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Chemically Mediated Prey-Approaching Behaviour of the Reduviid Predator *Rhynocoris fuscipes* (Fabricius) (Insecta: Heteroptera: Reduviidae) by Y-arm Olfactometer

K. Nagarajan and D.P. Ambrose

Entomology Research Unit, St. Xavier's College, Palayamkottai-627 002, Tamil Nadu, India

Abstract: The reduviid predator, *Rhynocoris fuscipes* (Fabricius) (Heteroptera: Reduviidae) is a potential predator inhabiting diverse agroecosystems and preying upon about 50 minor as well as major insect pests. The prey approaching behaviour of *R. fuscipes* (Fabricius) to the hexane extracts of insect pests viz., *Helicoverpa armigera* Hubner, *Spodoptera litura* (Fabricius), *Achaea janata* Linnaeus, *Dysdercus cingulatus* Fabricius and *Mylabris indica* Thunberg was assessed in Y-shaped olfactometer in terms of Excess Proportion Index (EPI). Ether in hexane fraction extracts of *S. litura* stimulated higher rostral protruding activity (6.53 ± 1.56 min) than that of *H. armigera* (4.61 ± 1.29 min) followed by *A. janata* (3.17 ± 1.11) and *D. cingulatus* (2.95 ± 1.12 min). The lowest response was observed to the hexane extract of *M. indica* (1.30 ± 0.63 min). The order of excitement in behavioral response of *R. fuscipes* to the tested body extract of five insect pests was ranked as follows: *S. litura* > *H. armigera* > *A. janata* > *D. cingulatus* > *M. indica*. Thus the present study clearly reveals the host preference of *R. fuscipes* to the taxonomically diverse insect pests.

Key words: Biocontrol agent, chemical cues, predator-prey interaction, *Rhynocoris fuscipes*, Y-arm olfactometer

INTRODUCTION

Chemical ecology is the science that addresses the role of chemical cues in the interaction of organisms with their environment. The most important factors mediating the location of a host are semiochemicals through different sources. Animal communication is the exchange of information between individuals. In this exchange, one individual (signaler) transmits information to others (receivers), with both signaler and receivers being expected to benefit (Greenfield, 2002). In tritrophic interaction the semiochemicals produce different responses such as attraction, repulsion, arrest, deterrence and stimulation. The chemicals, which govern the prey and predator interaction, are generally called info-chemicals. It includes kairomones and allomones, which influence the prey-predator interaction (Ananathakrishnan, 2002). Cues can be physical such as colour, sound, shape and size as well as chemical and these may be useful for long or short-range attraction to prey (Hatano *et al.*, 2008).

Rhynocoris fuscipes (Fabricius), is a polyphagous predator inhabiting diverse agroecosystem. Prey record and pest suppression efficacy of *R. fuscipes* on various crops was extensively studied in India (Ambrose, 1995, 1999; Babu *et al.*, 1995; Ambrose and Claver, 2001; Nagarajan *et al.*, 2010). Information on its prey record, bioecology and ecophysiology and behaviour indicate

that it could be harnessed as an effective biocontrol agent in the Integrated Pest Management Programme (Ambrose, 1999). However, no tangible studies have been carried out to identify the chemical cues mediating the host seeking behaviour of any reduviid predators. Hence, the present study aims at investigating the behaviour of *R. fuscipes* to the chemical cues of *Helicoverpa armigera* Hubner, *Spodoptera litura* (Fabricius), *Achaea janata* Linnaeus, *Mylabris indica* Thunberg and *D. cingulatus* tested in the Y-arm olfactometer that elicit the host seeking response in *R. fuscipes* can be identified.

MATERIALS AND METHODS

Predator collection and maintenance: Life stages of *Rhynocoris fuscipes* (Fabricius) were collected from Muppanthal Scrub Jungle in Kanyakumarii District, Tamil Nadu. (altitude $77^{\circ} 21'$ and $8^{\circ} 7' N$) and reared in plastic containers (15×7 cm) in the laboratory under optimal conditions (temperature: $29 \pm 2^{\circ}C$; relative humidity: $80 \pm 5\%$; photoperiod 12 ± 1) on the head crushed larvae of rice meal moth *Corcyra cephalonica* (Stainton).

Pest collection and maintenance: The larvae of *Spodoptera litura* (Fabricius) and *Achea janata* (Linnaeus) (Lepidoptera) were collected from the castor fields and adults of *Mylabris indica* (Coleoptera) Thunberg were collected from pigeon pea fields in and

around Palayamkottai, Tirunelveli District, Tamil Nadu, South India. The larvae of *Helicoverpa armigera* (Hubner) (Lepidoptera) and *Dysdercus cingulatus* (Fabricius) (Hemiptera) were collected from lady's finger field near Sivanthipatti, Tirunelveli District. The larvae of *S. litura* and *A. janata* were reared in the plastic troughs (32×9 cm) on fresh castor leaves and *H. armigera* were reared in plastic containers (6×4 cm) on fresh fruits of lady's finger. The adults of *M. indica* were reared in the plastic troughs (32×9 cm) on fresh flowers of *Cassia auriculata* Linnaeus and the adults of *D. cingulatus* were reared on soaked cotton seeds in the iron field cage (2×1×1 m) covered with mosquito net. The rearing troughs or containers were examined daily and the faecal matter was removed to prevent the fungal contamination. The pests belonging to three different orders were chosen to understand the preference of the predators to any particular group of order. Moreover, these pests are inhabiting along with the predator *R. fuscipes* in cotton and pigeon pea agroecosystems.

Solvent extract of insect larvae: The body extracts of different insect pests were prepared following the methodology of Yasuda (1997). Hundred live fifth instars lepidopteran larvae of *S. litura*, *H. armigera* and *A. janata* and adults of coleopteran *M. indica* and hemipteran *D. cingulatus*, were kept in reagent bottles having 1:2 mixtures of hexane and acetone for 30 min at room temperatures, separately and subsequently stored in freezer for overnight. The solvent extracts were then filtered through a Whatman No. 1 filter paper. Thereafter, the filtered solvent extracts were evaporated in a vacuum desiccator under room temperature and the residues were dissolved in 100 mL of ether, separately. Then the ether was washed off with 50 mL of distilled water thrice. Then the ether-soluble layer was dried over sodium sulfate. Thereafter, the solvent was removed by using vacuum desiccators under room temperature and the residue was dissolved in hexane. The resultant extracts were stored at below -20°C until further use.

Bioassay for orientation/approaching behavior in Y-shaped olfactometer: A Y-shaped olfactometer made up of glass (main stem 20 cm length, two arms 15 cm length and 5 cm diameter, each and 90° angle between them.) was used for the bioassay studies. The two arms were connected to 6.5 cm diameter glass chambers (odour cells), in which the prey solvent extracts (odour sources) could be placed. Before starting the experiment the Y-shaped olfactometer with odour cells were cleaned with 70% alcohol followed by continuous blowing of air by an aerator for 15 min to remove the unwanted odour from the

odour cells. The air was blown into the two arms of the olfactometer using a small 'T' tube and the air was allowed to pass outside through the exit tubes of odour cells. A small piece of sterile cotton impregnated with body extract of insect pests (100 µL of a sample was used as test and cotton impregnated with hexane was used as control (100 µL of hexane). The 24 h-starved predators were introduced through the main stem and their predatory behaviour was observed for 30 min continuously. The predatory behaviour was observed in terms of approaching and sucking time. From these the handling time was calculated by summing up both (Cohen, 2000). The predators choose either the test chamber with body extract or the control chamber with hexane or neither. Predator chooses the test chamber or control chamber considered as positive choice or negative choice, respectively. If the predator chooses neither of the chambers, then it was considered as no choice. The experiment was replicated 12 times with 24 h starved and inexperienced predators on each body extract of insect pests, separately. The data were subjected to analyzed t-test by SPSS, 1998 9.0V.

The approaching time taken by the predators in the olfactometers to different body extracts was converted into an index called Excess Proportion Index (EPI) (Sakuma and Fukami, 1985) using the following formula:

$$EPI = \frac{NS-NC}{NS+NC}$$

where, NS is number of predators choosing the sample cell. NC is number of predators choosing the control cells, EPI values from +1 to -1. These terms simply express polarity of the directional choice. Positive values indicate a positive approach response. The assay for contact chemicals consists of counting antenation and probing frequencies towards each test sample, at given period of time.

RESULTS

The bioassay experiments were performed in the Y-shaped olfactometer and time spent by the predators *Rhynocoris fuscipes* (Fabricius) to the hexane extract of insect pest provided a clear representation of behavioural responses (Table 1).

When the predator *R. fuscipes* was released into the main chamber of the Y-shaped olfactometer, it oriented towards the odour source present in the sterile cotton with antennae directing towards the odour source. After getting perfect orientation the reduviid palpated its antennae, followed by rubbing their legs, rostral cleaning

and extended rostrum towards the odour source. Once the predator entered the sample cell it exhibited quick walking and approaching with antennae, wings and legs cleaning and rostral protrusion.

The hexane fractions of all the insect pests elicited a positive approaching response in *R. fuscipes*. *R. fuscipes* showed the highest responses to the hexane extract of *S. litura* (6.53±1.56 min) followed by *H. armigera* (4.61±1.29 min), *A. janata* (3.17±1.11 min) and *D. cingulatus* (2.95±1.12 min) and the least response to the hexane extract of *M. indica* (1.30±0.63 min). The preference to *S. litura* and *H. armigera* body extracts was significantly greater ($p < 0.05$) than to *A. janata*, *D. cingulatus* and *M. indica* (Table 1).

The handling time of *R. fuscipes* (in terms of duration of sucking time of insect pests body extracts) to the body extracts of insect pests is shown in Table 3. The predator *R. fuscipes* exhibited the highest handling time in *S. litura* extract (4.84±1.45 min) followed by *H. armigera* (2.08±1.27 min), *A. janata* (1.88±0.65 min), *M. indica* (1.30±0.63 min) and *D. cingulatus* (1.04±0.42 min) (Table 3).

The EPI values of *R. fuscipes* showed positive response to the *S. litura* (0.50), *H. armigera* (0.16) and *A. janata* (0.09) larval extracts and negative responses to *M. pustulata* (-0.27) and *D. cingulatus* (-0.16) (Table 2).

DISCUSSION

The cues stimulate receptors generating sensory inputs and finally behavioural responses. The approaching response and handling time of *Rhynocoris fuscipes* (Fabricius) to the hexane body extracts of *Spodoptera litura* (Fabricius), *Helicoverpa armigera* Hubner, *Achaea janata* Linnaeus, *Dysdercus cingulatus* Fabricius and *Mylabris pustulata* Thunberg are

Table 1: Approaching responses (min) of adult *R. fuscipes* to hexane extracts of different insect pests (n = 12; X±SE)

<i>S. litura</i>	<i>H. armigera</i>	<i>A. janata</i>	<i>M. indica</i>	<i>D. cingulatus</i>
6.53±1.56*	4.61±1.29*	3.17±1.11*	2.02±0.95	2.95±1.12

*t-test significant at $p < 0.05$

Table 2: The EPI value of adult *R. fuscipes* to hexane extracts of different insect pests

Excess Proportion Index (EPI) value				
<i>S. litura</i>	<i>H. armigera</i>	<i>A. janata</i>	<i>M. indica</i>	<i>D. cingulatus</i>
0.5	0.16	0.09	-0.27	-0.16

Table 3: Handling time (min) of *R. fuscipes* to the hexane extracts of different insect pests (X±SE)

<i>S. litura</i>		<i>H. armigera</i>		<i>A. janata</i>		<i>M. indica</i>		<i>D. cingulatus</i>	
Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
0.36±0.24	4.84±1.45*	0.16±0.11	2.08±1.27*	0.61±0.27	1.88±0.65*	-	1.30±0.63	0.36±0.25	1.04±0.42

*t-test significant at $p < 0.05$

presented in Table 1-3. The predatory behavioural pattern of *R. fuscipes* is arousal-approach-rostral probing behaviour-injecting toxic saliva-paralyzing-sucking and post predatory behaviour. *R. fuscipes* oriented towards the prey with facing antenna, after getting perfect orientation, the predator palpated its antennae, then aroused and subsequently showed the other behavioral responses (Ambrose, 1999) as observed in this reduviid predator.

The handling time of *R. fuscipes* on the info-chemicals from the five pests studied could be supported by the findings Maran (1999) in different reduviids such as *Rhynocoris kumarii* Ambrose and Livingstone and *Rhynocoris marginatus* (Fabricius) and by those of Yasuda and Wakamura (1996). They postulated that the chemical cues or kairomones of the prey stimulate the predators to respond towards them. It was very clear and from observations that *R. fuscipes* exhibited approaching, rostrum protrusion and handling the body extracts on the cotton swabs. Also reported similar kinds of observation by Yasuda and Wakamura (1996) and Yasuda (1997) with the predatory bug *Eocanthecona furcellata* (Wolff) (Heteroptera: Pentatomidae) was attracted to the larval extracts of *S. litura* from a distance and protruded their proboscis when they were approach to the odour source. *R. fuscipes* approached the test chamber raster than the control a chamber except the body extracts of *M. pustulata* and *D. cingulatus* (Table 1).

A number of saturated hydrocarbons were identified in the scales as well as whole body wash of many lepidopteran insects and their kairomonal activity has also been demonstrated (Jones *et al.*, 1973; Madhu *et al.*, 1997).

Kairomones involved in the foraging behaviour of organisms have been published and reviewed during the past decades (Ruther *et al.*, 2002). It includes chemical cues exploited by predators (Hendrichs *et al.*, 1994; Mendel *et al.*, 1995; Dejean and Beugnon, 1996; Kieley *et al.*, 1996) as well as parasitoids (Vinson, 1976; Godfray, 1994; Leprince *et al.*, 1994; Carroll *et al.*, 1995; Carroll *et al.*, 1998; Dougherty *et al.*, 1999) during their search for food or oviposition sites or both.

The involvement of antennae in the perception of volatile chemicals was proved by Crocker (1977). In his experiment, the detection of *Tricoplusia ni* (Hubner) eggs by the big eyed bug *Geocoris punctipes* (Say) was

reduced, if the distal half of the antennae was removed. It was further proved by the antennectomy studies by Ambrose *et al.* (1991) in *Acanthaspis siva* (Distant); Ambrose *et al.* (1994) in *Euagoras plagiatu*s (Bermeister) and Ambrose and Mayamuthu (1994) in *Rhynocoris fuscipes* (Fabricius), in which the antennectomized predator exhibited delayed arousal, approach capturing, paralyzing and sucking suggesting the role of antennae in predation.

The variation in the behaviour of *R. fuscipes* to the five insect pests might be due to the different volatile chemicals of the pests as explained by Ananathakrishnan (2002). In the present observation, though *R. fuscipes* responded to all the insect pest extracts it highly responded to the lepidopteran larval extracts viz., *S. litura*, *H. armigera*, *A. janata* and least preference was observed to the insect pests *M. indica* and *D. cingulatus* as observed by Maran (1999) in *Rhynocoris* spp. Interacting with *S. litura*, *M. indica* and *D. cingulatus* body extracts. Because reduviid predators generally prefer lepidopteran larvae (Ambrose, 1999). McMahan (1983) noted that reduviids, preference for one prey to another in choice test might be influenced by the noxious smell or unpleasant taste of the prey.

CONCLUSION

The present study suggests that the body extracts of *S. litura*, *H. armigera*, *A. janata* *M. indica* and *D. cingulatus* containing kairomonal compounds attracting *R. fuscipes* revealed that the predator preferred lepidopteran prey over hemipteran and coleopteran prey. Among the three lepidopterans, *R. fuscipes* exhibited an order of preference. Such an understanding will have far reaching applied value in terms of utilizing the biocontrol agent against insect pests. The result clearly indicates that *R. fuscipes* could be a better biological control agent against the lepidopteran pests than hemipteran and coleopteran pests. Among lepidopteran insect pests *R. fuscipes* could effectively control *S. litura* and *H. armigera* than *A. janata* indicated that it could be considered as a biocontrol agent against in these pests as part of Integrated pest Management Programme (IPM).

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