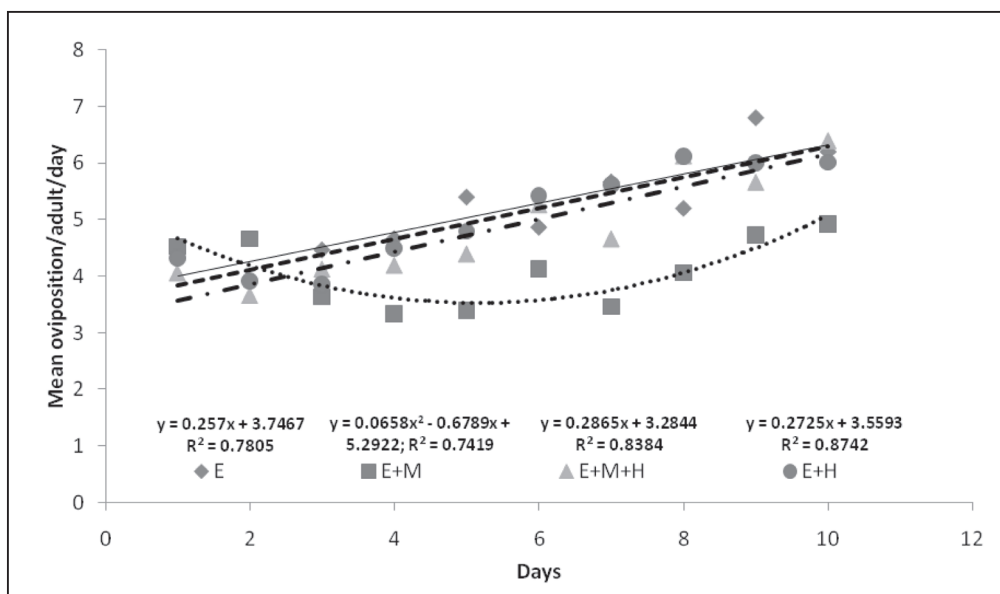


Fig. 2. Relationship between mean oviposition in adult *C. montrouzieri* under different conditions (A: adult mealybugs alone; E: mealybug eggs alone; E+M: mealybug eggs and exuviae; E+H: mealybug eggs and honeydew; E+M+H: mealybug eggs with exuviae and honeydew)

et al., 1993). The correlation analysis also showed a high significant positive relationship with the treatments comprising the prey eggs as well as honeydew. Nevertheless, the treatment comprising the prey eggs and mealy covering did not show any significant relationship with the clutch size in *C. montrouzieri* as days progressed. Further, this treatment combination (prey eggs and exuviae) brought down the clutch size indicating its negative influence on the ovipositing *C. montrouzieri*, probably inhibiting egg laying or even egg formation. This is also suggestive of a probable hormonal trigger for reproductive development in the presence of prey eggs and honeydew. This physiological angle is a future line of work. This aspect is reinforced by the fact that the addition of honeydew to the above treatment indicated synergistic effect through significant increase in the clutch size.

Dixon and Guo (1993) reported that prey abundance affects the potential egg production in *Coccinella septempunctata* L. Michael (2009) reported that food-based cues indicate the quantity or quality of a food item that directly influence a female's decision to oviposit and particularly the coccinellids exhibit high ovipositional preference for the sites with abundant essential prey. Honeydew is considered as an arrestant during coccinellid foraging as it often indicates the presence of live prey (Carter and Dixon, 1984; Ide *et al.*, 2007; Michael, 2009). On the contrary, some coccinellids may avoid ovipositing near sticky substances, possibly because of a large amount of honeydew, often associated with declining colonies (Iperti and Prudent, 1986; Johki *et al.*, 1988). However, Evans and Dixon (1986) observed that small amounts of honeydew along with aphid odours can elicit oviposition of gravid aphidophagous coccinellids even when aphids are absent. In the present study also only a small quantity of honeydew was used on filter paper as cue, would have served as positive stimuli to the gravid *C. montrouzieri*. Thus, the titre and volume of honeydew and source of (prey) honeydew may all have the effect on egg production and opens an array of new studies. In the present study, presence of honeydew enhanced the clutch size and addition of mealy covering or exuviae exhibited negative impact on the clutch size of *C. montrouzieri*. The simple linear regression equations explained maximum variability in the clutch size of *C. montrouzieri* in the presence of prey eggs alone as well as prey eggs along with mealy covering and honeydew explaining the relationship between these cues and clutch size is simple and straight line fit. However, when gravid beetles were exposed to a combination of prey eggs and mealy covering, instead of linear regression, the polynomial order (2), a quadratic function gave the

best fit explaining the maximum variability in the clutch size hinting at trade-offs between the cues viz., the dominating negative effect of mealy covering over prey eggs. Therefore, the present study clearly establishes that the prey specific cues viz., prey eggs and honeydew serves as positive cues for oviposition and enhances the clutch size in predatory coccinellid, *C. montrouzieri*.

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