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Behaviour of *Rhynocoris marginatus* (Fabricius) (Heteroptera: Reduviidae) to chemical cues from three lepidopteran pests

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ABSTRACT: **A laboratory experiment was conducted to find out the excess proportion index (EPI) and frequency of prey location (rostrum protrusion) behaviour of a reduviid predator, *Rhynocoris marginatus* (Fabr.) to solvent extracts of three groundnut pests, *Aproaerema modicella* Dev., *Helicoverpa armigera* (Hübner) and *Spodoptera litura* (Fabr.), using six-arm glass olfactometers. Fourth, 5th instar and adult stages of the predator were attracted by all the larval extracts. The frequency of prey location behaviour was high to *S. litura* larval extracts for fourth (10.43 minutes), fifth (12.07 minutes) instar nymphs and adults (15.38 minutes) of the predator, though statistically not significant. In general, adult predators exhibited high frequency of prey location behaviour for all the tested pests.**

KEY WORDS: *Aproarema modicella*, *Helicoverpa armigera*, prey location, *Rhynocoris marginatus*, *Spodoptera litura*

The possibilities of predatory insects as pest control agents in Integrated Pest Management (IPM) have been investigated in many papers. Reduviid bugs have been used as biocontrol agents against *Helicoverpa armigera* (Hübner) and *Spodoptera litura* (Fabricius) in field condition (Sahayaraj, 1999a). Natural enemies utilize a variety of stimuli to locate and identify their hosts based on chemical cues emanating from the host's body. Herbivorous prey may influence predators because the herbivores are chemically (allelochemicals) defended to some degree. These allelochemicals are present in the gut, haemolymph and nutrient storage sites (Duffey, 1980). The release of homogenates of lepidopterous larvae has been shown to increase retention of many generalised predators (Gross *et al.*, 1985; Senrayan, 1989). Although the role of allelochemicals in the relationship between the parasitoid, predator and its host has been described in many papers (Weseloh, 1981; Yasuda and Wakamura, 1996; Yasuda, 1997; Ananthakrishnan, 1999), no studies have dealt with the allelochemical relationship between reduviids and their prey insects. In this study, we investigated the behavioural response (prey searching and rostrum protruding behaviour) of the different life stages of *Rhynocoris marginatus* to solvent extracts of *Aproaerema modicella*, *H. armigera* and *S. litura* larval stages in order to reveal any chemical cue (s) that might elicit prey stage location behaviour.

MATERIALS AND METHODS

Predator

A colony of the predator, *R. marginatus* was maintained on larvae of *A. modicella*, *H. armigera* and *S. litura* in laboratory conditions (28 ± 2°C; L 13: D11 photoperiod and relative humidity 73

± 4%) as per methods developed by Sahayaraj (1995). One day old adults and fourth and fifth instar nymphs were used for this study.

Pests

Different larval instars of *A. modicella, H. armigera* and *S. litura* were collected from groundnut fields in and around Palayamkottai and reared on groundnut plant under laboratory conditions. For the assay, legated larvae were used in order to remove any possible influence of feaces or regurgitation of the larvae.

Solvent extracts of pest larvae

Fifty fourth instar *S. litura* larvae were extracted with 100ml of hexane and acetone (1: 2 mixture) for 12 hours at room temperature. The extract was filtered through a Whatmann No. 1 filter paper. The solvent was removed and the residues were dissolved with one ml of acetone and used for the experiment. Same procedure was followed for other instars of *S. litura* and also other pests studied.

Glass Olfactometer

A six-armed glass olfactometer designed earlier in the laboratory was used to find out the orientation of the predators towards the kairomones of different pest larvae. The glass olfactometer consists of a central chamber (6 cm diameter and 3cm height) from which six glass tubes (20cm length and 2.5cm diameter) project outwards. The angle between two tubes is 60°. The middle chamber has a opening (2.5cm diameter) at the top. The distal end of each arm is fitted to a glass beaker (9 cm height and 7 cm diameter).

Behaviour observation

For test material, a small piece of filter paper (Whatmann No. 1) (5 x 2 cm) was impregnated with the fourth instar *S. litura* larval extracts (100 µl) and placed inside three beakers alternatively. In the remaining three beakers, a small piece of filter paper (5 x 2 cm) with 100µl acetone was placed and treated as control. Then the beakers were closed with muslin cloth. Ten 24h starved fourth instar predator nymphs were introduced into the central tube and plugged tightly with plastic lid. Behavioural observation was recorded for 30 minutes and repeated 10 times using different individuals. The rostrum-protruding response of *R. marginatus* was scored for every 30 minutes on different instars of *S. litura* larval extracts. Similar procedure was followed for other pests studied here.

Computing of index and statistical analysis

We assessed the approaching response observed in the glass olfactometer and defined it in terms of an excess proportion index (EPI), according to Sakuma and Fukami (1985) and Yasuda and Wakamura (1996). The EPI is calculated as follows:

$$EPI = (NS - NC)/ (NS + NC)$$

Where NS - number of animals choosing the sample side and NC - number of animal choosing the control side. Analysis of variance technique was followed and means were separated by Duncan's New Multiple Range Test (DMRT) at 5 per cent level of significance (Duncan, 1955).

RESULTS AND DISCUSSION

Approaching behaviour

Rhynocoris marginatus nymphs and adults were found attracted to the solvent extracts of different pest larvae studied. Once the predator approached the filter paper, then protruded its rostrum towards the filter paper and inserted the rostrum into the paper placed on the beaker. However, the predators did not approach the filter paper which was treated by acetone alone (control). These observations suggested that any volatile chemical (s) attracted the bugs and stimulated them to protrude the rostrum and prey-location behaviour of the reduviid bug. Irrespective of the pest larvae, adult predators continued this behaviour for more than 9 minutes followed by fifth (7min) and fourth instars (5 min) of *R. marginatus* to *A. modicella* larval extract

Table 1. Frequency (in minutes) of prey-location behaviour of *R. marginatus* to three groundnut pests larvae

Predator Stage	Odour source			
	A. modicella	*H. armigera*	*S. litura*	Control
Fourth instar	5.13 ± 0.54^{oc}	8.51 ± 1.03^{cB}	10.43 ± 0.47^{cA}	0.0^{ab}
Fifth instar	7.46 ± 0.27^{bc}	11.36 ± 0.58^{bB}	12.07 ± 0.53^{bA}	0.0^{aD}
Adult	9.08 ± 0.44^{aC}	13.07 ± 0.47^{aB}	15.38 ± 1.77^{aA}	0.0^{aD}

Values carrying the same lower case letter in a column and upper case letter in a row are not statistically significant at $p < 0.05$, using the Duncan's Multiple Range Test (DMRT).

(Table 1). The duration of rostrum protrusion to the *S. litura* larval extracts was higher than the other larval extracts and their differences were statistically significant ($p < 0.05$). The bugs showed the prey-location behaviour only to the larval extract and the difference in the frequency of prey location towards different larval extracts was significant at 5 per cent level by DMRT. Some chemicals were identified as "kairomones" in predators (Gross *et al.*, 1985; Yasuda and Wakamura, 1996; Yasuda, 1997). The results indicated that all the life stages of the predator spent more time when they were encountered with *S. litura* larval extracts followed by *H. armigera* and *A. modicella* and confirm the visual observation of Sahayaraj (1999b).

The EPI values showed that all the larval extracts had attractant activity and ranged from 0.44 to 0.89 (Table 2). The results indicated that *R. marginatus* fourth (0.79) and fifth nymphal (0.89) instars preferred *H. armigera,* followed by *S. litura* (0.72 and 0.78 for fourth and fifth nymphal instars, respectively) and *A. modicella.* The DMRT analysis showed that preference of predator stages to *A. modicella* was significantly lower ($p < 0.05$) than that of *H. armigera* and *S. litura.* The adult predators highly preferred

Table 2. Impact of different pest larval extracts on the EPI value of *R. marginatus*

Predator Stage	Preference (in %)		
	A. modicella	*H. armigera*	*S. litura*
Fourth instar	0.56^{aB} (fifth instar)	0.79^{aA} (Fourth instar)	0.72^{aA} (Fourth instar)
Fifth instar	0.57^{aB} (fifth instar)	0.89^{aA} (Fifth instar)	0.78^{aA} (Fifth instar)
Adult	0.44 (fifth instar)	0.67^{bB} (Fifth instar)	0.80^{aA} (Sixth instar)

Values carrying the same lower case letter in a column and upper case letter in a row are not statistically significant at $p < 0.05$, using Duncan's Multiple Range Test (DMRT).

S. litura followed by *H. armigera* and *A. modicella* and it was statistically significant ($p < 0.05$) by DMRT. Table 2 shows that larger predator accepted the larger-sized prey and the smaller predator accepted the smaller-sized prey (except *A. modicella*) and so the timely release of the predator is very important in biological control programme.

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