International Research Journal of Insect Sciences
2014 Vol.1, No.1, pp.12-19
ISSN(e): 2408-9281
ISSN(p): 2412-3439

OLFACTORY RESPONSE OF AN ASSASSIN BUG, *RHYNOCORIS LONGIFRONS* (INSECTA: HEMIPTERA: REDUVIIDAE) TO THE HEXANE EXTRACTS OF DIFFERENT AGRICULTURAL INSECT PESTS

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ABSTRACT

The predator-prey interaction of five prey species with the assassin bug, Rhynocoris longifrons (Stål) (Hemiptera: Reduviidae) was assessed in a Y-shaped olfactometer and the prey preference was assessed in six-arm olfactometer provided with the bodyextracts in hexane. Although R. longifrons responded to all the hexane extracts of testedinsect pests, R. longifrons showed maximum response to the lepidopterans Spodoptera litura (F.) (6.67±1.18 min), Helicoverpa armigera (Hübner) (5.17±0.89 min) and Achaea janata (L.) (4.42±1.04 min) followed by the coleopteran Mylabris indica (Thunberg) (3.00±0.82 min) and the least response to the hemipteran Dysdercus cingulatus (F.) (2.42±0.76 min).Thus, the present study clearly reveals the order of the host preference of R. longifrons to the tested hexane extracts of the taxonomically diverse insect pests.

Keywords: Assassin bug, *Rhynocoris longifrons*, Kairomonal ecology, Olfactometer, *Spodoptera litura, Helicoverpa armigera, Achaea janata, Mylabris indica, Dysdercus cingulatus.*

Contribution/ Originality

This paper contributes the first hand information on the allelochemical interaction of Rhynocoris longifrons with its prey species Spodoptera litura, Helicoverpa armigera, Achea janata, Dysdercus cingulatus and Mylabris indica and reveals its prey preference. It also enables one to employ R. longifrons as a biocontrol agent against these insect pests.

1. INTRODUCTION

The Reduviidae is the largest family of predaceous land Hemiptera and many of its members are found to be potential predators of a number of insect pests [1]. Since they are polyphagous they may not be effective on specific pests, but they are valuable predators in situations where a variety of insect pests occur. *Rhynocoris longifrons* (Stål) (Hemiptera: Reduviidae) is an harpactorine assassin bug predating upon insect pests such as cotton bollworm *Helicoverpa*

armigera (Hübner), plume moth *Exelastis atomosa* Walsingham, tur pod bug *Clavigralla gibbosa* Spinola, green stink bug *Nezara viridula* (Linnaeus) andpod bug *Riptortus pedestris* (Fabricius) [2].

Chemical and/or physical cues from the host, the substrate and associated material and/or organisms play a fundamental role in mediating the different steps of host selection [3-5]. Volatiles are important cues for many arthropods. Use of chemicals emanating from the host and its by-products which enhance the behavioural dynamics of entomophages increasing their effectiveness were advocated [6-8]. Semiochemicals help natural enemies to locate and recognize their hosts or prey [9, 10] and elicit behavioural and physiological responses in the receiver, which results in the interaction between them [11].

Behavioural chemicals that provide cues for orientation of predators in the prey finding sequence includes secondary plant metabolites and chemicals directly associated with the host prey [12]. Antennectomy studies by various authors on the predatory behaviour of reduviids revealed that, these predators use the prey chemical cues to locate their prey[13][14], [15]. Thus, it seemed likely that the reduviids utilize chemicals released by their prey as kairomone in prey location. Only few studies have been undertaken on the allelochemical relationship between reduviids and their prey location behaviour. Mere augmenting and releasing the biocontrol agents into the agroecosystems will not be successful unless the behavioural dynamics of reduviid predators to chemical cues of the prey is known. Hence, the behavioural responses of *R. longifrons* to the solvent extracts of tobacco leaf armyworm *Spodoptera litura* (Fabricius), *H. armigera*, castor semilooper *Achea janata* (Linnaeus), red cotton bug *Dysdercus cingulatus* (Fabricius) and blister beetle *Mylabris indica* Thunberg were investigated since these are major insect pest of cotton, in order to understand the prey preference and prey-location behaviour of the predator using kairomonal cues, if any, that elicit from prey belonging to diverse orders.

2. MATERIALS AND METHODS

2.1. Predator Collection and Maintenance

The adults of *R. longifrons* were collected from Muppandal Scrub Jungle (77°31' E and 8°22' N), Tamil Nadu, South India and were reared in the laboratory under optimal conditions (temperature 30 ± 1° C; RH 75 ± 5 %; Photoperiod 12 ± 1 hr.) in plastic containers on the larvae of *Corcyra cephalonica* Stainton *ad libitum.*

2.2. Pest Collection and Maintenance

Lepidopteran insect pests viz., *S. litura*, *H. armigera* and *A. janata* and a hemipteran pest *D. cingulatus* and a coleopteran pest *M. indica* were used in this study as these are economically important and collected from cotton, lady'sfinger and castor agroecosystems in and around Palayamkottai (77°73'E and 8°72'N), Alankulam (77°54'E and 8°93'N) and Pavoorchatram (77°55'E and 8°10'N) agroecosystems. The *H. armigera* larvae were maintained on fresh lady'sfinger fruits, cotton bolls and flowers in plastic containers (7 x 7 cm) separate. The *S. litura* and *A. janata* larvae were maintained on fresh cotton and castor leaves in the plastic troughs (15 x

30 cm). The adults of *M. indica* were maintained on fresh lady'sfinger and cotton flowers in a mesh cage (5 x 2x 3 ft). The adults of *D. cingulatus* were maintained on wet cotton seeds in a mesh cage (5 x 2x 3 ft).

2.3. Solvent Extracts of Prey Species

The whole body extracts of different prey species were prepared following the methodology of Yasuda [16]. Hundredlive 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 minutes at room temperatures, separately and subsequently stored in a freezer 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. Thereafter, the ether-soluble layer was dried over sodium sulfate. Then the solvent was removed by using vacuum desiccator at room temperature and the residue was dissolved in hexane. The resultant extracts were stored at below -20^{o} C until further use.

2.4. Behavioral Bioassay

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^{o} between them.) was used for the bioassay studies i.e., to test the predator response to individual extracts. 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 minutes 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 24hr starved predators were introduced through the main stem and their predatory behaviour was observed for 30 mins continuously. The predatory behaviour was observed in terms of approaching and sucking time. From these observations, the handling time was calculated by summing up both [17]. 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 hour starved and inexperienced predators on each body extract of insect pests, separately with new impregnated cotton for each replicate.

The approaching time taken by the predators in the olfactometers to different body extracts was converted into an index called Excess Proportion Index (EPI) [18] using the following formula:

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

Where,

NS- Number of predators choosing the sample cell.

NC- Number of predators choosing the control cells

EPI values are ranging 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 consist of counting antennation and probing frequencies towards each test sample, at given period of time.

2.5. Six-Arm Olfactometer

The six arm (each arm 20cm long) olfactometerwas used to compare predator attraction to all five extracts, made up of glass had six arms, each arm terminating with an odour cell. The arms met at a central cell. At a time five odour cells can be used as test cells, each with one prey extract (20µl) impregnated in sterile cotton and the sixth cell as control cell with hexane alone (20µl) impregnated in sterile cotton. Before the introduction of predators, air was blown with aerator into the central cell and from there to the arms and odour cells to remove the odour, if any in the olfactometer.

Chemically mediated host preference of *R. longifrons* to different body extracts of insect pest were assessed in terms of percentage preference of host insects (pests) extracts. The 24 hrs starved and inexperienced adult *R. longifrons* was introduced into the central cell of the six-arm olfactometers.

When the predator was released into the central chamber, the predator exhibited behavioural responses, due to chemical cues elicited from the cotton impregnated with body extracts of pests viz., *S. litura*, *H. armigera*, *A. janata*, *D. cingulatus*and *M. indica*. Due to the chemical cues from the samples the predator moved towards the respective odour cell. The procedure was repeated 15 times against the body extract of insect pests.

The data were subjected to analyze the by one-way analysis of variance (ANOVA) and means were compared by Tukey test using the software OriginPro 7.5 version (Origin Laboratory, USA).

3. RESULTS

3.1. Y-Shaped Olfactometer

The bioassay experiments performed in the Y-shaped olfactometer and time spent by the predators*R. longifrons* to the hexane extracts of insect pests suggested behavioural responses of predators. When the predator *R. longifrons*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 it palpated the antennae, followed by rubbing legs, rostral cleaning, and extending towards the odour source. Once the predator entered the sample cell it quickly approached the odour cell with extended rostrum.

The positive approaching responses were exhibited by *R. longifrons* to the hexane fractions of all the insect pests (Table 1). The highest response of 6.67±1.18 min. was observed to *S. litura* which is followed by 5.17±0.89, 4.42±1.04, 3.00±0.82 and 2.42±0.76 min. for *H. armigera*, *A. janata*, *M. india* and *D. cingulatus* respectively.

The EPI values of *R. longifrons* showed positive response to the *S. litura*(0.67), *H. armigera*(0.33) and *A. janata*(0.17) larval extracts and negative responses to *M. indica* (-0.33) and *D. cingulatus* (-0.17) (Table 1).

The handling time of *R. longifrons* (in terms of duration of sucking time of insect pests body extracts) to the body extracts of insect pests is shown in Table 1. The predator *R. longifrons* exhibited the highest handling time to *S. litura* extract (6.33±1.25 min.) followed by *H. armigera* (5.58±1.11min.), *A. janata* (2.92±0.76 min.), *M. indica* (2.50±0.65 min) and *D. cingulatus* (1.75±0.72 min.) (Table 1).

3.2. Six-Arm Olfactometer

Rhynocoris longifrons highly preferred the body extract of *S. litura* (41.67 %) followed by *H. armigera* (25.00 %) and *A. janata* (16.67 %) and the least preference were observed to the body extracts of *M. indica* (8.33 %) and *D. cingulatus* (8.33 %) (Table 2).

4. DISCUSSION

In the present investigation, *R. longifrons* responded to the hexane extracts of *S. litura*, *H. armigera*, *A. janata*, *M. indica* and *D. cingulatus*, which was inferred by the approaching response of *R. longifrons* to the sample cells than the control cell. Moreover the predator extended its rostrum towards the sample loaded cotton and rubbed it, suggesting that volatile chemicals attracted the predator and stimulated it to extend its rostrum. At first the inactive predators oriented toward the odour source with antennae [19, 20].

Harpactorine reduviids oriented towards the prey with extended and palpating antennae at arousal and subsequently approach the prey as observed in this harpactorine reduviid [21]. The hydrocarbons in the hexane fractions of *S. litura* caterpillars, which contained n-tetradecane (C_{14}), n-pentadecane (C_{15}), n-heptadecane (C_{17}), n-heptacosane (C_{27}), squalene, n-nonacosane (C_{29}), n-hentriacotane (C_{31}) 2, 6, 10, 15, 19, 23 - hexamethyl - 2, 6, 10, 14, 18, 22 - tetracosahexane (squalene) and Ephytol [16]. Among these hydrocarbons n-pentadecane (C_{15}) attracted the predator *Eocanthecona furcellata* (Wolff) and E-phytol induced proboscis extension. The chemical perception in these predators depends on the chemosensory system conveying the requisite quantity of information about prey [20].

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 [22, 23].

Kairomones involved in the foraging behaviour of organisms have been published and reviewed during the past decades [24]. The kairomones in moth scales probably act as sign stimuli, eliciting intensified searching behaviour, rather than as a guiding cue attracting and directing the parasitoid to the host [25].

In the present observation though the predator *R. longifrons* responded to all the insect pest extracts, it highly responded to the lepidopteran larval extracts viz., *S. litura*, *H. armigera*, and *A. janata* and the least preference was observed for the coleopteran and hemipteran insect pests *M. indica* and *D. cingulatus*.

The reduviid predators generally prefer lepidopteran larvae [1]. McMahan [26] 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.

The present study shows that the body extracts of *H. armigera*, *S. litura*, *A. janata*, *M. indica*, and *D. cingulatus* containing kairomonal compounds that attracted *R. longifrons* revealed that the predator preferred lepidopteran prey than the hemipteran and coleopteran prey and exhibited an order of preference among lepidopteran prey.

However, the manner in which kairomones will be used for insect control is even less clear than for pheromones. Moreover, if these chemicals used for manipulating the action of natural or released biological control agents, will no doubt involve the direct application of the chemicals to crops where the natural agents and the insect hosts occur. They might also be applied to parasites or predators to stimulate host seeing when released in augmentation systems [27]. Further investigations are required to assess the nature of kairomones and their effects on the predatory behaviour of *R. longifrons*.

5. ACKNOWLEDGEMENTS

The authors are grateful to the authorities of St. Xavier's College (Autonomous) for providing the facilities and encouragements.

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