Prey consumption by the mealybug predator *Spalgis epius* on pink hibiscus mealybug (*Maconellicoccus hirsutus*)

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Abstract The pink hibiscus mealybug *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae) is a major pest of economically important crops. The apefly *Spalgis epius* (Westwood) (Lepidoptera: Lycaenidae) is a potential predator of various species of mealybugs. Studies of its preying potential and preference for prey stages on *M. hirsutus* are incomplete. An investigation was undertaken to determine the daily prey consumption and preference for prey stages by different larval instars of *S. epius* reared on *M. hirsutus* in the laboratory. During the 8-day larval development period with four larval instars of *S. epius*, the daily prey consumption increased from the first to the seventh day and decreased on the eighth day prior to the prepupal stage. Generally, there was a significant difference in the prey consumption on different days. The 1st to 4th instar larvae of *S. epius* consumed, respectively, a mean of 181.3, 679.1, 1770.4 and 4333.0 eggs or 19.1, 67.7, 153.0 and 639.0 nymphs or 2.72, 6.26, 13.8 and 32.1 adults of *M. hirsutus*. When an *S. epius* larva was fed on *M. hirsutus* eggs, nymphs and adults separately, it consumed a mean of 6952.6 eggs, 878.8 nymphs or 53.9 adults during its entire development. A single larva of *S. epius* consumed 2358.3 eggs, 151.2 nymphs and 34.3 adults of *M. hirsutus* during its

entire development when the prey stages were offered all together. The study revealed that *S. epius* is a voracious predator of *M. hirsutus* and thus could be utilized as a potential biological control agent.

Keywords Apefly · Biocontrol agent · Lycaenidae · Prey stages

Introduction

Different species of mealybugs are serious pests of economically important crops worldwide (Browning 1992; Franco et al. 2001). Of them, *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae) is a major pest of numerous economically important crops such as grapes, citrus, avocado, carambola, fig, guava, mango, sugarcane and mulberry; vegetable crops including asparagus, beans, beets, cabbage, peanuts, pigeon pea, cucumber, lettuce, pepper, pumpkin, and tomato; forest trees, many species of ornamental plants; and various fruits and shade trees in both tropical and subtropical countries (Hoy et al. 2002; Kairo et al. 2000; Reddy et al. 2009; Roltsch et al. 2006). *M. hirsutus* adults lay 300–600 eggs and complete the life cycle in 23–30 days with three (female) and four (male) nymphal instars (Hoy et al. 2002). *M. hirsutus* is the only species in the genus that has a worldwide distribution (Meyerdirk et al. 2002). Annual economic losses due to this pest have been estimated at approximately \$2.5 million in

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Grenada, \$5.1 million in Trinidad and \$750 million in the United States, and to cause severe economic losses in Egypt and India (Kairo et al. 2000). Reddy et al. (2009) studied interactions of *M. hirsutus* and its natural enemies in Guam. Roltsch et al. (2006) reported that two species of parasitoids reduced 98% of *M. hirsutus* density in southern California.

The apefly *Spalgis epius* (Lepidoptera: Lycaenidae: Miletinae) has been recorded as a predator of various species of mealybugs, viz., *Dactylopius* sp., *Planococcus virgatus* (Ckll.), *P. lilacinus* (Ckll.), *P. citri* (Risso), *Ferrisia virgata* (Ckll.), and *Paracoccus marginatus* Williams and Granara de Willink (Homoptera: Pseudococcidae), and *M. hirsutus* (Dinesh et al. 2010; Thangamalar et al. 2010); and a scale insect *Chloropulvinaria polygonata* (Ckll.) (Homoptera: Coccidae) (Ali 1978). Of the several insect predators of mealybugs in India, the larva of *S. epius* has been reported as a potential predator of *P. citri* and *P. lilacinus* in coffee plantations (Chacko et al. 1977; Rahiman and Vijayalakshmi 1998), and *M. hirsutus* in mulberry gardens (Gowda et al. 1996). Long ago, India imported an important predator *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) for the control of different species of mealybugs (Rao et al. 1971).

Spalgis epius occurs in India, Burma and Sri Lanka (Bingham 1907), Philippines and Java (Le Pelley 1943), Bangladesh (Ali 1978), and Thailand (Lohman and Samarita 2009). The morphology, development, life history and behavior of *S. epius* has been studied (De Niceville 1890; Vinod Kumar et al. 2006; Dinesh et al. 2010; Thangamalar et al. 2010). Observations on the behavior of *S. epius* and its interaction with ants have been made in the field (Venkatesha et al. 2004; Venkatesha 2005; Vinod Kumar et al. 2008a). Vinod Kumar et al. (2008b) and Thangamalar et al. (2010) observed the predatory potential of *S. epius* on *P. citri* and *P. marginatus*, respectively, by offering all the prey stages together. Furthermore, brief field observations have been made of the feeding activities of *S. epius* on *P. citri* and *P. lilacinus* in coffee *Coffea canephora* Pierre ex Froehner (Rahiman and Vijayalakshmi 1998), of *M. hirsutus* in mulberry *Morus alba* L. (Pushpaveni et al. 1973), and of *P. lilacinus* in the ber crop *Zizyphus mauritiana* L. (Mani and Krishnamoorthy 1996) in India.

Although *S. epius* is a potential predator of *M. hirsutus*, no detailed study has been made of the daily

prey consumption by developing *S. epius* larvae on different prey stages of the pest offered together and separately. Hence, the present study was undertaken to assess its daily and instar-wise prey consumption, and preference for different prey stages such as eggs, nymphs and adults of *M. hirsutus* in the laboratory. This information is essential to utilize *S. epius* as a major biological control agent of *M. hirsutus* in the field.

Materials and methods

Lab rearing of prey *Maconellicoccus hirsutus* was reared in the laboratory following the method used for rearing *Planococcus citri* (Anon. 1995). The medium sized (≈ 70 cm diam and 20 cm height) ripe pumpkins (*Cucurbita maxima* Duchesne) with ridges and grooves, with a small stalk for handling, were selected for rearing *M. hirsutus*. The pumpkins were thoroughly washed with tap water and then dipped in 0.1% fungicide (Bovistin) to kill the fungal pathogens. The eggs and nymphs of *M. hirsutus* were collected with a camel hair brush from hibiscus plants, *Hibiscus rosa sinensis* L. (Malvaceae), in the field of Bangalore University campus, Bengaluru, India (latitude 12°58'N, longitude 77°35'E, elevation 921 masl) and transferred onto cleaned pumpkins in the laboratory. The *M. hirsutus*-infested pumpkins were maintained individually in a nylon rearing cage (30×30×30 cm) and reared. For regular availability of prey, fresh pumpkins were infested with *M. hirsutus* whenever needed.

Lab rearing of predator *Spalgis epius* larvae from the laboratory culture were reared on the pumpkins infested with *M. hirsutus* and completed their development on those pumpkins. Emerged adults of *S. epius* were allowed to mate and the gravid females of *S. epius* were provided with fresh mealybug-infested pumpkins for egg deposition. The laboratory cultured population of *S. epius* was used for the experiment.

Prey consumption The experiments on prey consumption by *S. epius* were conducted in the laboratory at 28.0±1.0°C, 65±5% r.h., and photoperiod 12L:12D in an environmental chamber. The prey consumption of *S. epius* was assessed by

offering different *M. hirsutus* stages (i.e., eggs, 2nd instar nymphs and adults) separately and together.

In order to determine the prey consumption potential of *S. epius* larva, the eggs of *S. epius* laid in the mass of mealybugs on the pumpkin were collected carefully with a fine camel hair brush and kept individually in petri dishes (5 cm diam). Newly emerged larvae from these eggs were maintained in the same petri dishes and provided with a daily known number of egg masses, nymphs and adults of *M. hirsutus* separately as food until they reached the prepupal stage. The prey eggs, nymphs and adults were counted under a stereo zoom microscope. Based on the preliminary laboratory observations, the prey stages were offered to *S. epius* larvae daily in quantities pre-determined according to the predator age to ensure that the available prey-food exceeded the amount that the predator could consume in a day. The number of eggs, nymphs and adults of the prey consumed by *S. epius* larva was recorded at 24-h intervals by counting the remaining prey stages in the petri dish. The larval excreta and left-over prey stages were removed daily and the predator was provided with a fresh batch of the prey stages. In a separate experiment, the larval instar-wise prey consumption was assessed by examining the petri dishes daily for exuviae and head capsules of *S. epius* larvae and the amount of prey consumed during each larval instar was recorded.

Preference for prey stages The preference for the prey stages and amount of each prey stage consumed when the stages were offered together was determined by providing eggs, 2nd instar nymphs and adults of *M. hirsutus* simultaneously to the freshly hatched *S. epius* larvae in a petri dish. Daily and instar-wise prey consumption by *S. epius* larvae fed on different prey stages was recorded. For each of the above experiments 30 *S. epius* larvae were maintained individually on separate petri dishes.

Data analysis To test the difference in the daily and instar-wise prey consumption by the predator during its larval development, one-way ANOVA was conducted and significant differences were determined utilizing the Tukey HSD test at $P \leq 5\%$ (SPSS Inc. 2001). For each experiment, there were six replications with five larvae per replication.

Results

Prey consumption *Spalgis epius* larvae completed their development in 8 days with four larval instars in the laboratory. Newly hatched 1st instar larvae of *S. epius* consumed a mean ($\pm$ SE) of 85.5 ± 1.4 eggs of *M. hirsutus* on the first day and its egg consumption increased thereafter as the development progressed. The larvae consumed the highest number of prey eggs (2416.5 ± 24.1) on the seventh day, which decreased to 1916.5 ± 53.5 eggs on the eighth day (Fig. 1). There was a significant difference in mean number of eggs consumed on different days ($F=1734.7$; $df=7, 40$; $P<0.0001$) except between the first and second days ($P>0.05$).

Fig. 1 Mean daily prey consumption by *Spalgis epius* larvae feeding on *Maconellicoccus hirsutus* stages offered separately. Bars with different letters indicate a significant difference at $P < 0.05$ (one way ANOVA—Tukey HSD test) between the days within the same prey stage (vertical line indicates the SE mean prey consumption)

Fig. 2 Mean larval instar-wise prey consumption by *Spalgis epius* feeding on *Maconellicoccus hirsutus* stages offered separately. Bars with different letters indicate a significant difference at $P < 0.05$ (one way ANOVA—Tukey HSD test) between different larval instars within the same prey stage (vertical line indicates the SE of mean prey consumption)

When the 2nd instar nymphs of *M. hirsutus* were provided as a prey, mean prey consumption by a newly hatched *S. epius* larva on the first day was 8.8 ± 0.2 nymphs and consumption increased thereafter. The predatory larvae consumed a maximum number of nymphs (333.0 ± 4.4) on the seventh day, which decreased to 298.0 ± 3.2 nymphs on the eighth day. There was a significant difference in mean number of nymphs consumed on different days ($F = 4518.1$; $df = 7, 40$; $P < 0.0001$) except between the first and second days ($P > 0.05$) (Fig. 1).

When *M. hirsutus* adults were offered as a prey, freshly hatched *S. epius* larvae consumed a mean of 1.2 ± 0.08 adults on the first day and consumption increased thereafter to the highest mean of 18.4 ± 0.5 adults on the seventh day and decreased to 13.6 ± 0.1

adults on the eighth day. There was a significant difference in mean number of *M. hirsutus* adults consumed on different days ($F = 493.0$; $df = 7, 40$; $P < 0.0001$) except between the first and second, and the third and fourth days ($P > 0.05$) (Fig. 1). Overall, a single *S. epius* larva consumed a mean of 6952.6 ± 64.7 eggs, 878.8 ± 6.6 nymphs or 53.9 ± 0.7 adults of *M. hirsutus* during its entire larval development period.

With respect to larval instar-wise prey consumption, the prey consumption increased from the first to fourth larval instars of *S. epius* (Fig. 2). The mean number of prey eggs ($F = 4154.9$; $df = 3, 20$; $P < 0.0001$), nymphs ($F = 14259.9$; $df = 3, 20$; $P < 0.0001$) and adults ($F = 710.1$; $df = 3, 20$; $P < 0.0001$) consumed was significantly different among the four larval instars of *S. epius*.

Preference for prey stages When the prey stages were offered together, a mean minimum of prey consumed (79.3 ± 0.07 eggs, 0.1 ± 0.07 nymphs and 0.1 ± 0.06 adults) on the first day, with a mean maximum consumption (514.0 ± 32.7 eggs, 38.9 ± 1.3 nymphs and 11.0 ± 0.5 adults) on the seventh day. Prey consumption on the eighth day decreased to 491.1 ± 31.7 eggs, 36.67 ± 1.6 nymphs and 10.3 ± 0.5 adults and did not differ significantly from that on the seventh day ($F = 105.6$; $df = 7, 40$; $P = 0.966$) (Fig. 3). The first two days, *S. epius* larvae generally preferred eggs to nymphs and adults of *M. hirsutus*, whereas 3- to 4-day-old larvae fed on eggs and a few nymphs as well. From the fifth day onwards, in addition to the eggs and nymphs, the larvae consumed adults of the prey. Mean prey consumption during the entire larval

Fig. 3 Mean daily prey consumption by *Spalgis epius* larvae feeding on *Maconellicoccus hirsutus* stages offered together

Fig. 4 Mean larval instar-wise prey consumption by *Spalgis epius* feeding on *Maconellicoccus hirsutus* stages offered together

development period of *S. epius* was 2358.3 ± 36.7 eggs, 151.2 ± 6.8 nymphs and 34.3 ± 1.7 adults when the prey stages were offered all together.

The prey consumption increased significantly from the 1st to 4th instar larvae of *S. epius* ($F=293.2$; $df=7, 40$; $P<0.0001$) (Fig. 4). The 1st and 2nd instar larvae usually preferred eggs to nymphs and adults of *M. hirsutus*, whereas 3rd and 4th larval instars fed on all stages of the prey. The 1st and 2nd instar larvae of *S. epius* were almost sedentary, whereas the 3rd and 4th instar larvae crawl while feeding.

Discussion

Spalgis epius larvae were able to prey successfully on the large number of *M. hirsutus* eggs, nymphs and adults. A mean daily consumption of prey stages by the developing larvae of *S. epius* increased significantly on the seventh day but decreased significantly on the eighth day as the larva slows down its feeding and prepares for the prepupal stage. In general, there was a significant difference in prey consumption by *S. epius* larvae on different days. Preliminary observations made in the field on *S. epius* revealed that a fully grown larva consumes 300 nymphs of *M. hirsutus* per day (Pushpaveni et al. 1973); a mean of 132 eggs, 260 nymphs and 75 adults of *M. hirsutus* during the final stage of development (Gowda et al. 1996); and 22.3 ovisacs and 121.6 nymphs and adults of *P. marginatus* (Thangamalar et al. 2010). In the present study, when the different prey stages were offered either separately or together, the 4th instar larvae of *S. epius* consumed more eggs than nymphs and adults of *M. hirsutus*. This could be due to the size of the eggs,

which are very small compared with that of the nymphs and adults, as observed in the case of predation by *C. montrouzieri* on *M. hirsutus* (Mani and Thontadarya 1987). The daily and larval instar-wise prey consumption of *S. epius* increased with their progressing development, as reported with *C. montrouzieri* preying on *M. hirsutus* (Mani and Thontadarya 1987).

Vinod Kumar et al. (2008b) and Thangamalar et al. (2010) reported that a single *S. epius* larva consumed 416.4 eggs, 449.2 nymphs and 158.3 adults of *P. citri*; and 48.1 ovisacs and 210.9 nymphs and adults of *P. marginatus* when all the prey stages were offered together. *S. epius* larva consumed more eggs and fewer nymphs and adults of *M. hirsutus* when compared with that of *P. citri* and *P. marginatus*. On the whole, an *S. epius* larva consumed about 2543 *M. hirsutus* stages, which is more than that of *P. citri* and *P. marginatus*. Hence, it could be used as a major predator of *M. hirsutus*. In the case of *C. montrouzieri*, a single grub during its entire development consumes 881.3 eggs or 295 nymphs or 27.5 adults of *M. hirsutus* (Mani and Thontadarya 1987) or preys exclusively on 1325 or 3330 *P. citri* eggs in the laboratory (Oncuer and Bayhan 1982; Whitecomb 1940). Again, the overall consumption of prey stages by *S. epius* is more than that of *C. montrouzieri*. Furthermore, *S. epius* larvae fed only on eggs, and nymphs or adults of *M. hirsutus* were able to complete their normal development successfully, whereas *C. montrouzieri* fed only on the 1st instar nymphs of *P. citri*, which was unable to complete its normal development (Oncuer and Bayhan 1982). The normal development of *S. epius* on any stages of *M. hirsutus* indicated that it is able to survive successfully and bring down the prey population effectively.

The completely sedentary early stage larvae of *S. epius* (i.e., the 1st and 2nd instar larvae) in the feeding chamber constructed within the ovisac of the prey, preferred eggs to nymphs and adults of *M. hirsutus* as reported by Dinesh et al. (2010). This could be due to the constant movement of young *M. hirsutus* nymphs, which normally escape from predation; and the almost sedentary older nymphs and adults of *M. hirsutus* are usually not available around the feeding chamber. While feeding on the eggs, the young larvae sometimes nibbled the nymphs and adults of *M. hirsutus* and such injured prey were killed. However, if the prey eggs were not available for feeding, the

young larvae preyed on the nymphs and adults of the prey and could complete their development. From the fifth day onwards (*i.e.*, the 3rd and 4th instar larvae), the well developed and large sized predatory larva (6.6–11.4 mm) consumed all stages of *M. hirsutus*.

Our observations on the feeding potential of *S. epius* indicated that the predator fed voraciously on all stages of the prey. It was reported that at the rate of 3.7 *S. epius* larvae per shoot, *S. epius* was able to bring down a *P. lilacinus* population from 45.50 to 0.4 bugs per shoot in the ber crop within a month (Mani and Krishnamoorthy 1996). It was also reported that 13–20 *S. epius* larvae reduced 54.78–68.38% of different species of mealybug (*P. citri*, *P. lilacinus* and *F. virgata*) populations in coffee in 10 days (Rahiman and Vijayalakshmi 1998).

Successful biological control of any pest species depends on at what rate and extent a natural enemy is able to suppress the pest population. Our study showed that a single *S. epius* larva during its 8-day development preyed successfully upon a large number of *M. hirsutus* stages. In addition, *S. epius* larva feeding on a large number of eggs of *M. hirsutus* not only bring down the pest population but also reduce drastically the subsequent damage caused by developing nymphs and adults of the pest. Furthermore, lycaenids feeding on Hemiptera live among their prey without provoking an escape response or an attack response from the ant mutualists of Hemiptera (Pierce et al. 2002). Similarly, *S. epius* larvae successfully feed on mealybugs although some species of mealybug-tending ants are hostile to the predatory larvae (Venkatesha 2005; Vinod Kumar et al. 2008a). Mealybug-mutualist ants hinder the activities of *C. montrouzieri* in the field (Le Pelley 1968). In this study it was determined that *S. epius* can consume a large number of *M. hirsutus* and thus could be utilized as a major biological control agent of *M. hirsutus* in India and worldwide.

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