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Full Length Article

Life traits and predatory potential of *Antilochus coqueberti* (Fab.) (Heteroptera: Pyrrhocoridae) against *Dysdercus koenigii* Fab.

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ABSTRACT

The red cotton bug, *Dysdercus koenigii* Fab., and its specialized predator *Antilochus coquebertii* (Fab.), are among the most abundant insects in Asian cotton agro-ecosystems. To gauge the potential of using *A. coquebertii* to control *D. koenigii* in cotton, we tested the role of feeding on cotton leaves in development of the predator, prey stage preference and characterized the functional response of the predator to prey density under laboratory conditions. *Antilochus coquebertii* exhibited an active hunting strategy indicative of using both olfactory and visual orientation. Immature stages of the predator successfully developed and reproduced when offered *D. koenigii* or cotton leaves with *D. koenigii*. However, *A. coquebertii* nymphs failed to develop past the third instar when feeding on cotton leaves alone. The food regime did not significantly affect body size of the predator. Mated male and female adults live long when fed with *D. koenigii*. Total number of prey consumed by an adult predator during 15-days observation reveals not much deviation when offered *D. koenigii* or cotton leaves with *D. koenigii* thill d a maximum of six *D. koenigii* adults per day, and were preying on *D. koenigii* populations in a density dependent manner (showed a type II functional response). We argue that *A. coquebertii* has considerable potential for the biological control of the red cotton bug *D. koenigii*.

Introduction

Heteropteran predators can be important natural enemies of phytophagous insects and have thus potential in biological control of crop pests. However, like many arthropod predators, predatory bugs are often omnivores consuming also non-prey foods such as phloem sap, pollen, nectar, honeydew and fungi (Sanchez and Lacasa, 2008; Lundgren, 2009) and some omnivores may live on a purely phytophagous diet albeit at lower population densities (Garay et al., 2012). Their plant feeding behavior may in part explain why omnivores are often neglected in biological control (Sanchez and Lacasa, 2008). Therefore, the role of plant tissue in the nutrition of predators that may be omnivorous has to be addressed when assessing their potential in biological control of pests.

The red cotton bug, *Dysdercus koenigii* Fab., and its natural predator *Antilochus coquebertii* (Fab.) (Heteroptera: Pyrrhocoridae), are among the most abundant insects in Asian cotton agro-ecosystems, suggesting the importance of *A. coquebertii* in controlling *D. koenigii* (Schaefer and Ahmad, 2000). *A. coquebertii* first instar nymphs develop without any food (Kohno et al., 2002), and other life stages are reported as predators of cotton stainers, *Dysdercus* spp. (Pyrrhocoridae) (Chauthani and Misra, 1966; Iwata, 1975, 1978a, 1978b; Quayum and Nahar, 1980;

Dhiman, 1985; Kohno et al., 2002; Kohno, 2003; Muthupandi et al., 2014). The feeding of *A. coquebertii* was studied in detail in the field and under laboratory condition (25 °C) by Kohno et al. (2004). Both nymphs and adults are obligate predators of *Dysdercus cingulatus* (Fab.) (Kohno, 2003; Kohno et al., 2002, 2004) and *Dysdercus decussatus* Boisduval (Kohno et al., 2002). In India, the species was observed to feed on *Dysdercus koengii* (Kamble, 1974). However, *A. cogueberti* has also been reported as an herbivore, attacking malvaceous plants like *Hibiscus titiaceus* (L.) and cotton (Kawasawa and Kawamura, 1977; Singh and Tomar, 1977).

Pest suppression by a predator species depends strongly on two major components of predator-prey interactions: the predator's numerical and functional response. To understand the predator-prey dynamics, it is important to have insight into density dependent interactions. In predatory bugs, predation rate tend to reach and asymptote when more prey was offered per day and this can be understood as a Type II Functional response. Such functional response was recorded in predatory reduviid bugs in the genus *Rhynocoris* such as *R. fuscipes* (Fab.) (Claver and Ambrose, 2002), *R. longifrons* Stål (Sahayaraj et al., 2012) and *R. kumarii* (Sahayaraj and Asha, 2010; Sahayaraj et al., 2015) and also in *A. coquebertii* (Kohno et al., 2004). Functional responses of predatory hemipteran bugs can be influenced by the prey's

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developmental stage (dos Santos et al., 2016), prey species (Sharifian et al., 2016), prey density (Queiroz et al., 2015), and type of plants (Chao et al., 2015). However, influence of host plant on the functional response of *A. coquebertii* has not been studied so far.

Therefore we aimed to clarify to what extent *A. coquebertii* is predacious, and to quantify its efficiency against the natural prey *D. koenigii*. Hence, it was hypothesis whether addition of cotton leaf along with red cotton bug altered the life traits and bioefficacy of *A. cogueberti* or not. First we made detailed observations of *A. cogueberti* preference on *D. koenigii*. Second, we measured life history parameters of *A. coquebertii* on diet of exclusively cotton leaves or *D. koenigii* or a combination. Lastly, we estimated the functional responses of *A. coquebertii* in the presence versus absence of cotton leaves by offering *D. koenigii* prey in different densities and observing prey consumption, search time and handling time.

Materials and methods

Insect collection and maintenance

A mixture of the different life stages of A. coquebertii was collected from cotton fields in the Tirunelveli (8°05' and 9°30' N latitude and 77°05' and 78°25' E longitude) and Thoothukudi (8.53°N 78.36°E) districts in the state of Tamil Nadu, India and maintained individually in plastic vials (5.5 \times 4.4 cm with perforated lids) under laboratory conditions at 30.3 \pm 0.2 °C, 71.6 \pm 0.4 RH and 11 L: 13D h on a mixture of nymphs and adults of D. koenigii. The prey was replenished every day with those of the same age and size mixture. After emergence, 50 adult *A. coquebertii* (mean weight = 106.2 ± 5.0 mg) were kept for one month in pairs using similar plastic vials. The adults were kept in the same conditions as above on red cotton bugs, with the prey being replenished every second day. The prey, D. koenigii adults and nymphs were collected from the same cotton fields from which predators were collected. The collected insects were maintained in the above mentioned laboratory conditions. D. koenigii were group-reared in transparent plastic containers (20 cm \times 10 cm \times 15 cm) and provided with water soaked cotton seeds.

Diet experiment

Food requirements of the predator were tested under three conditions; 1) Predators were offered cotton leaves alone, 2) red cotton bugs alone, 3) or cotton leaves and red cotton bugs. A total of 246 A. coquebertii first-instar nymphs were randomly assigned to one of the three food categories with a total of 60 nymphs held on cotton leaves, 126 with D. koenigii prey alone and 60 nymphs on cotton leaves and prey. Three predators in a container (8 cm \times 7 cm) were maintained under laboratory conditions as mentioned above. A ball of moist cotton was placed on each container to keep the cotton leaf hydrated and as a water source for the predator. The cotton ball was moistened daily. Attempting to standardize prey size, only second and third instar red cotton nymphs were provided daily at an equal ratio to second and third instar A. coquebertii, whereas other nymphal instars and adults were provided with both fifth instar and adult red cotton bugs daily in equal proportions. Cotton leaves were provided as a food source for the nymphs in each container. For the 'D. koenigii alone' treatment, a moist cotton ball was suspended in the center as a water source for A. coquebertii. The tops of the containers were covered with a fine mesh net for ventilation.

Nymphal development

Each *A. coquebertii* nymph was monitored daily for development, survival and predation rate (number of prey killed/predator/day) until they became adults. Developmental time in each instar or stage was then calculated as the time between two molts. Emerged adults were

sexed and their sex ratio was calculated as female sex ratio = Number of females Emerged/Total number of adults emerged. Cannibalism was observed in first, second and third instars were also recorded in per cent.

Adult longevity and reproduction

When adults emerged, allow them to copulate naturally, paired partners were carefully transferred in to a new container and provided with 8-preys (four each of fifth instar nymph and adult red cotton bug) on alternative days. The adults were kept on the same diet regime which they had been given during nymphal development. The longevity, fecundity, and predation rate (number of prey killed/predator/ day) were recorded daily. From each pair, all eggs were collected daily and isolated in Petri dishes until females died. The eggs were held for 6-10-day under the same laboratory conditions as the nymphs and adults and the number hatching was recorded. In another experiment, 10-15 newly emerged adult males and females were separately maintained till their death by providing adult red cotton bugs (without discriminating sex).

Body size

After molting of each nymphal instar (2nd to 5th), as well as the adult stage, weight of each individual was recorded using an electronic monopan balance [Dhona Instruments (P) Ltd., Kolkata] with 0.1 mg accuracy. A total of 10-15 individual insects were used for this purpose. Dead nymphal stages (fourth and fifth nymphal instar) and adults were preserved in 70% ethanol for morphometry analysis using stage and ocular micrometers (AMBA Electro Mec Devices, Haryana, India; Model: Optic AE/116699). Length of the head, antenna, thorax, tibia (fore tibia, mid-tibia, hind tibia), wings, abdomen, and also the width of head, thorax, wings (fore wing and hind wing) and abdomen were recorded.

Functional response

Before conducting the biocontrol potential evolution tests, the prey stage preferences of *A. coquebertii* was recorded with a prey-choice test. Two each of second, third, fourth and fifth instar nymphs, and adults were released into a transparent plastic box (8 cm depth \times 7 cm width). Subsequently, one 24 h starved second instar predator was introduced into the experimental arena, and we closed the box with a perforated lid. The first captured prey stage was recorded visually and taken as the preference and the same prey was offered to various life stages of the predator to evaluate the functional response.

We used the same set-up as in Kohno et al. (2004) who recorded the bioefficacy of A. coquebertii nymphal stages for one day without cotton leaves in the experimental arena at 25 $^\circ \text{C}.$ Thus, each experimental arena consisted of a plastic container (8 cm \times 7 cm) lined with a moist cotton layer, with a ventilation hole in the polystyrene lid. Cotton plants (variety SVP2) selected for the leaf disc-assay were healthy, young, vigorous and free of arthropod pests. Leaf discs (7 cm diameter) were cut with scissors from detached cotton leaves at the center of the blade beginning near the intersection between the petiole and the edge of the leaf. Leaf discs were placed with the abaxial surface on the cotton layer in the arenas. Different allotted numbers of third instar red cotton bugs, 1, 2, 3, 4, 5, 6, 8, or 10 were placed together for each density in separate Petri dishes, and left undisturbed to settle for 1-h. Once red cotton bugs had settled on the cotton leaf-disc, a single male or female adult A. coquebertii was introduced into each dish. Feeding behavior was continuously recorded for three hours. The number of prey killed by different predatory life stages of A. coquebertii was recorded every 24-h for 15-days. To maintain the same number of prey in each density category in the arena, similar aged prey replaced dead prey after every 24 h observation period. Predatory life stages with the allotted number

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