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Stage Preference and Functional Response of *Rhynocoris longifrons* (Stål) (Hemiptera: Reduviidae) on Three Hemipteran Cotton Pests

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ABSTRACT

In this work, the stage preference and functional response of the indigenous reduviid bug Rhynocoris longifrons *feeding on five different densities of the cotton aphid* Aphis gossypii, Phenacoccus solenopsis, *and* Dysdercus cingulatus *was examined in Petri dish arenas containing cotton leaves under laboratory conditions. The reduviid predator exhibited a Type II functional response at all hemipteran pests evaluated when data were fit to Holling's disc equation. Predatory rate gradually increased while the predator grew older and adults consumed maximum number of* D. cingulatus *and* P. solenopsis. *An opposite trend was observed, while the reduviid was provided with* Aphis gossypii. *The rate of attack on* P. solenopsis *was quite low but fairly consistent, with the different life stages of the predator generally more effective. Further investigation of the biological control potential of* R. longifrons *against cotton pests under pot and controlled filed should be done due to the predator's ability to kill adult stages of all prey species evaluated. These results indicated that* R. longifrons *could eat more aphids at high prey densities; however, predators also considerably reduced other cotton pests too so it could be considered a prospective candidate for use as a commercial biological control agent for cotton hemipteran pests in India.*

Key words: Cotton pests, functional response, reduviid predator, stage preference

INTRODUCTION

Dysdercus cingulatus(Fab.) (Pyrrhocoridae), *Phenacoccus solenopsis* (Tinsley) (Pseudococcidae) and *Aphis gossypii* (Glover) (Aphididae) are representative species of the three key economically important Hemipteran genera. Mealy bug, *P. solenopsis* is the most widely distributed species in tropical, subtropical and warm regions. *P. solenopsis* attacks the roots just below the level of the soil, especially where the root and the stem meet (Patel et al. 2010). Red cotton bug, native to Asia, is similarly widespread although it has not yet attained pest status in Central and South America, Europe, or North Africa. The red cotton bug or cotton stainer, *D. cingulatus* in particular causes serious damage by feeding on developing cotton bolls and ripe cotton seeds and transmitting fungi (Iwata 1975). It is difficult to control by insecticides because it is a highly mobile, polyphagous and polymorphic pest (Sahayaraj and Ilayaraja 2008) of many malvaceae crops. The cotton aphid *A. gossypii* is a polyphagous pest with worldwide distribution in tropical, subtropical, and warm temperate regions (Isikber 2005). This aphid is a vector of more than 30 plant viruses and has been observed feeding on more than 80 plant families (Blackman and Estop 1984; Ebert and Cartwright 1997; Ghabeish et al. 2010).

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Reduviid predators are considered as potential biocontrol agents against many insect pests (Sahayaraj 2007; Grundy and Maelzer 2000; Grundy 2007) and have been suggested to integrate in Bio-intensive Integrated Pest Management (BIPM). *Rhynocoris longifrons* (Stal) (Hemiptera: Reduviidae) is a voracious harpactorine reduviid predator (Ambrose et al. 2003) mainly distributed in India. This reduviid has been found in cotton ecosystems and predating on many insects pets (unpublished data). *Rhynocoris longifrons* is largely effective in predating upon the larval stages of cotton pests, such as *Helicoverpa armigera* that typically develop within the flowers and leaves (Ravichandran et al. 2003). Records of specific associations between *R. longifrons* and cotton sucking pests are limited.

Functional response characterizes the relationship between the number of prey consumed by the individual predators and the density of available prey (Solomon 1949; Holling 1959 a,b). The potential biocontrol efficacy of candidate agents can be extrapolated by quantifying the functional response, which serves as a predictor of attainable top-down, density-dependent regulation of a given pest species (Murdoch and Oaten 1975). Although some studies have investigated the functional response of *R. longifrons* against *Odontotermes obesus* Rambur (Kumar and Ambrose 1996), *Clavigralla gibbosa* Spinola (Claver et al. 2002), *Helicoverpa armigera* (Hübner) (Ravichandran et al. 2003), none of them has addressed the effect of hemipteran cotton pests on the functional response of the predator. Published accounts of reduviid functional response to cotton insect pests have thus far excluded the evaluations of the predator's potential to control the hemipteran prey (Grundy and Maelzer 2000; Grundy 2007). Functional response is an appropriate way to characterize the interaction of *R. longifrons* a number of three different hemipteran prey species and stages in a highly simplified environment. The current study investigated the stage preference and functional response of *R. longifrons* against three cotton pests with a view to optimize the biological control of these economically important sucking pests.

MATERIALS AND METHODS

Life stages of *R. longifrons* were collected from the scrub jungle bordering cotton agroecosystem of Kanyakumari district, Tamil Nadu. *R.longifrons* was maintained in the control temperature room at 32 ^{o}C and 75±5 % RH in plastic containers (one litre capacity) in a photo period of 11 h L and 13 h D. The pest *D. cingulatus*, *P. solenopsis* and *A. gossypii* were collected from the cotton agro-ecosystem of Tirunelveli district. They were maintained in the laboratory condition as mentioned above in plastic troughs (3 litre capacity). The predator was reared for one generation on an *ad libitum* supply of mixed life stages of these pests before starting the functional response study.

Stage preference

Stage preference studies were conducted in third, fourth, fifth nymphal instars and adult of *R. longifrons* against the life stages of *D. cingulatus* (second, third, and fourth nymphal instars), *P. solenopsis* (first, second, third nymphal instars and adult). To standardize the response, predators were starved for 24 h in plastic boxes before release into the test arena. The experimental arena consisted of a glass Petri dish (14 cm in diameter) lined with paper towel. Each dish contained a cotton leaf with its petioles inserted into an Eppendorf tube (2 ml) filled with sucrose water (1 %). The average leaf area (both sides) was estimated to be approximately 20-25 cm^2 (n = 5). Preys (2 in each stage) were gently transferred by a fine camel hair brush from plants of the stock culture to the leaves in the test arenas. The preys were allowed to settle and a third instar predator was introduced in each Petri dish. At each prey type, there were ten replicates for predator treatments and five controls (i.e., arenas without a predator). The preferred stage of the predator was recorded visually; similar procedure was followed for other life stages. Successfully preferred stage of the prey was used to record the functional response study.

Functional response studies

Experimental arena was prepared as mentioned for the stage preference studies. Second, third nymphal instars *D. cingulatus* (for nymphs and adult predator); adults of *P. solenopsis* and *A. gossypii* (all life stages of the predator) were used as prey in the experiment. The experiment was performed at five different densities of *Aphis gossypii* (5,10,20,30,40), *P. solenopsis* (2,4,6,8,10), and *D. cingulatus* (1,2,4,8,16). Appropriate numbers of aphids were gently transferred by a fine camel hair brush from the plants of the stock culture to the cotton leaves in the test arenas. The aphids were allowed to settle and a third instar predator was introduced in each Petri dish. At each prey density, there were ten replicates for predator treatments and controls (i.e. arenas without a predator). The total number of prey killed during a 24-h period was recorded. Killed preys were replaced during the experiment. No mortality was recorded in the control category. Holling ‘disc’ equation (Holling 1965) was used to describe the functional response of *R. longifrons.*

Data analyses

Data were analyzed using SPSS (version 11.5) for the analysis of variance (ANOVA) and t-test, SAS for the analysis of functional response. Data were submitted to a two-way ANOVA 137 for the significance of the main effects of prey density and temperature on predation and their interaction. In the present study, the prey densities changed during the experimental period with each consumption event. To account for this prey depletion during the experiments, a generalized model of Rogers’s random predator equation (Rogers, 1972) was used.

RESULTS

Stage preference

Results showed that third, fourth and fifth nymphal instars of *R.longifrons* significantly preferred second instar nymphs of *D. cingulatus* (df3,18; F= 8.70; P = 0.05), whereas the adult preferred third instar nymphs (df3,18; F= 8.68; P = 0.05) (Fig. 1A). However, nymphal instars and adult of *R. longifrons* selected adults of *P. solenopsis* (df3,18; F= 8.69; P = 0.05) (Fig. 1B) and *A. gossypii* (100%) (df3,18; F = 26.80; P = 0.01).

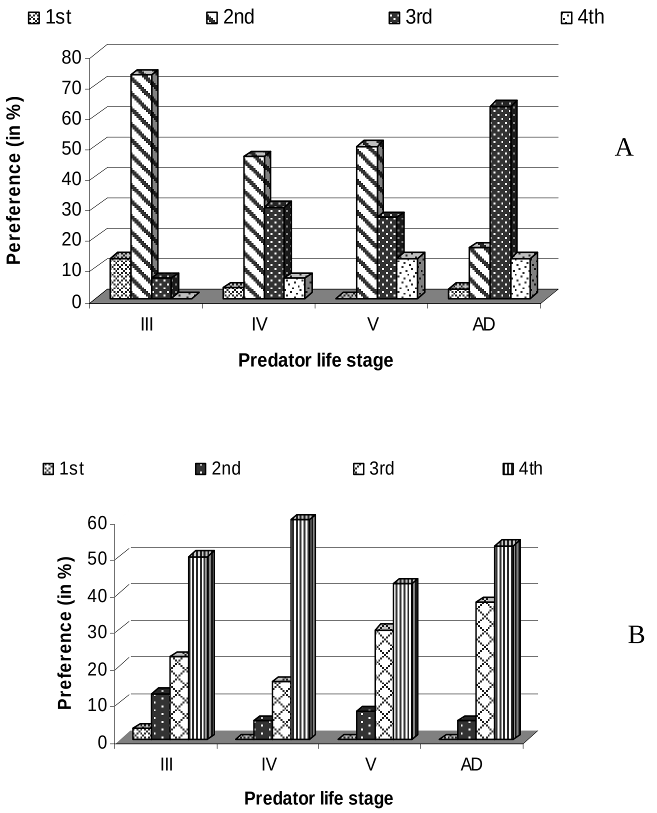

Figure 1 - Stage preference of *R .longifrons* on *D .cingulatus* (A), *P. solenopsis* (B).

Functional response

The proportion of prey consumed by the predator declined with increasing prey density. The coefficients of determination (R^2) indicated not much variation in predation rates against the tested preys (Table 1). Generally, searching efficiency

(E) gradually diminished while the prey density increased. When *D. cingulatus* (Table 2) and *P. solenopsis* (Table 3) were provided as preys, *R. longifrons* nymphs quickly searched rather than the adults. An opposite trend was observed when *A. gossypii* was offered as prey (Table 4). Attack rate decreased as prey density increased from 1 to 16 (*D. cingulatus*) or 5 to 40 (*Aphis gossypii*) or 2 to 10 (*P. solenopsis*) preys. At higher *D. cingulatus* (0.68 h^{-1}) and *P. solenopsis* (0.57 h^{-1}) densities, attack rate of adult predator was higher than that of third instar predator. An opposite response was observed while *A. gossypii* was provided as a prey. Maximum prey consumption (Na Maximum) gradually diminished as the predator grew older while offered with *Aphis gossypii*. However, the prey consumption increased when the predator was provided with *P. solenopsis* and *D. cingulatus.*

Table 1 - Correlation coefficient (R^2) between numbers of prey offered (N) and number of prey consumed (Na) by *R. longifrons* provided with three hemipteran pests of cotton

Life stage of the reduviid	**Cotton Pests**		
	D. cingulatus	***Aphis gossypii***	***P. solenopsis***
Third instar	0.92628	0.94034	0.94387
Fourth Instar	0.93105	0.976623	0.982511
Fifth instar	0.99294	0.97888	0.88211
Adult	0.97930	0.91317	0.98196

Table 2 - Functional response parameters recorded for the life stages of *R. longifrons* on *D. cingulatus***.**

Predator life stages	**N**	**Na**	**E**	**T_h**	**Total Th**	**Ts**	**Na Maximum**	**a'**
III	1	1.0	1.0	0.29	0.29	0.71		1.40
	2	2.0	1.0	0.25	0.50	0.75		1.33
	4	2.3	0.57	0.37	0.85	0.63		0.90
	8	3.1	0.38	0.33	1.02	0.67		0.56
	16	3.8	0.23	0.12	0.45	0.88	3.8	0.26
IV	1	1.0	1.0	0.40	0.40	0.60		1.66
	2	1.2	0.60	0.27	0.34	0.73		0.82
	4	1.8	0.45	0.43	0.77	0.57		0.78
	8	3.4	0.42	0.43	1.46	0.57		0.73
	16	3.8	0.23	0.41	1.56	0.59	3.8	0.38
V	1	1.0	1.0	0.36	0.36	0.64		1.56
	2	1.6	0.80	0.07	0.11	0.93		0.86
	4	2.4	0.60	0.52	1.25	0.48		1.25
	8	3.2	0.40	0.22	0.70	0.78		0.51
	16	5.4	0.33	0.30	1.62	0.70	5.4	0.47
Adult	1	1.0	1.0	0.25	0.25	0.75		1.33
	2	1.6	0.80	0.25	0.40	0.75		1.06
	4	2.3	0.57	0.12	0.28	0.88		0.64
	8	3.0	0.37	0.20	0.60	0.80		0.46
	16	8.0	0.50	0.27	2.16	0.73	8.0	0.68

Functional response parameters: N = Prey densities, Na = No. of prey consumed, E = Searching Efficiency, T_h = Handling time, Total Th = Na x Th, Ts = time of searching, Na Maximum, a = rate of discovery.

Table 3 - Functional response parameters recorded for the life stages of *R. longifrons* on *P. solenopsis.*

Predator life stages	N	Na	E	T_h	Total Th	Ts	Na Maximum	a’
III	2	1.0	0.50	0.15	0.15	0.85		0.58
	4	1.3	0.32	0.4	0.52	0.59		0.54
	6	2.3	0.38	0.06	0.13	0.94		0.40
	8	2.3	0.28	0.20	0.40	0.80		0.35
	10	2.6	0.26	0.17	0.44	0.83	2.6	0.31
IV	2	1.4	0.70	0.38	0.53	0.62		1.12
	4	1.8	0.45	0.53	0.95	0.47		0.95
	6	2.6	0.43	0.49	1.27	0.51		0.84
	8	2.8	0.35	0.21	0.58	0.79		0.44
	10	3.2	0.32	0.64	2.0	0.36	3.2	0.88
V	2	1.6	0.80	0.12	0.19	0.88		0.90
	4	1.6	0.40	0.05	0.08	0.95		0.42
	6	3.0	0.50	0.12	0.36	0.88		0.56
	8	2.6	0.32	0.19	0.49	0.81		0.39
	10	3.3	0.33	0.15	0.49	0.85	3.3	0.38
Adult	2	1.3	0.65	0.04	0.05	0.96		0.67
	4	1.6	0.40	0.16	0.26	0.84		0.47
	6	2.6	0.43	0.19	0.49	0.81		0.53
	8	4.0	0.50	0.09	0.36	0.91		0.54
	10	5.0	0.50	0.13	0.65	0.87	5.0	0.57

Functional response parameters: N = Prey densities, Na = No. of prey consumed, E = Searching Efficiency, T_h = Handling time, Total Th = Na x Th, Ts = time of searching, Na Maximum, a = rate of discovery

Table 4 - Functional response parameters recorded for the life stages of *R. longifrons* on *Aphis gossypii.*

Predator life stages	N	Na	E	T_h	Total Th	Ts	Na Maximum	a’
III	5	1.8	0.36	0.31	0.56	0.69		0.52
	10	3.0	0.30	0.27	0.81	0.73		0.41
	20	5.8	0.29	0.37	2.14	0.63		0.46
	30	5.4	0.18	0.26	1.40	0.74		0.24
	40	10.6	0.26	0.16	1.69	0.84	10.6	0.30
IV	5	2.4	0.48	0.15	0.36	0.85		0.56
	10	5.0	0.50	0.44	2.2	0.56		0.89
	20	6.0	0.30	0.15	0.90	0.85		0.35
	30	7.6	0.25	0.24	1.82	0.76		0.32
	40	10.4	0.26	0.37	3.85	0.63	10.4	0.41
V	5	1.0	0.20	0.16	0.16	0.84		0.23
	10	2.6	0.26	0.24	0.62	0.76		0.34
	20	5.0	0.25	0.09	0.45	0.91		0.27
	30	5.6	0.18	0.36	2.01	0.64		0.28
	40	7.6	0.19	0.30	2.28	0.70	7.6	0.27
Adult	5	1.0	0.20	0.40	0.40	0.60		0.33
	10	3.8	0.38	0.19	0.72	0.81		0.46
	20	3.6	0.18	0.37	1.33	0.63		0.28
	30	5.8	0.19	0.15	0.87	0.85		0.22
	40	6.2	0.15	0.20	1.24	0.80	6.2	0.18

Functional response parameters: N = Prey densities, Na = No. of prey consumed, E = Searching Efficiency, T_h = Handling time, Total Th = Na x Th, Ts = time of searching, Na Maximum, a = rate of discovery

DISCUSSION

Rhynocoris longifrons is a generalist predator; it occurs from India through the central Tamil Nadu. This species is commonly found in agro-ecosystems in India. Few studies have examined the effect of prey size on predator responses. Sahayaraj (1995a), Ambrose and Sahayaraj (1993), Sahayaraj and Ambrose (1994), Sahayaraj and Ambrose (1995), Cogni et al. (2002), Claver and Ambrose (2002) reported that small size reduviids preferred small size preys whereas large size predator preferred large size prey. As a rule, it could be supposed that larger preys were easier to be detected by a predator (Bell 1990). Similarly in the present study, all life stages of *R. longifrons* preferred only the adults of *P. solenopsis* and *A. gossypii*. However, nymphs and adults of *R. longifrons* preferred second and third instar nymphs of *D. cingulatus*. When compared to the predator body mass, all the tested prey's body was comparatively less, and hence predators invariable preferred stages were the largest size among the tested prey stages.

Although most predators attack the largest available individuals of their prey species, those species are generally smaller in body size than the predator. Predatory arthropods are known to be an exception to this limiting predator: prey relative body size ratios, because maximum prey size can be increased through the use of venoms, traps, or group hunting (Sabelis 1992). The results of the present study indicated that *R. longifrons* was capable of low level but fairly consistent success in killing its larger hemipteran prey. The results indicated that the percentage of hemipteran life stages of tested prey attacked by *R. longifrons* decreased as prey availability increased, typifying a Type II density independent functional response (Holling 1959, 1965; Gotelli 1995). A similar Type II functional response curves have been reported in a number of other reduviids (Sahayaraj 1995; Ambrose and Sahayaraj 1996; Claver et al. 2002; Ambrose et al. 2008, 2009, 2010; Sahayaraj and Asha 2010). However, Holling (1965) stated that predators showing a type III response were theoretically more capable of suppressing prey populations. It, therefore, could be expected that the equilibrium in predator: prey population dynamics, the theoretical hallmark of pest population regulation through the biological control, would not be attained following the release of *R. longifrons* in cotton field. But Schenk and Bacher (2002) reported that the evaluations performed under restrictive conditions (cages; single prey species) routinely indicated a Type II functional response in generalist insect predators. Van Alebeek et al. (1996) suggested that the constraints of experimental design might actually obfuscate the true nature of the functional response curve in the context of invertebrate predators, specifically citing how in a confined arena the increased chance of prey discovery might exaggerate the steepness of the response curve at the lowest prey densities. Finally, significant discrepancies in the outcome of laboratory vs. field evaluations of functional response have been reported (Schenk and Bacher 2002). Although the predator's response to life stages of *D. cingulatus* was particularly encouraging, the results presented here suggested that further evaluations of the predatory response of *R. longifrons* to hemipteran pest under more complexes experimental conditions should be done.

Functional responses may provide important information on the voracity of a biological control agent, and on the effects of abiotic (e.g. temperature) or biotic (e.g., host insect) factors on its foraging efficiency (Mohaghegh et al. 2001; Skirvin and Fenlon 2001; Mahdian et al. 2006; Li et al. 2007). However, functional response studies have been criticized because they are often performed in small artificial arenas using unrealistic prey densities and do not consider spatial habitat complexities or multispecies prey situations (Murdoch 1983; O'Neil 1989; Kareiva 1990; Wiedenmann and O'Neil 1991; Hardman et al. 1999). Furthermore, functional responses do not consider crucial life history parameters of a predator that may affect its value as a biological control agent.

Biological control programs should consider that although *R. longifrons* uses a large range of prey size, this predator prefers aphids and mealy bug adults with less than its own mass. This is important information to decide which part of the moth life-cycle could be more efficiently suppressed in the field by this predator. However, more field studies are needed to understand the foraging behavior *R. longifrons* in different cropping systems, in order to design the practical release strategies for this reduviid.

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