

Approaching and rostrum protrusion behaviours of *Rhynocoris marginatus* on three prey chemical cues

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Abstract

Rhynocoris marginatus (F.) (Heteroptera Reduviidae) is a polyphagous predator predominantly found in agroecosystems, and their bordering ecosystems like scrub jungles, semi-arid zones and forests in India. Although *R. marginatus* is a polyphagous predator, it exhibited a certain degree of host specificity. Due to its predatory potential, *R. marginatus* has been used as an important biological control agent in India. Laboratory and field trials showed that *R. marginatus* feeds mainly on lepidopteran pests followed by coleopteran pests. *R. marginatus* locates the preys by the chemical cues emanating from them. Approaching and rostrum protrusion behaviours of *R. marginatus* life stages on hexane extract of three groundnut pests, *Helicoverpa armigera* (Hubner) (Lepidoptera Noctuidae), *Spodoptera litura* (F.) (Lepidoptera Noctuidae) and *Mylabris pustulata* (Thunberg) (Coleoptera Meloidae). Significantly *R. marginatus* adult was found to be more responsive to the chemical cues of *S. litura* (62.5%) followed by *H. armigera* (60%) and *M. pustulata* (40%). *R. marginatus* showed minimal preference to *M. pustulata* chemical cues as compared to *H. armigera* and *S. litura* chemical cues. The prey's chemical cues elicited a quicker approaching behaviour of the predator than the control. Moreover the rostrum protrusion time was also higher in the prey chemical cues categories as compared to the control. The results clearly show the role of prey chemical cues in prey-predator interaction. GC-MS analysis of the prey chemical cues indicated *H. armigera*, *M. pustulata* and *S. litura* contains eight, eight and five saturated hydrocarbons, respectively.

Key words: *Rhynocoris marginatus*, biological control agent, *Helicoverpa armigera*, *Spodoptera litura*, *Mylabris pustulata*, chemical cues.

Introduction

Inoculative release or augmentation of natural enemies as biological control agents of insect pests in Integrated Pest Management (IPM) is gaining more importance due to the adverse effects of synthetic pesticides. Insects use chemical signals to orient, survive and reproduce in their specific environments. As stated by Duffey (1980), these chemical cues are present in the gut, hemolymph and nutrient storage sites of the prey species. *Rhynocoris marginatus* (F.) (Heteroptera Reduviidae), a predatory reduviid is found to be an important natural enemy of several economically important pests including *Helicoverpa armigera* (Hubner) (Lepidoptera Noctuidae), *Spodoptera litura* (F.) (Lepidoptera Noctuidae) and *Amsacta albistriga* (Walker) (Lepidoptera Arctiidae) (Sahayaraj, 2002b; Sahayaraj and Ravi, 2007). Recently Sahayaraj and Raju (2006) reported that *R. marginatus* was the top predator than *Menochilus sexmaculatus* (F.). Studies on the host searching behaviour of this heteropteran predator to the prey kairomones were carried out (Maran, 1999; Sahayaraj and Paulraj, 2001; Sahayaraj and Delma, 2004). Several studies have been carried out to study the role of kairomones from lepidopteran pests in eliciting a host searching behaviour of many generalist predators (McLain, 1979; Yasuda and Wakamura, 1996; Yasuda, 1997; Bakthavatsalam *et al.*, 1999; Bakthavatsalam *et al.*, 2000; Sahayaraj and Paulraj 2001; Singh *et al.*, 2002). However, no concrete studies have been carried out to identify the volatile chemicals mediating the host seeking behaviour of any reduviid predators. Hence the present study aims at investigating the behaviour of *R. marginatus* life stages to the chemical cues of *H. ar-*

migera, *Mylabris pustulata* (Thunberg) (Coleoptera Meloidae) and *S. litura* and identifying the compounds that elicit the host-seeking response of *R. marginatus*.

Materials and methods

Insect rearing

Life stages of *R. marginatus* were collected from groundnut ecosystem, Palayamkottai, Tamil Nadu, India and maintained on *Corcyra cephalonica* Stainton larvae under laboratory conditions (28 ± 2 °C, L13: D11 photoperiod and relative humidity 73 ± 4%) by the method developed by Sahayaraj (2002a). Different life stages of *S. litura* and *H. armigera* and adult *M. pustulata* were also collected from the same locality, reared on groundnut leaves and flowers, respectively under the same laboratory conditions. Laboratory emerged fifth instar larvae of *S. litura* and *H. armigera* and adults of *M. pustulata* (both male and female) were used for this study.

Hexane extracts of the prey

M. pustulata, *S. litura* and *H. armigera* were extracted using hexane (Singh *et al.*, 2002) with slight modification of Sahayaraj and Paulraj (2001). Hundred live *M. pustulata* adults were weighed and placed in a stopper bottle and add 75 ml of hexane. The bottle was shaken very often at room temperature (28 °C) for 2 hours and kept at 50 °C in water bath for 20 minutes. The extract was filtered through Whatmann no.1 filter paper, then fractionated into hexane-soluble and water-soluble fractions and the hexane fraction was used for the experiment. The same procedure was followed for other preys.

Behavioural bioassay

A two-armed glass olfactometer designed by Sahayaraj and Paulraj (2001) was used to study the approaching and rostrum protrusion behaviours of *R. marginatus*. A small piece of filter paper (1x1 cm) impregnated with the hexane fraction of *H. armigera* extract was used as test and filter paper impregnated with hexane was used as control. Ninety-six hours starved second instar nymphs of *R. marginatus* were released separately into the central chamber and their behaviour (approaching and rostrum protrusion) was observed for one hour continuously. Similar procedure was carried out for III, IV, and V nymphal instars and adult *R. marginatus* with both *M. pustulata* and *S. litura* extracts separately. The predators chose either test chamber or control chamber or neither. Predator chose the test or control chamber were considered as positive choice or negative choice respectively. If the predator chose neither of the chambers, then it was considered that predator made no choice. The approaching behaviour of the predator between the hexane fraction of the prey extracts and hexane was determined by χ^2 test and the significance was expressed at 5% level. The predatory behaviour was observed in terms of approaching and rostrum protrusion time. A student's 't' test was performed to determine the differences in the approaching and protrusion time between the control (hexane) and test (hexane fraction of the prey). The significances were determined and expressed at 1%.

Gas chromatograph - mass spectrometer (GC - MS)

GC - MS analyses were conducted with GCMS - QP5000 Shimadzu combined gas chromatograph - mass spectrometer using the column CBP - 5 (25m x 0.25 mm I.D x 0.2 μ m film thickness). The prey extracts were concentrated to 0.5 ml and 0.1 μ l was injected into

the column. The column temperature was first set at 70 °C with 5 min standing time and programmed to increase to 260 °C at a rate of 10 °C / min. The final temperature of 260 °C was held for 15 min. The carrier gas was helium at a column pressure of 70 KPa with a flow rate of 1 ml / min. The compounds present in the extract were identified by comparing the extract with the compounds from WILEY library based on their retention time and mass spectra.

Results and discussion

Behaviour

The response of *R. marginatus* to the hexane soluble fractions of *M. pustulata*, *S. litura* and *H. armigera* extracts is shown in tables 1, 2 and 3. The response was higher for *H. armigera* extract (50, 70, 50, 50 and 60% of II, III, IV, V and adult respectively) as compared to *S. litura* (40, 12.5, 37.5, 12.5 and 62.5% of II, III, IV, V and adult respectively) and *M. pustulata* (40, 37.5, 25, 10 and 40% of II, III, IV, V and adult respectively) extracts. Similarly when water fraction of *H. armigera*, *S. litura* and *M. pustulata* were provided as chemical cues sources to *R. marginatus*, it preferred *H. armigera* (Sahayaraj and Delma, 2004). However, *S. litura* elicited maximum response in *R. marginatus* than *M. pustulata* extract. The response of *R. marginatus* towards the prey chemical cues can be visualized by exploration and probing behaviour exhibited by the predators due to their chemosensory perception as observed by Bilgrami and Pervez (2001). Once the predators approached the filter paper impregnated with the prey extract, they clumped around it, extended their rostrum and started probing the filter paper. Sahayaraj and Paulraj (2001) also showed similar observations in reduviids. Several

Table 1. Approaching behaviour of *R. marginatus* to hexane fraction of *H. armigera*.

Response	Life stages of predator				
	II (10)	III (10)	IV (10)	V (10)	Adult (10)
Positive choice	5	7	5	5	6
Negative choice	-	1	2	3	3
No choice	5	2	3	2	1
χ^2	5.004	6.205	2.241	1.403.802	0.198
Significance	N.S.	*	N.S.	N.S.	*

* = Significant at 5% level, N.S. = not significant.

Table 2. Approaching behaviour of *R. marginatus* to hexane fraction of *S. litura*.

Response	Life stages of predator				
	II (10)	III (8)	IV (8)	V (8)	Adult (8)
Positive choice	4	1	3	1	5
Negative choice	2	5	4	3	1
No choice	4	2	1	4	2
χ^2	0.799	3.245	1.746	1.746	3.245
Significance	N.S.	N.S.	N.S.	N.S.	*

* = Significant at 5% level, N.S. = not significant.

Table 3. Approaching behaviour of *R. marginatus* to hexane fraction of *M. pustulata*.

Response	Life stages of predator				
	II (10)	III (8)	IV (8)	V (10)	Adult (10)
Positive choice	4	3	2	1	4
Negative choice	5	4	1	2	3
No choice	1	1	5	7	3
χ^2	2.601	1.746	3.245	6.205	0.198
Significance	N.S.	N.S.	N.S.	N.S.	*

* = Significant at 5% level, N.S. = not significant.

studies were also carried out in this regard in other predatory insects (Yasuda and Wakamura, 1996; Yasuda, 1997; Jhansilakshmi *et al.*, 2000; Singh and Paul, 2002). They postulated that the chemical cues or kairomones of the preys stimulate the predators to respond towards them.

It was very clear from observations that *R. marginatus* exhibited only approaching and rostrum protruding behaviours. Similar kind of observations were also recorded by Yasuda and Wakamura (1996) and Yasuda (1997) who had shown that the predatory stinkbug *Eo-canthecona furcellata* (Wolff) (Heteroptera Pentatomidae) was attracted to the larval extracts of *S. litura* from a distance and protruded their proboscis when they were close to the odour source. Tested life stages of *R. marginatus* approached the test chamber faster than the control chamber (figure 1) except the second instar nymphs for *M. pustulata* chemical cues (2.1 and 3.79 minutes for control and test category respectively) and IV instar and adult predators on *S. litura* (4.45 and 7.48 minutes for control and test category respectively) and adult predators for *H. armigera* (13.02 and 15.43 minutes control and test category respectively) chemical cues. However the difference in the approaching time between control and test categories were statistically in-

significant except for III ($t = 2.035$, $df = 4$, $p < 0.1$) and V ($t = 2.120$, $df = 2$, $p < 0.1$) nymphal instars and adult ($t = 1.622$, $df = 4$, $p < 0.1$) with respect to *M. pustulata* extract and III instar ($t = 1.733$, $df = 6$, $p < 0.1$) on *H. armigera* chemical cues.

Once the predator approached the prey chemical cues impregnated filter paper, immediately extend its rostrum and probe the paper. From the figure 2, it was very clear that predators probe the filter paper which contain prey chemical cues longer period than in the control chamber except the adult predator on *S. litura* chemical cues. In all the cases, the difference between the control and the test category was found to be insignificant at 1% level. Third (0.2 minutes), fourth (0.089 minutes) and fifth (0.074 minutes) nymphal instars of *R. marginatus* exhibited maximum rostrum protrusion response to *S. litura*, *M. pustulata* and *H. armigera* chemical cues. The rostrum protrusion time of *R. marginatus* adults was maximum on *M. pustulata* (0.038 minutes) followed by *S. litura* (0.035 minutes) and *H. armigera* (0.031 minutes) chemical cues. Results indicate that *R. marginatus* life stages are active decision makers when they were provided with prey chemical cues and in accordance with the findings of Kotikal and Sengonca (1999).

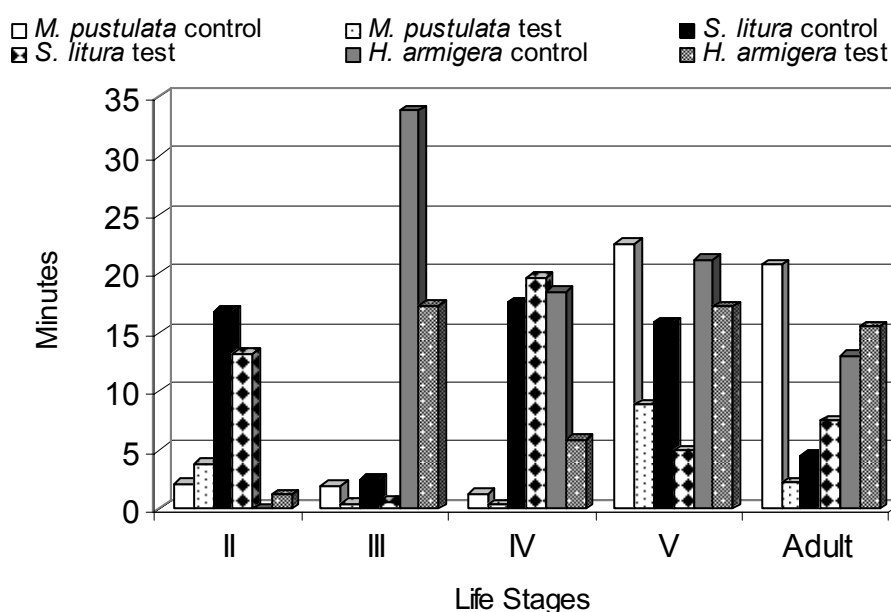


Figure 1. Approaching time of *R. marginatus* towards the prey chemical cues.

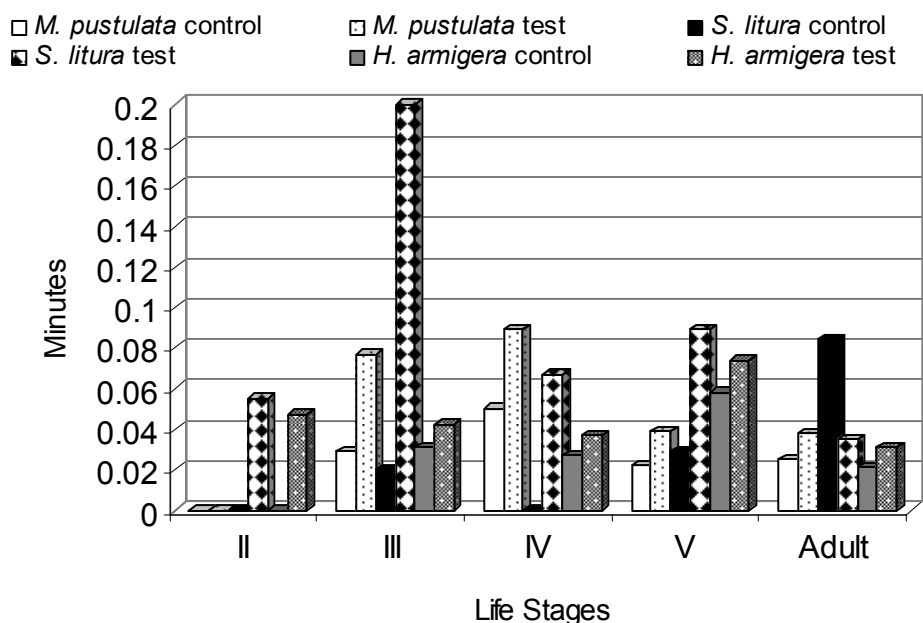


Figure 2. Rostrum protrusion time of *R. marginatus* towards the prey chemical cues.

GC - MS Analysis of prey extracts

The chemicals present in the hexane fraction of the prey extracts were identified using GC - MS. Compounds like tridecane, octacosane, 1-iododecane, octodecane, eicosane, pentacosane, heptacosane and dotriacontane were identified from the hexane fraction of *H. armigera* extract in accordance with the results of Ananthakrishnan *et al.*, (1991) and Singh *et al.*, (2002). According to them, the higher response of *R. marginatus* to *H. armigera* may be attributed to the presence of these saturated hydrocarbons. The analysis of *S. litura* extract showed that the kairomone extract had di-n-octyl phthalate, 1,2-benzene dicarboxylic acid, diisooctyl ester, 3,3-dimethyl octane, 4-methyl decane and bis (2-ethylhexyl) phthalate. However, Yasuda (1997) had reported that hexane fraction of *S. litura* obtained from hexane acetone extract was found to contain n-tetradecane, n-pentadecane, n-heptadecane, n-heptacosane, squalene, n-nonacosane and n-hentriacontane. Nevertheless, hexane fraction of *S. litura* extract elicited a positive approaching behaviour in *R. marginatus*. Eight compounds were identified from *M. pustulata* extract which include 3-hexyne 2, 5-diol, (E,Z)-2-hexane-1-ol, acetate, (E,Z)-6,10-dimethyl-5,9-undecadien-2-one, 1,2-benzenedicarboxylic acid, diisooctyl phthalate, 3,5-dimethyl octane and 2-methyl heptadecane.

The chemical cues compounds from various preys that elicit a host-searching behaviour in other predatory insects and spiders were identified by several workers (Bakthavatsalam *et al.*, 1999; Dunkelblum *et al.*, 2000; Boo *et al.*, 1998; Zhu *et al.*, 1999; Reddy *et al.*, 2002). Singh and Paul (2002) have also reported the response of *Chrysoperla carnea* (Stephens) (Neuroptera Chrysopidae) towards 26 saturated hydrocarbons. All these results and the present investigation reveal that the semiochemicals emitted from the prey species indeed elicit an intense host searching behaviour in the predators. Al-

though all the three prey extracts tested in the present study elicit a positive response in *R. marginatus* the results tabulated show that the predator prefers lepidopteran pests, *H. armigera* and *S. litura* to the coleopteran pest, *M. pustulata*. Thus this investigation offers new strategies for the chemical cues - mediated pest management in agroecosystems.

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