EUROPEAN JOURNAL OF ENTOMOLOGY
ISSN (online): 1802-8829
http://www.eje.cz
Eur. J. Entomol. **113**: 29–36, 2016
doi: 10.14411/eje.2016.003
ORIGINAL ARTICLE

Response of the reduviid bug, *Rhynocoris marginatus* (Heteroptera: Reduviidae) to six different species of cotton pests

KITHERIAN SAHAYARAJ[1], SUBRAMANIAN MUTHU KUMAR[2] and ANNIE ENKEGAARD[3]

[1]Crop Protection Research Centre, St. Xavier's College, Palayamkottai - 627 002, Tamil Nadu, India; e-mail: ksraj48@gmail.com

[2]UPASI Tea Research Foundation, Tea Research Institute, Nirar Dam (PO), Valparai - 642 127, Coimbatore District, Tamil Nadu, India; e-mail: smkkurichi@gmail.com

[3]Aarhus University, Science and Technology, Department of Agroecology, Research Centre Flakkebjerg, DK-4200 Slagelse, Denmark, e-mail: annie.enkegaard@agro.au.dk

Key words. Heteroptera, Reduviidae, *Rhynocoris marginatus*, macromolecular profiles, kairomone, feeding behaviour, haemocyte levels

Abstract. In Indian agro-ecosystems *Rhynocoris marginatus* (F.) is one of the most abundant predatory arthropods and feeds on a wide range of insect pests. We investigated the responses of *R. marginatus* to six species of cotton pests: *Spodoptera litura* (F.), *Sylepta derogata* (F.), *Pericallia ricini* (F.), *Mylabris indica* (Thunberg), *Mylabris pustulata* (Thunberg) and *Dysdercus cingulatus* (F.), in terms of its predatory behaviour (approach and handling times), weight gain, macromolecular profile (content of carbohydrates, proteins, free aminoacids and lipids) and haemocytic profile. We also determined the predator's reliance on kairomones from different species of prey. Larvae of the species of Lepidoptera studied were approached and captured more quickly than adults of the two meloid coleopteran and one heteropteran pest and were more beneficial to the predator in terms of weight gain. Predators had a higher total protein content when reared on larvae of the three lepidopteran species, higher lipid content when reared on adults of the two meloid coleopteran species and a higher carbohydrate content when reared on adults of one heteropteran species. The number of haemocytes was greater in predators reared on larvae of the Lepidoptera studied, followed by those reared on adults of the heteropteran and lowest in those reared on adults of the two meloid coleopterans. Response to kairomones was strongest for *S. litura* followed by *S. derogata* and *M. pustulata*. We conclude that the Lepidoptera studied tended to be, for this predator, superior prey, with *S. litura* being especially beneficial and the prey species for which *R. marginatus* has the highest kairomonal preference. Moreover, we propose that *R. marginatus* may be useful as a biocontrol agent against lepidopteran cotton pests.

INTRODUCTION

In India, 160 species of insects are known to feed on cotton. The economic effects on cotton production of infestations by bollworms, including *Spodoptera litura* (F.) (Lepidoptera: Noctuidae), *Sylepta derogata* (F.) (Lepidoptera: Pyralidae) and *Pericallia ricini* (F.) (Lepidoptera: Arctiidae), are particularly significant (David & Anathakrishnan, 2004). Other serious pests of Indian cotton are *Mylabris indica* (Thunberg (Coleoptera: Meloidae), *M. pustulata* (Thunberg) (Coleoptera: Meloidae) and *Dysdercus cingulatus* (F.) (Heteroptera: Pyrrhocoridae) (CICR, 2011).

Rhynocoris marginatus (F.) (Heteroptera: Reduviidae), a polyphagous reduviid predator, is present in most Indian agro-ecosystems including groundnut, cotton, soybean (Sahayaraj, 1995, 2000, 2007, 2014), sugarcane (Easwaramoorthy et al., 1994) and pigeon pea (Ambrose & Claver, 2001). Based on laboratory and field studies of pest suppression efficiency, we know that *R. marginatus* consumes a broad range of prey (Sahayaraj, 2006) and that it specifically suppresses pests like *S. litura*, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) and *D. cingulatus* in the field (Sahayaraj, 1999; Sahayaraj & Martin, 2003; Sahayaraj & Ravi, 2007).

However, in order to determine whether this predator can be exploited for biological control, we need a more complete understanding of its performance as a predator of different pest species and whether it prefers particular species of prey. Although a few other studies have addressed this predator's acceptance of different species of prey and potential preferences (Sahayaraj & Paulraj, 2001; Sahayaraj & Delma, 2004; Sahayaraj et al., 2004), these studies were not on the above mentioned economically important pest species. In this study, we therefore evaluated the prey suitability for *R. marginatus* of *S. litura*, *S. derogata*, *P. ricini*, *M. indica*, *M. pustulata* and *D. cingulatus* based on its behaviour, increase in weight, macromolecular profile (free amino acids, total carbohydrates, proteins and lipids) and haemocyte levels. As a step towards determining whether

R. marginatus prefers particular species of prey, we also investigated whether it responds to the kairomones of the different species of prey and whether the response is similar for all these prey species. As a reference species for the studies on the responses of *R. marginatus* to the cotton pests mentioned above, we included the rice moth, *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) in our studies. The suitability of this species as prey and for rearing *R. marginatus* is recorded (Sahayaraj, 2002; Sahayaraj & Sathiamoorthi, 2002).

MATERIAL AND METHODS

Collection and maintenance of insects

A mixture of the different life stages of *R. marginatus* was collected from cotton fields in the Tirunelveli district in the state of Tamil Nadu and maintained individually in plastic vials (5.5 by 4.4 cm with perforated lids) under laboratory conditions at 27–29°C, 60–80% RH and 11L : 13D h on a mixture of larvae of *S. litura*, *S. derogata*, *P. ricini* and adults of *M. indica* and *M. pustulata*, and fifth stadium nymph of *D. cingulatus* and larvae of *C. cephalonica* in equal amounts. The prey was replenished every second day. After emergence, 30 adult predators (139 ± 2 mg and 181 ± 3 mg for male and female, respectively) were maintained individually in similar plastic vials as above for one month to standardize their physiological state. The adults were kept in the same conditions as above on either just one of the mentioned species of pests ("single-pest rearing") or on a mixture of the six pest species in equal amounts ("mixed-pest rearing") with the prey being replenished every second day. After one month, the predators from the single-pest rearing were killed and used to estimate their content of free amino acids and total protein, carbohydrate and lipid

Except for *C. cephalonica* all pest species were collected from the same cotton field from which the reduviids were collected. Larvae of the three noctuid pests *S. litura*, *S. derogata* and *P. ricini* were maintained in groups in plastic troughs (300 ml) on young cotton leaves, whereas the adults of two meloid coleopterans, *M. indica* and *M. pustulata*, and the adults of the heteropteran, *D. cingulatus*, were reared on flowers and cotton bolls in plastic vials (500 ml). *C. cephalonica* eggs were purchased from the Agriculture Office, Palayamkottai, and maintained in plastic troughs using crushed wheat and groundnuts as food for the emerging larvae. All species were kept at the same conditions as above. Laboratory reared fifth instar larvae of *S. litura* (374 ± 10 mg), *S. derogata* (393 ± 2 mg), *P. ricini* (415 ± 14 mg) and *C. cephalonica* (42 ± 1 mg) and adults of *D. cingulatus* (106 ± 5 mg), *M. indica* (323 ± 2 mg) and *M. pustulata* (344 ± 3 mg) were used in the experiments.

Predatory behaviour

Three pre-weighed individuals of the same species were introduced into a plastic container (300 ml) and allowed to move undisturbed for five minutes. Predators were randomly selected from the mixed-pest rearing and starved for 24 h by confining them individually in an empty Petri dish (9-cm in diameter) at 27–29°C, 60–80% RH and 11L : 13D. Predators were subsequently introduced singly into the Petri dishes, and the sequence of feeding events were recorded and timed visually until feeding ceased. The sequential pattern in predatory behaviour is (see Sahayaraj, 2007, for details) : (1) arousal – the predator closely watches the movement of the prey, is restless and has straightened legs, (2) approach – the predator moves towards the prey, pointing its rostrum and antennae forward, (3) capture – the predator adapts its rate of movement to that of the prey, closes in on the prey and captures and holds it tightly by its forelegs, (4) rostral probing – the predator probes various parts of the body of the prey with its rostrum, (5) paralysing the prey – the predator withdraws its rostrum; the prey becomes immobile indicating that paralysis has occurred, (6) feeding – the predator transports the prey to a secluded place and sucks out the body content by inserting its rostrum at one or more places and (7) post-predatory behaviour – the predator drops the carcass and cleans its rostrum, antennae and forelegs. The behavioural elements 1–2 imply that *R. marginatus* is able to detect and identify its prey from a distance as stated by Haridass et al. (1988) and Claver & Ambrose (2003). The time that the predators spend in carrying out each of the first three events (1–3) was summed and used as a collective measure of the time spent locating and capturing the prey (termed approach time, AT). Similarly, the time that the predators spent in each of the subsequent three events (4–6) was summed and used as a collective measure of time spent handling and eating the prey (termed handling time, HT). These observations were recorded in the laboratory at 27–29°C, 60–80% RH. After 24 h, the weight of the prey was recorded and the difference in weight of the prey was used as a measure of the weight gained by the predator. Weight gains were corrected by predator weight loss due to desiccation as determined by weighing 6 predators kept individually in separate plastic containers without prey for 24 h. For each prey, the behaviour was recorded separately for ten male and female reduviids.

Prey extracts and predator choice

To test for possible kairomonal responses of *R. marginatus* to the different species of prey, compounds from each of them were extracted using hexane, following the method used by Singh & Paul (2002) and modified by Sahayaraj et al. (2003) and Sahayaraj (2008). Two pieces (1 cm^2) of filter paper (Whatmann No. 1) was each impregnated with extracts from either *M. pustulata* or *M. indica* (treatment "TC-1"). A third piece of filter paper was impregnated with hexane and served as a control. The three pieces of filter paper were arranged in a triangle in a Petri dish (9 cm in diameter). Subsequently one predator (starved for 24 h as described above) from the mixed-pest rearing was randomly selected and introduced into the centre of the Petri dish and the lid gently replaced. The piece of filter paper first approached by the predator was recorded. Observations took place in the laboratory at 27–29°C, 60–80% RH. Ten predators were tested and the preferred prey extracts determined were those first approached by the majority of the individuals tested. The preferred extracts were subsequently tested in another 10 replicates in a similar way with a piece of filter paper with extracts of *D. cingulatus* (treatment "TC-2") and the five other prey species in "TC-3", "TC-4", "TC-5" and "TC-6" (refer to Table 3 for the prey extracts tested in each test). No predator was tested more than once in these experiments. From the results, the percentage of predators preferring the extracts of each pest species was calculated (Sahayaraj, 2008).

Sample preparation for macromolecule estimation

Ten predators from each of the single-pest rearings were randomly selected, pre-weighed separately, killed and dried in a hot-air oven at 50°C for 6 h. The weight of the dried insects was recorded after which the insects were powdered using a mortar and pestle and the total content of carbohydrates (Sadasivam & Manickam, 1997), proteins (Lowry et al., 1951), lipids (Bragdon, 1951) and total free amino acids (Palanivelu, 2001) determined.

Table 1. Mean (± SE) approach time (AT), handling time (HT), weight gain (WG) and weight gain efficiency (WGE) of *Rhynocoris marginatus* males and females when fed different cotton pests.

Pest species	Sex	AT (min)	HT (min)	WG (mg)	WGE (mg/min)
Corcyra cephalonica	Male	5.14 ± 0.18^{a}	187.4 ± 1.18^{a}	31.7 ± 0.98^{a}	0.17 ± 0.01^{a}
	Female	6.21 ± 0.56^{A}	212.6 ± 2.01^{A*}	22.0 ± 0.90^{A*}	0.11 ± 0.01^{A}
Pericallia ricini	Male	4.18 ± 0.38^{b}	60.2 ± 4.75^{b}	45.4 ± 3.63^{b}	0.71 ± 0.02^{b}
	Female	4.06 ± 0.58^{B}	50.4 ± 3.797^{B*}	68.8 ± 4.81^{B*}	1.27 ± 0.03^{B*}
Sylepta derogata	Male	4.20 ± 0.59^{bc}	76.3 ± 4.36^{c}	90.5 ± 2.83^{c}	1.13 ± 0.01^{c}
	Female	4.13 ± 0.39^{BC}	31.1 ± 3.58^{C*}	88.5 ± 2.41^{C*}	2.44 ± 0.02^{C*}
Spodoptera litura	Male	2.88 ± 0.33^{d}	110.0 ± 5.77^{d}	54.0 ± 4.08^{d}	0.48 ± 0.01^{d}
	Female	2.54 ± 0.74^{D*}	130.1 ± 1.66^{D*}	68.0 ± 5.27^{BD*}	0.52 ± 0.01^{E}
Mylabris pustulata	Male	6.70 ± 2.22^{e}	92.1 ± 3.39^{e}	10.6 ± 1.38^{e}	0.11 ± 0.01^{e}
	Female	5.36 ± 0.33^{E*}	147.2 ± 4.19^{E*}	11.0 ± 1.95^{E}	0.07 ± 0.00^{E}
Mylabris indica	Male	5.62 ± 0.57^{a}	79.5 ± 9.43^{f}	40.2 ± 4.06^{bf}	0.47 ± 0.01^{f}
	Female	5.18 ± 0.55^{EF}	212.5 ± 5.47^{AF*}	27.3 ± 3.11^{F*}	0.12 ± 0.01^{F*}
Dysdercus cingulatus	Male	6.16 ± 0.73^{eg}	65.2 ± 2.83^{g}	23.2 ± 2.59^{g}	0.33 ± 0.01^{g}
	Female	5.19 ± 0.38^{FG*}	50.3 ± 4.26^{BG*}	20.2 ± 2.84^{A*}	0.38 ± 0.01^{G}

Different lower case (for male) and upper case (for female) letters within a column indicate significant differences at 5% level. Significant differences between male and female predators feeding on the same species are indicated by *.

Haemocytes

Ten predators from each of the single-pest rearings were randomly selected and used to determine the total number of haemocytes per mm^3 (total haemocyte count, THC) and the percentages of the different types of haemocytes (differential haemocyte count, DHC). The foreleg of a predator was amputated with a pair of fine sterilized scissors and up to 0.5 µl of the haemolymph was directly drawn in to a WBC pipette (Naubauer's haemocytometer). The haemolymph was then diluted with an acidified physiological saline (NaCl – 4.65 g, KCl – 0.5 g, $CaCl_2$ – 0.11 g, Gentian violet – 0.005 g, acetic acid – 1.25 ml diluted in 100 ml distilled water). The diluting fluid was drawn into the pipette up to mark 1.1 giving 20 times dilution. The inner surface of the WBC pipette was rinsed several times with diluting fluid before the haemolymph was drawn. After at least one gentle stirring to ensure complete dispersion of the cells and prevent agglutination and plasma clot formation, the haemocyte population (cells/mm³) (Jones, 1962) was counted under microscope. For differential haemocyte counts, the haemolymph that oozed-out of the foreleg was spread out on a clean slide and a neat thin smear was prepared with a glass rod or a cover slip. It was then fixed with 1 ml of 100% methanol or acetic acid, stained with 4% Giemsa stain and allowed to dry out in the air. The preparations were observed under a microscope. The haemocytes were categorised based on the identification keys of Gupta (1985) and Ambrose (1999) as prohaemocytes, plasmatocytes, granulocytes, cystocytes and oencytoids.

Statistical analysis

Individual data on approach time, handling time, weight gain, total carbohydrate, protein and lipid level and various haemocyte levels in male and female predators fed on different species of prey were subjected to two-way ANOVA and Tukey's Test with significance recorded at the 5% level. Various relationships between the parameters measured were analyzed using linear regression. The percentage preference of the predator for extracts of the different species of prey was compared among treatments using chi-square tests of independence. All analyses were performed using SPSS Version 13.0 (SPSS, 2004).

RESULTS

Approach time (AT) and handling time (HT)

Generally, *R. marginatus* became aroused, approached and captured its prey quickly with AT values varying between 2.5 and 6.7 min (Table 1). The shortest ATs were recorded for both male and female reduviids attacking *S. litura* and the longest for females attacking the reference prey *C. cephalonica* and for males attacking *D. cingulatus* and *M. pustulata* (Table 1). Generally both males and females had shorter ATs for larvae of the three lepidopteran cotton pests, with shorter times recorded for larvae of the noctuid *S. litura* than for the adults of one heteropteran and two coleopteran pests (Table 1). There was not a significant relationship between AT and the weight of the prey species (df 6, 68; $F = 4.15$; $P > 0.05$; $r^2 = -0.544$).

The time (HT) required for the reduviids to probe, paralyse and eat the different species of prey varied greatly from about half an hour for females attacking larvae of the noctuid *S. derogata* to more than 200 min for those attacking *C. cephalonica* and *M. indica* (Table 1). There were no consistent differences in HTs of females and males, with the latter having the shortest HT when attacking *P. ricini* and the longest when attacking *C. cephalonica* (Table 1). There were no significant relationships between HT and AT for either male or females reduviids (males: df 6, 62; $F = 4.00$; $P > 0.05$; $r^2 = -0.070$; females: df 6, 60; $F = 4.01$; $P > 0.05$; $r^2 = 0.466$). Similarly, there were no significant relationships between HT and the weight of the prey (df 6, 65; $F = 4.08$; $P > 0.05$; $r^2 = -0.401$).

Weight gain

The weight gained by *R. marginatus* after feeding on the different species of prey differed greatly with values ranging between 10.6 and 90.5 mg, with the highest and lowest weight gain recorded when the reduviids (both males

Table 2. Percentages of *Rhynocoris marginatus* that approached extracts of the six cotton pests used in this study.

Test choice	Prey extract	First approach (%)	χ^2	Significance
TC-1	Hexane	0		
	Mylabris pustulata	90	3.28	$P < 0.05$
	Mylabris indica	10	11.20	$P > 0.05$
TC-2	Hexane	0	12.28	$P > 0.05$
	Mylabris pustulata	80	0.09	$P < 0.05$
	Dysdercus cingulatus	20	9.01	$P > 0.05$
TC-3	Hexane	0	12.28	$P > 0.05$
	Mylabris pustulata	90	3.28	$P < 0.05$
	Pericallia ricini	10	11.20	$P > 0.05$
TC-4	Hexane	0	12.28	$P > 0.05$
	Mylabris pustulata	10	11.20	$P > 0.05$
	Sylepta derogata	90	3.28	$P < 0.05$
TC-5	Hexane	0	12.28	$P > 0.05$
	Sylepta derogata	20	9.01	$P > 0.05$
	Spodoptera litura	80	0.09	$P < 0.05$
TC-6	Hexane	0	12.28	$P > 0.05$
	Mylabris pustulata	30	8.06	$P > 0.05$
	Spodoptera litura	70	0.60	$P < 0.05$

and females) ate larvae of the noctuid *S. derogata* and adults of the meloid coleopteran, *M. pustulata*, respectively (Table 1). Although the weight gain of males and females that ate the same species of prey often differed significantly, there was no consistent difference (Table 1). There was no relationship between weight gain and prey body weight for either males or females (males: df 6, 65; F = 4.07; $P > 0.05$; $r^2 = 0.467$; females: df 6, 66; F = 4.07; $P > 0.05$; $r^2 = 0.631$).

For each prey species considered separately, except for *C. cephalonica* (t = 2.829; $P > 0.05$), there was a significant positive relationship between the quantity of body mass consumed and HT (*P. ricini*: t = 3.960, $P < 0.0005$; $r^2 = 0.898$; *S. derogata*: t = 3.962, $P < 0.0005$; $r^2 = 0.912$; *S. litura*: t = 2.820; $P < 0.005$; $r^2 = 0.819$; *M. pustulata*: t = 2.829; $P < 0.005$; $r^2 = 0.453$; *M. indica*: t = 1.734; $P < 0.05$; $r^2 = 0.817$; *D. cingulatus*: t = 1.730; $P < 0.05$; $r^2 = 0.769$). However, for all the prey species combined, there was no correlation between consumed body mass and HT for either males or females (males: df 6, 65; F = 4.02; $P > 0.05$; $r^2 = –0.166$; females: df 6, 64; F = 4.05; $P > 0.05$; $r^2 = –0.586$). There was a significant relationship between the quantity of body mass consumed and AT when the prey species were considered separately for *M. indica* (t = 1.733; $P < 0.05$; $r^2 = 0.869$), *S. derogata* (t = 1.734; $P < 0.005$; $r^2 = 0.823$) and *S. litura* (t = 1.730; $P < 0.005$) but not for *P. ricini* (t = 2.550; $P \geq 0.05$; $r^2 = 0.116$), *M. pustulata* (t = 2.551; $P \geq 0.05$; $r^2 = 0.339$) and *D. cingulatus* (t = 2.552; $P \geq 0.05$; $r^2 = 0.216$). For all species combined there was a significant negative correlation between consumed body mass and AT for both males and females (males: df 6, 67; F = 4.15; $P < 0.0054$; $r^2 = –0.424$; females: df 6, 65; F = 4.16; $P < 0.00051$; $r^2 = –0.689$).

The reduviid efficiency in obtaining food from prey in relation to the time invested in capturing and feeding varied between prey species from 0.07 to 2.4 mg per minute (Table 1). The highest efficiencies were recorded for both males and females attacking larvae of *S. derogata* followed by *P. ricini* and *S. litura*, and the lowest when attacking *M. pustulata* (Table 1). Except for *M. indica* and *D. cingulatus* the order in the efficiencies was the same for both males and females (Table 1).

Prey extracts and predator choice

When the reduviids were exposed to prey extracts, they showed the same initial behaviour as recorded in the sequence of behaviour normally leading to capture of prey (Sahayaraj, 2008), i.e. they moved towards the paper with their rostrum and antennae pointing forward, protruded the rostrum and tried to obtain food from the filter paper. In the first three tests ("TC-1" to "TC-3"), *R. marginatus* invariably chose extracts of *M. pustulata* over those of *M. indica*, *D. cingulatus* and *P. ricini* (Table 2). In contrast, in the fourth test, *R. marginatus* chose extracts of larvae of the lepidopteran, *S. derogata*, over those of the meloid coleopteran, *M. pustulata*, and in the remaining two choices the reduviid chose extracts of *S. litura* over those of *S. derogata* and *M. pustulata*.

Macromolecular profile

The prey species used as food in the single-species rearing of *R. marginatus* had a significant effect on their macromolecular composition (Table 3). Compared with predators reared on the reference prey, predators reared on larvae of either of the two lepidopteran species, *S. litura* and *S. derogata*, had a significantly higher content of free amino acids followed by those reared on *P. ricini* or *M. pustulata*. In contrast the amino acid content of predators reared on *M. indica* or *D. cingulatus* was not different from that of predators reared on the reference prey. These differences were partly reflected in the protein content, which was significantly higher for predators reared on larvae of either of the three lepidopteran species, *S. litura*, *S. derogata* and *P. ricini*, compared with predators in the reference group. However, the protein content of predators

Table 3. Mean (± SE) macromolecular content of *Rhynocoris marginatus* fed on different pest species.

Pest species	Protein		Carbohydrate (mg/mg)	Lipid (mg/mg)
	Free amino acids (μg/mg)	Total protein (mg/mg)		
Corcyra cephalonica	40.0 ± 1.2[a]	0.46 ± 0.01[a]	0.43 ± 0.01[a]	0.08 ± 0.01[a]
Pericallia ricini	63.0 ± 0.6[b]	0.53 ± 0.01[b]	0.35 ± 0.01[b]	0.09 ± 0.01[a]
Sylepta derogata	81.2 ± 0.7[c]	0.58 ± 0.01[b]	0.38 ± 0.01[b]	0.02 ± 0.01[b]
Spodoptera litura	85.1 ± 0.5[cd]	0.62 ± 0.01[d]	0.28 ± 0.01[d]	0.04 ± 0.01[b]
Mylabris pustulata	58.2 ± 0.4[b]	0.28 ± 0.01[e]	0.23 ± 0.01[e]	0.45 ± 0.02[d]
Mylabris indica	42.0 ± 0.7[a]	0.23 ± 0.01[f]	0.25 ± 0.02[ef]	0.49 ± 0.01[d]
Dysdercus cingulatus	38.2 ± 0.6[a]	0.35 ± 0.01[g]	0.50 ± 0.01[g]	0.12 ± 0.02[f]

Different letters within a column indicate significant differences at 5% level.

reared on *M. pustulata*, *M. indica* or *D. cingulatus* was significantly lower than that of the predators in the reference group. Regarding the carbohydrate content, this was significantly higher for predators reared on *D. cingulatus* compared with the predators in the reference group, whereas it was significantly reduced compared to the reference group for predators reared on the remaining prey species (Table 3). Compared with predators reared on the reference prey, total lipid content was higher for predators reared on the two meloid coleopterans (*M. pustulata*, *M. indica*) or adults of the heteropteran, *D. cingulatus*, being highest for coleopteran-reared *R. marginatus* and lowest for those reared on larvae of either of the two lepidopteran species, *S. derogata* and *S. litura* (Table 3).

THC and DHC of *R. marginatus*

Total haemocyte count for predators from the single-species rearing is shown in Table 4. THC of the predators reared on *S. litura*, *S. derogata*, *P. ricini* or *D. cingulatus* was significantly higher than that of the predators in the reference group. In contrast, THC of predators reared on *M. indica* was not significantly different from that recorded for the reference group, and predators reared on *M. pustulata* had a significantly lower THC than those reared on the reference prey group. Generally, the THC level was higher in predators reared on larvae of the three lepidopteran species and adults of the heteropteran pest compared with those reared on the two meloid Coleoptera. Regression analyses between total protein content and THC ($r^2 = 0.621$; df 1, 5; $t = 2.230$; $P < 0.05$) and also between total lipid content and THC ($r^2 = 0.597$; df 1, 5; $t = 2.230$; $P < 0.05$) were significant.

Results of the differential haemocyte count (DHC) (Table 4) revealed that prohaemocyte and plasmatocyte levels were higher in predators reared on *S. litura* or *S. derogata* than for those reared on the reference prey. For predators reared on *M. pustulata*, *M. indica* or *D. cingulatus* only the prohaemocyte level was higher in comparison with predators reared on the reference prey, whereas plasmatocyte levels were the same or lower. Compared with predators reared on the reference prey, the level of cystocytes was higher for predators reared on all pest species except *S. litura*, whereas the oenocytoid level was higher compared to the reference prey group for predators reared on *P. ricini*, *M. pustulata*, *M. indica* or *D. cingulatus* but lower for predators reared on *S. derogata* or *S. litura.*

DISCUSSION

There were significant differences in how fast *R. marginatus* became aroused, approached and captured (approach time, AT) the different species of prey studied, which presumably reflect combinations of differences in prey size, mobility and speed of movement, prey defence and/or prey chemical cues (Ambrose, 1999; Sahayaraj, 1991, 2006).

Arousal, approach and capture were generally faster for larvae of the three species of lepidopteran pests (except the reference prey *C. cephalonica*) than the small heteropteran (*D. cingulatus*) and the two somewhat smaller meloid coleopterans (*M. indica*, *M. pustulata*). This is in accordance with previous findings for other reduviids presented with the same species of prey (Sahayaraj, 1991). The longer arousal, approach and capture time for the coleopteran and heteropteran prey is most likely due to the

Table 4. Differential (DHC in %) and total haemocyte (THC) counts of *Rhynocoris marginatus* fed on different species of cotton pests.

Pest species	Haemocyte types					Total cells/mm^3
	Prohaemocytes	Plasmatocytes	Granulocytes	Cystocytes	Oenocytoids	
Corcyra cephalonica	13.63[a]	28.51[a]	40.62[a]	7.91[a]	9.34[a]	13610[a]
Pericallia ricini	12.62[ab]	29.46[ab]	35.43[b]	9.50[b]	12.36[b]	14301[b]
Sylepta derogata	15.16[c]	33.17[c]	36.75[bc]	9.67[bc]	5.26[c]	14652[c]
Spodoptera litura	17.01[d]	40.12[d]	35.11[bd]	6.11[d]	1.58[d]	15020[d]
Mylabris pustulata	14.69[ce]	27.17[ae]	37.17[ce]	8.19[e]	12.78[be]	12460[e]
Mylabris indica	14.65[cef]	26.33[ef]	36.02[cf]	10.21[cf]	12.79[bef]	13639[af]
Dysdercus cingulatus	16.79[g]	25.15[fg]	38.02[eg]	9.88[bcg]	10.17[ag]	14150[g]

Different letters within a column indicate significant differences at 5% level.

fact that they were offered as adults, whereas the lepidopterans were offered as fifth instar larvae, which are presumably less mobile and thus easier to capture. However, it is possible that *R. marginatus* was more motivated and/or more able to capture prey that provide high nutritional rewards more quickly, based on the negative relationship between weight gain and AT. An additional explanation for the long arousal, approach and capture time for the two meloid coleopterans is evidently their release of a defensive viscous fluid, which could delay reduviid approach and/or impede their attack (Sahayaraj, 1991). Cantharidin, a common defensive terpenoid present in the defensive viscous fluid of Meloidae, is toxic to the enemies of these beetles (Hashimoto & Hayashi, 2014). Although, *S. litura* is known to produce a defensive fluid (Kumaraswami, 1991), this was not observed in the present study.

Compared to the three Lepidoptera studied the AT recorded for the much smaller lepidopteran reference prey was relatively longer, although it might be assumed that it was more easily captured. It may be speculated that this reflects a lower interest of the reduviid in this species of prey, perhaps due to a perception of a smaller reward or of a longer handling time, which was the longest recorded, longer even than that recorded for the meloid coleopteran, *M. indica*. Apart from this possible link between AT and HT, no overall correlation was found between AT and HT, which indicates that quickly approached and captured species of prey were not also probed, paralyzed and eaten more quickly than prey approached and captured more slowly.

There were no consistent differences between male and female reduviids in the approach and capture time of the different species of prey, although females in three cases approached their prey faster than males. Similarly, although the time spent probing, paralyzing and eating differed significantly between the sexes for all the different species of prey, there was no consistency in one sex being faster in handling prey than the other. This is in contrast to the results of other studies on reduviids in which males both approach and handle prey faster than females (Sahayaraj & Ambrose, 1992, 1993, 1994; Rocha & Redaelli, 2004). Although other authors have shown that female reduviids consume more food than males (Ambrose, 1980; Kumaraswami, 1991; Sahayaraj, 1991, 2008), presumably to meet their reproductive needs, as demonstrated for Pentatomids, in which fecundity increases with body mass (Evans, 1962; Phoofolo et al., 1995; Grundy & Maelzer, 2000), we did not find similar differences in the weight gains of males and females; only when they fed on two of the species of prey (*P. ricini* and *S. litura*) did females gain more weight than males. The reason for this inconsistency is unknown.

There was no consistent pattern in the HTs associated with the different species of prey; relatively long HTs were recorded for both meloid coleopterans with hard cuticles (Sahayaraj, 2008) and the soft-bodied larvae of the lepidopteran reference prey. The reason for these differences in HT for the different prey species is not known but may be due to a longer time needed for paralyzing or liquefying the body contents, the viscosity of the liquefied prey or the amount ingested (Cohen, 1990), although the latter is not supported by the present result of no correlation between reduviid weight gain and HT.

Compared with the weight gained when they fed on the two adult meloid Coleoptera and the heteropteran, *R. marginatus* gained more weight when reared on the larvae of three Lepidoptera, *P. ricini*, *S. derogata* and *S. litura*. This most likely reflects a nutritionally superiority of this type of prey, resulting in a higher content of amino acids and proteins in the bodies of predators reared solely on either of these three species, and that larval prey are more easily digested than adult prey. However, the weight gained from feeding on the larvae of the lepidopteran reference prey, *C. cephalonica*, was, despite the longer handling time, considerably lower than that gained from the other Lepidoptera. This indicates that *C. cephalonica* is an inferior prey for *R. marginatus*, presumably due to its nutritional quality, since predators reared solely on this prey had a lower content of amino acids and proteins compared with those that fed on the other Lepidoptera. The differences in weights gained illustrate that long handling times do not necessarily result in high amounts of food being ingested but also point to the fact that considerable weight gains can be achieved from rather short handling times as seen when the reduviids were reared on *S. derogata*.

Our study has demonstrated that compounds or mixtures of compounds extracted from the different cotton pests examined here function as kairomonal stimulants in eliciting probing behaviour in *R. marginatus.* This supports previous findings for the same and other reduviid species (Cohen, 1990; Sahayaraj & Paulraj, 2001; Tebayashi et al., 2003; Sahayaraj & Delma, 2004; Sahayaraj, 2008; Sujatha et al., 2012). *R. marginatus* responded differently to the extracts from the different species of prey, which presumably reflect differences in volatile components and composition of the extracts (Ananthakrishnan, 1996), but showed a clear preference for *S. litura.* The seemingly general notion that reduviids prefer lepidopteran larvae to other types of prey (Ambrose, 1980; Kumaraswami, 1991; Sahayaraj, 1991, 2008) was, however, not fully supported by the present study in which the predator preferred the extract from the meloid coleopteran pest, *M. pustulata*, over that from the lepidopteran *P. ricini.* This may be due to the hairy appearance of *P. ricini* larvae, a noxious smell or unpleasant taste (McMahan, 1983), or to the presence of toxic allelochemicals as reported by Lamdin et al. (2000) for *Lagoacris pata* Packard (Lepidoptera: Megalopygidae).

The prey species had a significant influence on the macromolecular profile of *R. marginatus*, which had higher total protein content when reared on the three lepidopteran larvae, higher lipid content when reared on two adult meloid coleopterans and higher carbohydrate content when reared on the adult heteropteran prey, relative to that recorded when reared on the reference prey. This suggests that different metabolic pathways are activated by ingestion of these prey, with the increased synthesis of amino acids and proteins being of particular interest, as the biological control potential of reduviids increases logarithmically in response to the protein content of their prey (O'Neill et al., 2008). Also the number of haemocytes, taken as an index of the physiological state of the predator (Ambrose & George, 1996), was higher in predators reared on the lepidopteran larvae than on the reference prey, also a lepidopteran, followed by predators reared on one species of heteropteran prey, with a positive correlation between the number of haemocytes and content of amino acids and total lipids. The differences recorded here in the number of haemocytes in predators reared on different species of prey support the notion that total haemocytes counts, among other things, are influenced by nutrition (Jones, 1962; Wheeler, 1963). The regression analysis between total protein and lipids with THC was highly significant supporting the views of Wheeler (1963) and Jones (1962).

Haemocytes are involved in cellular immunity (Lavine & Strand, 2002) and protein storage (Geiger et al., 1977), transport of nutrients and hormones (Patton, 1983) and histo-morphological changes during development (Hazarika & Gupta, 1987). The especially high content of amino acids and protein in predators reared on larvae of the noctuid *S. litura* together with the high number of haemocytes (with elevated proportions of prohaemocytes and plasmatocytes) indicate that this species of prey is important for *R. marginatus* not only in terms of direct nutrition but also in terms of increased immunity. This presumed essential character of *S. litura* was reflected in the higher kairomone-based preference for this pest over the other three lepidopteran species.

The present study has demonstrated significant differences in the response of *R. marginatus* to several species of pests. Among the six species of prey offered, the larvae of lepidopteran cotton pests were generally of superior quality in terms of arousing this predator's interest (approach time), weight gain, content of amino acids and protein and increasing haemocyte numbers. This finding indicates that *R. marginatus* might be used as a good biocontrol agent against lepidopteran cotton pests, especially the larvae of the noctuid *S. litura*. This study also indicates that the body extracts of the prey species tested contain kairomones, which elicit probing behaviour in *R. marginatus*. Further studies should be undertaken to investigate the prey preferences of *R. marginatus* in plants infested with one or several herbivores (Moayeri et al., 2007). Further studies will also be needed to investigate suitable strategies for the use of *R. marginatus* for controlling insect pests of cotton.

ACKNOWLEDGEMENTS. Our sincere thanks to the authorities of St. Xavier's College for letting us use their laboratory facilities. The senior author acknowledges a grant (SR/SO/AS-33/2006) from the Department of Science and Technology, Government of India to carry out this work. K. Jensen, Dept. of Agroecology, Aarhus University, is thanked for editorial and language assistance. An anonymous reviewer and an associated editor is thanked for their thorough and critical comments and useful suggestions on a previous manuscript, which greatly helped us to improve this paper.

REFERENCES

Ambrose D.P. 1980: Bioecology of an alate assassin bug, *Acanthapis quinquespinosa* Fab. (Heteroptera: Reduviidae). In Goel S.C. (ed.): *Proceedings of Symposium on Insect Ecology and Resources Management*, Muzafar Nagar, India, pp. 107–115.

Ambrose D.P. 1999: *Assassin Bugs.* Science Publishers, New Hampshire, USA and Oxford & IBH Publishing, New Delhi, India, 337 pp.

Ambrose D.P. & Claver M.A. 2001: Survey of reduviid predators in seven pigeon pea agroecosystems in Tirunelveli, Tamil Nadu, India. — *Int. Chickpea Pigeonpea Newslet*. **8**: 44–45.

Ambrose D.P. & George P.J.E. 1996: Total and differential haemocytes diversity in three morphs of *Rhynocoris marginatus* (Insecta: Heteroptera: Reduviidae). — *Fresen. Environ. Bull.* **5**: 202–206.

Ananthakrishnan T.N. 1996: Molecular messengers in insect biocommunication: Modality 459 and relevance in biological control. — *Curr. Sci.* **70**: 215–218.

Bragdon J.H. 1951: Colorimetric determination of blood lipids. — *J. Biol. Chem.* **190**: 513–517.

CICR – Central Institute for Cotton Research 2011: http://www.cicr.org.in/ accessed 30 November 2011.

Claver M.A. & Ambrose D.P. 2003: Influence of hunger level and prey density on searching behaviour of the reduviid predator *Rhynocoris marginatus* (Fabricius) (Heteroptera, Reduviidae). — *J. Appl. Entomol*. **127**: 42–45.

Cohen A.C. 1990: Feeding adaptation of some predaceous Heteroptera. — *Ann. Entomol. Soc. Am.* **83**: 1215–1223.

David B.V. & Ananthakrishnan T.N. 2004: *General and Applied Entomology.* Tata McGraw-Hill, New Delhi, 260 pp.

Easwaramoorthy S., David H., Kurup N.K. & Santhanalakshmi G. 1994: Studies on the spider fauna of sugarcane ecosystem in southern Peninsular. — *Indian J. Biol. Contr*. **8**: 85–93.

Evans D.E. 1962: The food requirements of *Phonoctonus nigrofacialis* Stal (Hemiptera). — *Entomol. Exp. Appl.* **5**: 33–39.

Geiger G.J., Krolak J.M. & Mills R.R. 1977: Possible involvement of cockroach haemocytes in the storage and synthesis of cuticle proteins. — *J. Insect Physiol.* **23**: 227–230.

Grundy P. & Maelzer D. 2000. Predation by the assassin bug *Pristhesancus plagipennis* (Walker) (Hemiptera: Reduviidae) of *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) and *Nezara viridula* (L.) (Hemiptera: Pentadomidae) in the laboratory. — *Aust. J. Entomol.* **39**: 280–282.

Gupta A.P. 1985: Hemocytes. In Kerkut G.A & Gilbert L.I. (eds): *Comprehensive Insect Physiology, Biochemistry and Pharmacology*. *Vol. 3*. Pergamon Press, Oxford, pp. 453–485.

Haridass E.T., Morrison M.N. & Balu A. 1988: Predatory behaviour of *Rhynocoris marginatus* (Fab.) (Harpactorinae: Reduviidae: Heteroptera: Insecta). — *Proc. Ind. Acad. Sci. (Anim. Sci.)* **97**: 41–48.

Hashimoto K. & Hayashi F. 2014: Cantharidin world in nature: a concealed arthropod assemblage with interactions via the terpenoid cantharidin. — *Entomol. Sci*. **17**: 388–395.

Hazarika L.K. & Gupta A.P. 1987: Variation in haemocyte populations during various developmental stages of *Blatella germanica*. — *Zool. Sci.* **4**: 307–313.

Jones J.C. 1962: Current concepts concerning insect haemocytes. — *Am. Zool*. **2**: 209–246.

Kumaraswami N.S. 1991: *Bioecology and Ethology of Chosen Predatory Bugs and their Potential in Biological Control.* Ph.D. Thesis, Madurai Kamaraj University, 181 pp.

Lamdin J.M., Howell D.E., Kocan K.M., Murphey D.R., Amold D.C., Fenton A.W., Odell G.V. & Ownby C.L. 2000: The venomous hair structure, venom and life cycle of *Lagoa crispata*, a puss caterpillar of Oklahoma. — *Toxicon* **38**: 1163–1189.

Lavine M.D. & Strand M.R. 2002: Insect hemocytes and their role in immunity. — *Insect Biochem. Mol*. **32**: 1295–1309.

Lowry O.H., Rosebrough N.J., Farra L. & Randell R.J. 1951: Protein measurement with Folin phenol reagent. — *J. Biol. Chem.* **193**: 265–275.

McMahan E.A. 1983: Adaptations, feeding preferences, and biometrics of a termite-baiting assassin bug (Hemiptera: Reduviidae). — *Ann. Entomol. Soc. Am.* **76**: 483–486.

Moayeri H.R.S., Ashouri A., Poll L. & Enkegaard A. 2007: Olfactory response of a predatory mirid to herbivore induced plant volatiles: multiple herbivory vs. single herbivory. — *J. Appl. Entomol.* **131**: 326–332.

O'Neill K.M., Blodgett S., Olson B.E. & Miller R.S. 2008: Impact of livestock grazing on abundance of Miridae and Reduviidae (Hemiptera) incrested wheatgrass pastures. — *J. Econ. Entomol.* **101**: 309–313.

Palanivelu P. 2001: *Analytical Biochemistry and Separation Techniques*. Palkalai Nagar, Madurai, pp. 60–62.

Patton R.L. 1983: *Introductory Insect Physiology.* W.B. Saunders, Philadelphia, 245 pp.

Phoofolo M.W., Obrycki J.J. & Krafsur E.S. 1995: Temperature dependent ovarian development in *Coccinella septempunctata* (Coleoptera: Coccinellidae). — *Ann. Entomol. Soc. Am.* **88**: 72–79.

Rocha L. & Redaelli L.R. 2004: Functional response of *Cosmoclopius nigroannulatus* (Hemiptera: Reduviidae) to different densities of *Spatocera dentiventris* (Hemiptera: Coreidae) nymphaea. — *Braz. J. Biol.* **64**: 309–316.

Sadasivam S. & Manickam A. 1997: *Biochemical Methods. 2nd ed.* New Age International, New Delhi, 257 pp.

Sahayaraj K. 1991: *Bioecology, Ecophysiology and Ethology of Chosen Predatory Hemipterans and their Potential in Biological Control (Insecta: Heteroptera: Reduviidae)*. Ph.D. Thesis, Madurai Kamaraj University, 303 pp.

Sahayaraj K. 1995: Bioefficacy and prey size suitability of *Rhynocoris marginatus* (Fab.) to *Helicoverpa armigera* (Hub.) of groundnut (Insecta: Heteroptera: Reduviidae). — *Fresen. Environ. Bull*. **4**: 270–278.

Sahayaraj K. 1999: Effect of prey and their age on the feeding preference of *Rhynocoris marginatus* (Fab.). — *Int. Arachis Newsl*. **19**: 39–41.

Sahayaraj K. 2000: Evaluation of biological control potential evaluation of *Rhynocoris marginatus* (Fab.) on four groundnut pests under laboratory condition. — *Int. Arachis Newsl.* **20**: 72–74.

Sahayaraj K. 2002: Small scale laboratory rearing of a reduviid predator, *Rhynocoris marginatus* Fab. (Hemiptera: Reduviidae) on *Corcyra cephalonica* Stainton larvae by larval card method. — *J. Centr. Eur. Agric*. **3**: 137–147.

Sahayaraj K. 2006: Ecological adaptive features of hunter reduviids (Heteroptera: Reduviidae: Reduviinae) and their biological control. In Guptha V.K. & Verma A.K. (eds): *Perspective in Animal Ecology and Reproduction*. *Vol. 3.* Daya Publishing House, New Delhi, pp. 22–49.

Sahayaraj K. 2007: *Pest Control Mechanism of Reduviids*. ABD Publisher, Narayan Niwas, Jaipur, 204 pp.

Sahayaraj K. 2008: Approaching and rostrum protrusion behaviours of *Rhynocoris marginatus* on three prey chemical cues. — *Bull. Insectol.* **61**: 233–237.

Sahayaraj K. 2014: Reduviids and their merits in biological control. In Sahayaraj K. (ed.): *Basic and Applied Aspects of Biopesticides*. Springer, India, pp. 195–214.

Sahayaraj K. & Ambrose D.P. 1992: Biology and predatory potential of *Endochus umbrinus* Reuter (Heteroptera: Reduviidae) from South India. — *Bull. Entomol*. **33**: 42–55.

Sahayaraj K. & Ambrose D.P. 1993: Biology and predatory potential of *Coranus nodulosus* Ambrose & Sahayaraj on *Dysdercus cingulatus* Fab. and *Oxycarenus hyalinipennis* Costa (Heteroptera: Reduviidae). — *Hexapoda* **5**: 16–22.

Sahayaraj K. & Ambrose D.P. 1994: Stage and host preference of the predator *Acanthaspis pedestris* Stal to four cotton pests. — *J. Biol. Contr.* **8**: 23–26.

Sahayaraj K. & Delma J.C.R. 2004: Chemically mediated behaviour of the predator *Rhynocoris marginatus* (Fab.) (Heteroptera: Reduviidae) searching insect prey. — *Belg. J. Entomol.* **6**: 75–81.

Sahayaraj K. & Martin P. 2003: Assessment of *Rhynocoris marginatus* (Fab.) (Hemiptera: Reduviidae) as augmented control in groundnut pests. — *J. Centr. Eur. Agric*. **4**: 103–110.

Sahayaraj K. & Paulraj M.G. 2001: Behaviour of *Rhynocoris marginatus* (Fab.) (Hemiptera: Reduviidae) to chemical cues from three lepidopteran pests. — *J. Biol. Contr.* **15**: 1–4.

Sahayaraj K. & Ravi C. 2007: Evaluation of reduviid predators and plant products against chosen groundnut pests. — *Arch. Phytopathol. Plant Prot*. **40**: 281–290.

Sahayaraj K. & Sathiamoorthi P. 2002: Influence of different diets of *Corcyra cephalonica* on life history of a reduviid predator *Rhynocoris marginatus* (Fab.). — *J. Centr. Eur. Agric*. **3**: 53–62.

Sahayaraj K., Delma J.C.R. & Martin P. 2003: Biological control potential of aphidophagous reduviid predator *Rhynocoris marginatus*. — *Int. Arachis Newsl.* **23**: 29–30.

Sahayaraj K., Thangarani S. & Delma J.C.R. 2004: Comparative prey suitability of *Helicoverpa armigera* and *Spodoptera litura* larvae for *Rhynocoris marginatus* (Fab.) (Hemiptera: Reduviidae). — *Belg. J. Entomol*. **6**: 383–392.

Singh P.B. & Paul A.V.N. 2002: Kairomonal effect of some saturated hydrocarbons and other chemicals on the larvae of *Chrysoperla carnea* in a multi-armed olfactometer. — *Ind. J. Entomol.* **64**: 518–523.

SPSS 2004: *SPSS 13.0 for Windows*. SPSS Inc, Chicago.

Sujatha S., Vidya L.S. & Sumi G.S. 2012: Prey-predator interaction and info-chemical behavior of *Rhynocoris fuscipes* (Fab.) on three agricultural pests (Heteroptera: Reduviidae). — *J. Entomol.* **9**: 130–136.

Tebayashi S., Kawahara T., Kim C.S., Nishi A., Takashi K., Miyanoshita A. & Horiike M. 2003: Feeding stimulants eliciting the probing behavior for *Peregrinator biannulipes* Montrouzier et Signore (Hemiptera: Reduviidae) from *Tribolium confusum* (Jacquelin du Val). — *Z. Naturforsch.* **58**: 295–299.

Wheeler R.E. 1963: Studies on the total haemocyte count and haemolymph volume in *Periplaneta americana* with special reference to last moulting cycle. — *J. Insect Physiol.* **9**: 223–235.

Received October 21, 2014; revised and accepted October 23, 2015
Published online January 8, 2016