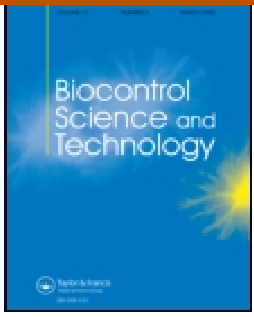

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RESEARCH PAPER

Predation of the apefly, *Spalgis epius* (Lepidoptera: Lycaenidae) on citrus mealybug, *Planococcus citri* (Hemiptera: Pseudococcidae)

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The citrus mealybug, *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae) is a serious pest of economically important crops worldwide. The apefly, *Spalgis epius* (Westwood) (Lepidoptera: Lycaenidae) is a potential predator of various species of mealybugs. Earlier investigation on its daily preying capacity and preference for prey stages on *P. citri* is incomplete. Hence, a study was conducted to find out the daily prey consumption ability and preference for prey stages by different larval instars of *S. epius* reared on *P. citri* in the laboratory. Through the 8-day developmental period with four larval instars, the daily prey consumption of *S. epius* 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. When the prey stages were offered separately, the first to fourth instar larva of *S. epius* consumed, respectively, a mean of 199.6, 722.6, 1908.8, and 4625.6 eggs or 21.5, 77.0, 168.5, and 670.5 nymphs or 3.2, 7.2, 16.0, and 35.1 adults of *P. citri.* When an *S. epius* larva was fed on *P. citri* eggs, nymphs and adults separately, it consumed a mean of 7456.7 eggs, 937.6 nymphs, or 62.3 adults during its entire development. When the prey stages were offered all together, a single *S. epius* larva consumed 2618.4 eggs, 170.4 nymphs, and 39.7 adults of *P. citri* throughout its entire development. The study revealed that *S. epius* is a voracious predator of *P. citri* and thus could be utilized as a major biological control agent.

Keywords: apefly; citrus mealybug; *Planococcus citri*; *Spalgis epius*; predation; prey stages

1. Introduction

Several species of mealybugs are serious pests of economically important crops worldwide (Browning 1992; Franco, Gross, Carvalho, Blumberg, and Mendal 2001). Of them, citrus mealybug *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae) attacks a wide range of plants (Murray 1982; Hill 1983; Blumberg, Klein, and Mendel 1995; Islam, Perera, and Copland 1997; Afifi, Arnaouty, Attia, and Abd Alla 2010; Bayer Crop Science 2010; Ben-Dov, Miller, and Gibson 2010). It has been an exceptionally severe pest of grapes *Vitis vinifera* L. in temperate areas and of coffee *Coffea canephora* Pierre ex Froehner and mango *Mangifera indica* L. in tropical regions (Clausen 1978). In greenhouses, the citrus mealybug is the most common pest on bulbs *Allium* spp., coleus *Solenostemon* spp., ferns *Nephrolepis* spp., and gardenias; yet, it may become abundant on other ornamentals as well

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(Blumberg and Van Driesche 2001). It is also a vector of some important plant viruses (Watson and Kubiriba 2005). Betrem (1936), Aziz (1969), Cox (1989), and Goldasteh et al. (2009) studied the biology of *P. citri*. The oviparous *P. citri* adults lay 300–600 eggs and complete the life cycle in about 30 days with three nymphal instars (Kerns, Wright, and Loghry 2002). Chemical control of the citrus mealybug is not easy because of the waxy material which covers the eggs and the adult females (Dean, Hart, and Ingle 1971). Satisfactory control of citrus mealybug has not been achieved hitherto with chemicals in India and elsewhere (Krishnamoorthy and Singh 1987). Owing to lack of effective control of *P. citri*, it causes crop loss of 80–90% in citrus plants (Uygun 2001). Long ago, India imported a predator *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) for the control of different species of mealybugs including *P. citri* and a parasitoid *Leptomastix dactylopii* Howard (Hymenoptera: Encyrtidae) exclusively for the control of *P. citri* (Rao, Ghani, Sankaran, and Mature 1971; Krishnamoorthy and Singh 1987) and they are moderately effective in the field (Krishnamoorthy and Singh 1987; Murulibaskaran, Srinivasan, Muthumeena, Muthulakshmi, and Mahadevan 2002; Mani and Krishnamoorthy 2007).

The apefly, *Spalgis epius* (Lepidoptera: Lycaenidae: Miletinae) has been recorded as a predator of various species of mealybugs e.g., *Ferrisia virgata* (Cockerell), *P. citri* (Risso), *P. lilacinus* (Cockerell.), *Paracoccus marginatus* Williams and Granara de Willink, and *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae), and scale insects *Chloropulvinaria polygonata* Cockerell (Hemiptera: Coccidae) and *Dactylopius* sp. (Hemiptera: Dactylopiidae) (see Dinesh and Venkatesha 2011). Of the several insect predators of mealybugs in India, the larvae of *S. epius* has been reported as a potential predator of *P. citri* and *P. lilacinus* in coffee plantations (Chacko, Bhat, and Ramanarayan 1977; Rahiman and Vijayalakshmi 1998), and of *M. hirsutus* in mulberry gardens (Gowda, Manjunath, Datta, and Kumar 1996).

S. epius occurs in India, Burma and Sri Lanka (De Niceville 1890), Philippines and Java (Le Pelley 1943), Bangladesh (Ali 1978), Thailand (Lohman and Samarita 2009) and Krakatau Island, Indonesia (New, Bush, Thornton, and Sudarman 1988). The morphology, development, life history and behavior of *S. epius* has been studied (Bingham 1907; Vinod Kumar, Vasudev, Seetharama, Irulandi, and Sreedharan 2006; Dinesh, Venkatesha, and Ramakrishna 2010; Thangamalar, Subramanian, and Mahalingam 2010). Observations have been made on the behaviour of *S. epius* and its interaction with ants in the field (Venkatesha, Shashikumar, and Gayathri Devi 2004; Venkatesha 2005; Vinod Kumar, Vasudev, Seetharama, Irulandi, and Sreedharan 2008a). Vinod Kumar, Vasudev, Seetharama, Irulandi, and Sreedharan (2008b) and Thangamalar et al. (2010) made a concise observation on the predatory potentiality of *S. epius* on *P. citri* and *P. marginatus*, respectively, by offering all the prey stages together. Furthermore, Dinesh and Venkatesha (2011) made a detailed observation on the daily and larval instar predatory capacity of *S. epius* on *M. hirsutus* stages offered individually and together. Brief field observations have been made on the feeding activities of *S. epius* on *P. citri* and *P. lilacinus* in coffee *C. canephora* (Rahiman and Vijayalakshmi 1998), on *M. hirsutus* in mulberry *Morus alba* L. (Pushpaveni, Rao, and Rao 1973), and on *P. lilacinus* in Indian jujube crop *Ziziphus mauritiana* Lam. (Mani and Krishnamoorthy 1996) in India.

Although Vinod kumar et al. (2008b) studied the feeding potential of *S. epius* on *P. citri* by offering all prey stages together and recorded the instar feeding potential, no detailed study has been made on 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 the daily and larval instar prey consumption of *S. epius*, and preference for different prey stages such as eggs, nymphs and adults of *P. citri* in the laboratory. This vital information is essential to utilize *S. epius* as a major biological control agent of *P. citri* in the field.

2. Materials and methods

2.1 Lab rearing of prey

P. citri was reared in the laboratory on medium sized ($\approx 70 \times 20$ cm, diameter $\times$ height) ripe pumpkins (*Cucurbita maxima* Duchesne) following Technology for Production of Natural Enemies (1995). Initially the eggs and nymphs of *P. citri* were collected with the help of a camel hair brush from croton plants (*Codiaeum* spp.) in the field at Bangalore University campus, Bengaluru, India (latitude 12°58′N, longitude 77°35′E, elevation 921 m above sea level) and transferred them onto cleaned pumpkins in the laboratory. The mealybug infested pumpkins were individually maintained in a nylon rearing cage ($30 \times 30 \times 30$ cm). For regular availability of the prey, fresh pumpkins were infested with *P. citri* whenever required.

2.2 Lab rearing of predator

S. epius larvae from the laboratory culture were reared on the pumpkins infested with *P. citri* following the method of Dinesh et al. (2010). Predator larvae completed their development on the mealybug-infested 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 lab cultured population of *S. epius* was used for the experiment.

2.3 Prey consumption

The experiments on prey consumption of *S. epius* were conducted in the laboratory at 28.0 ± 1.0°C, 65 ± 5% RH, and a 12 h L:12 h D photoperiod in an environmental chamber following the method of Dinesh and Venkatesha (2011).

To determine the daily prey consumption potential of *S. epius* larva, the eggs of *S. epius* laid in mass of mealybugs on pumpkin were carefully collected with help of a fine camel hair brush and kept individually in Petri dishes (5 cm diameter). 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 *P. citri* separately as food until they reached prepupal stage. Before providing the prey stages, eggs, nymphs and adults of the prey were counted under stereo zoom microscope. Based on 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 left over prey stages in the Petri dish. The larval excreta and left over prey stages were removed daily and the predator was fed with a fresh batch of prey stages. In a separate experiment, the instar prey consumption was studied 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 noted. The prey consumption of *S. epius* was assessed by offering different *P. citri* stages (i.e., eggs, second instar nymphs, and adults) separately and together. The first instar *P. citri* nymphs were not chosen as prey as they are highly mobile and thus the sedentary first and second instar *S. epius* larvae are not able to successfully prey on them.

2.4 Preference for prey stages

The preference for prey stages and amount of each prey stage consumed when they were offered together was determined by providing eggs, second instar nymphs and adults of *P. citri* all together to the freshly hatched *S. epius* larvae in a Petri dish. Daily and instar prey consumption of *S. epius* larva fed on different prey stages was recorded. For each of the above experiments, there were five replications with six *S. epius* larvae per replication.

2.5 Data analysis

To determine if there were differences in daily and larval instar prey consumption by the predator during its larval development, data were subjected to one-way ANOVA, and significant differences between treatment means were determined utilizing Tukey HSD test at $P \leq 5\%$ (SPSS Inc. 2008).

3. Results

3.1 Prey consumption

S. epius larvae with four larval instars completed their development in 8 days in the laboratory. Freshly hatched first instar larvae of *S. epius* consumed a mean of 96.8 ± 0.5 (Mean ± SE) eggs of *P. citri* on the first day and its egg consumption increased thereafter as the development progressed. The larvae consumed the highest number of prey eggs (2508.2 ± 10.0) on the seventh day and decreased to 2117.4 ± 27.7 eggs on the eighth day (Figure 1a). There was a significant difference in mean number of eggs consumed on different days ($F = 5446.3$; df = 7, 32; $P < 0.0001$) except between days 1 and 2 ($P > 0.05$).

When the second instar nymphs of *P. citri* were provided as prey, consumption by a newly hatched *S. epius* larva on the first day was 10.1 ± 0.1 nymphs, increasing thereafter. The predatory larvae consumed a maximum number of nymphs (357.8 ± 5.8) on the seventh day, which decreased to 312.7 ± 2.9 nymphs on the eighth day. There was a significant difference in mean number of nymphs consumed on different days ($F = 3064.6$; df = 7, 32; $P < 0.0001$) except between the first and second days ($P > 0.05$) (Figure 1b).

When *P. citri* adults were offered as a prey, freshly hatched *S. epius* larvae consumed a mean of 1.5 ± 0.05 adults on the first day and consumption increased thereafter to the highest mean of 20.9 ± 0.6 adults on the seventh day and decreased to 14.1 ± 0.1 adults on the eighth day. There was a significant difference in

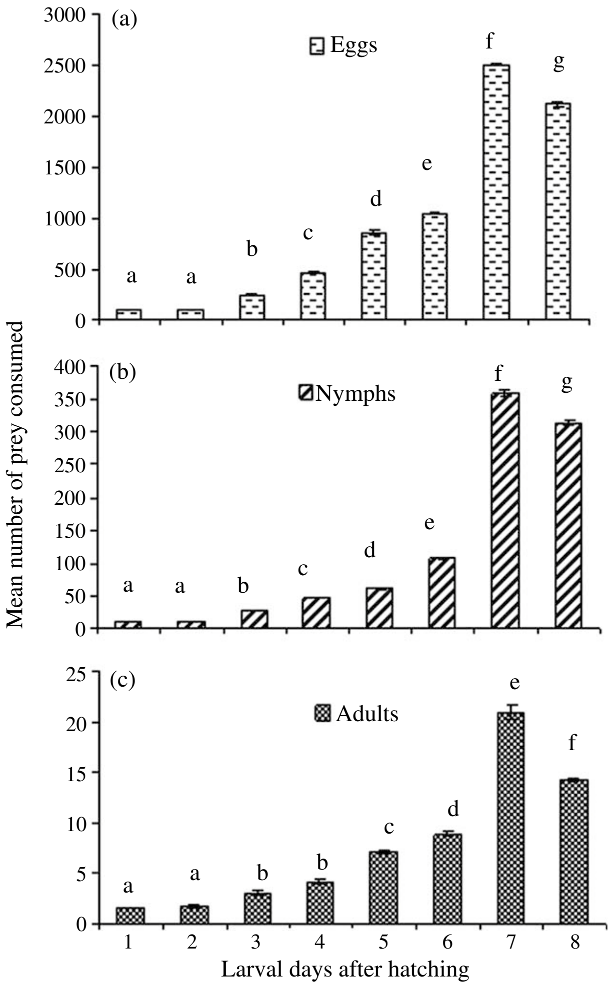

Figure 1. Daily mean prey consumption of *Spalgis epius* larvae on *Planococcus citri* (a) eggs, (b) nymphs, and (c) adults offered separately. Bars with different letters indicate the significant difference between the days within the same prey stage at $P < 0.05$ (one-way ANOVA – Tukey HSD test) (*vertical line indicates the SE mean prey consumption*).

mean number of *P. citri* adults consumed on different days ($F = 600.1$; df = 7, 32; $P < 0.0001$) except between the first and second and, the third and fourth days ($P > 0.05$) (Figure 1c). On average, a single *S. epius* larva consumed 7456.7 ± 50.7 eggs, 937.6 ± 2.3 nymphs, or 62.3 ± 1.1 adults of *P. citri* during its entire larval developmental period.

Prey consumption by *S. epius* increased as they aged, from the first to fourth larval instars. The mean number of the prey eggs ($F = 11456.9$; df = 3, 16; $P < 0.0001$), nymphs ($F = 18501.6$; df = 3, 16; $P < 0.0001$), and adults ($F = 1028.4$; df = 3, 16; $P < 0.0001$) consumed was significantly different among the four larval instars of *S. epius* (Figure 2). The first and second instar larvae of *S. epius* were almost sedentary, whereas the third and fourth instar larvae crawl while feeding.

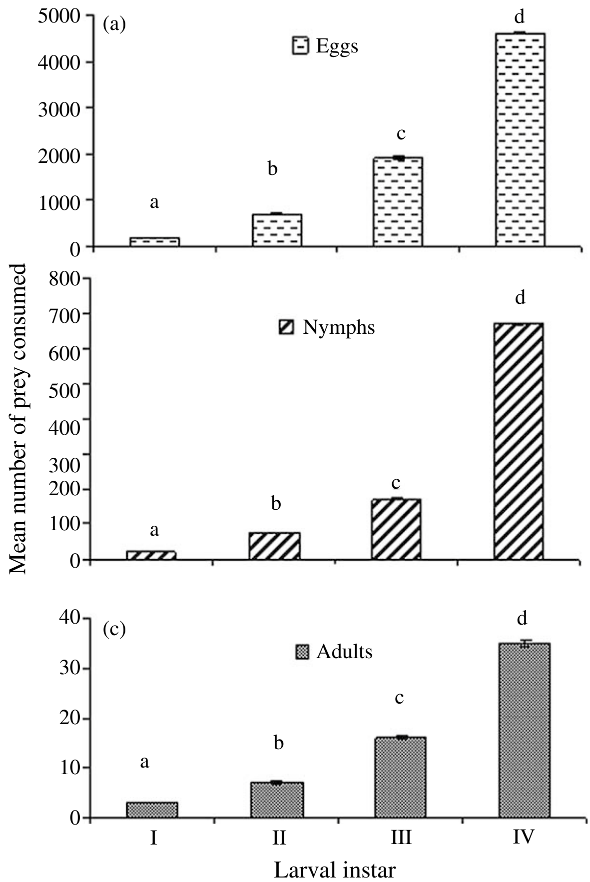

Figure 2. Mean prey consumption by *Spalgis epius* larval instars on *Planococcus citri* (a) eggs, (b) nymphs, and (c) adults offered separately. Bars with different letters indicate the significant difference between different larval instars within the same prey stage at $P < 0.05$ (one-way ANOVA – Tukey HSD test) (*vertical line indicates the SE mean prey consumption*).

3.2 Preference for prey stages

When the prey stages were offered together, an *S. epius* larva consumed a mean minimum of 87.8 ± 1.1 eggs, 0.6 ± 0.07 nymphs, and 0.4 ± 0.03 adults on the first day and a mean maximum consumption of 587.5 ± 13.5 eggs, 41.7 ± 1.8 nymphs, and 12.0 ± 0.4 adults on the seventh day. Although prey consumption on the eighth day decreased to 563.8 ± 28.0 eggs ($F = 219.5$; df = 7, 32; $P = 0.890$), 38.2 ± 1.0 nymphs ($F = 332.1$; df = 7, 32; $P = 0.091$), and 10.8 ± 0.7 adults ($F = 172.2$; df = 7, 32; $P = 0.178$), these were not different from that on the seventh day (Figure 3). The first 2 days, *S. epius* larvae generally preferred eggs to nymphs and adults of *P. citri*, but 3–4-day-old larvae fed on a few nymphs as well. From the fifth day onwards, in addition to the eggs and nymphs, the larvae consumed adults of the prey. During the entire larval development period of *S. epius*, a single larva consumed a mean of 2618.4 ± 23.8 eggs, 170.4 ± 1.7 nymphs, and 39.7 ± 1.2 adults of *P. citri* when the prey stages were offered all together.

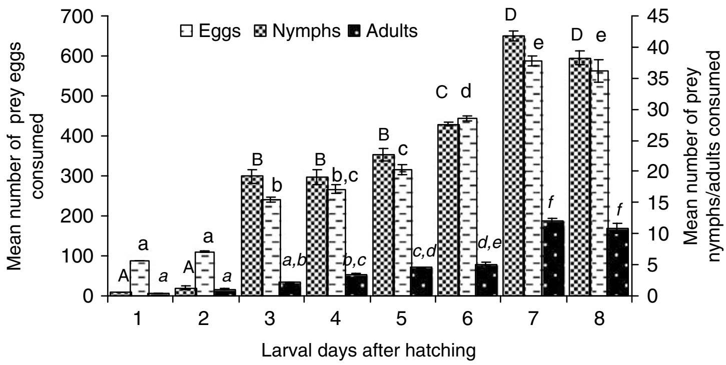

Figure 3. Daily mean prey consumption of *Spalgis epius* larvae on *Planococcus citri* stages offered together. Bars with different small (eggs), capital (nymphs), and italic small (adults) letters indicate the significant difference between the different days within the same prey stage at $P < 0.05$ (one-way ANOVA – Tukey HSD test) (*vertical line indicates the SE mean prey consumption*).

The prey consumption of *P. citri* eggs ($F = 487.5$; df = 7, 32; $P < 0.0001$), nymphs ($F = 1105.3$; df = 7, 32; $P < 0.0001$), and adults ($F = 326.0$; df = 7, 32; $P < 0.0001$) significantly increased from the first to fourth instar larva of *S. epius* (Figure 4). The first and second instar larvae preferred eggs to nymphs and adults of *P. citri*, whereas third and fourth larval instars fed on all stages of the prey.

4. Discussion

S. epius larvae preyed on *P. citri* eggs, nymphs and adults as reported by Vinod Kumar et al. (2008a). A mean daily consumption of prey stages by the developing larva of *S. epius* increased up to day 7, but decreased on the eighth day as the larva prepares for the prepupal stage as observed by Dinesh and Venkatesha (2011) in the

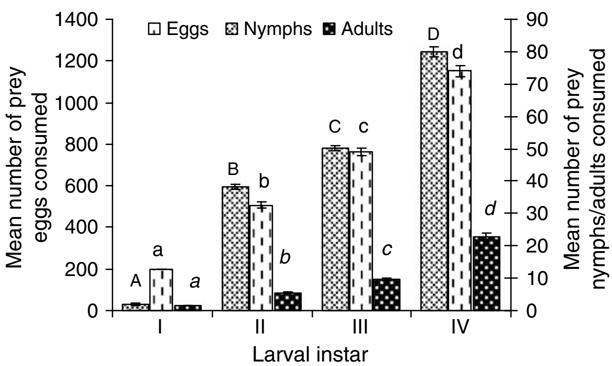

Figure 4. Mean prey consumption by *Spalgis epius* larval instars on *Planococcus citri* stages offered together. Bars with different small (eggs), capital (nymphs), and italic small (adults) letters indicate the significant difference between the different larval instars within the same prey stage at $P < 0.05$ (one-way ANOVA – Tukey HSD test) (*vertical line indicates the SE mean prey consumption*).

case of predation on *M. hirsutus*. In general, there was a significant difference in prey consumption of *S. epius* larvae on different days similar to its prey consumption on *M. hirsutus* (Dinesh and Venkatesha 2011). In the present study, when the different prey stages were offered either separately or together, the four larval instars of *S. epius* consumed more numbers of eggs than nymphs and adults of *P. citri* as observed with *M. hirsutus* (Dinesh and Venkatesha 2011). This could be owing to the size of the eggs, which are very small compared to that of nymphs and adults as reported in the case of predation of *C. montrouzieri* on *M. hirsutus* (Mani and Thontadarya 1987). The daily and larval instar prey consumption by *S. epius* on *P. citri* increased with their progressing development as reported with *C. montrouzieri* and *S. epius* preying on *M. hirsutus* (Mani and Thontadarya 1987; Dinesh and Venkatesha 2011).

Vinod Kumar et al. (2008b) reported that a single *S. epius* larva consumed 416.4 eggs, 449.2 nymphs, and 158.3 adults of *P. citri* when all the prey stages were offered together. However, in our study an *S. epius* larvae consumed greater number of eggs compared to the nymphs and adults of *P. citri*, similar to its preying on *M. hirsutus* (Dinesh and Venkatesha 2011). Differences observed in prey consumption by *S. epius* on *P. citri* between the present and previous studies could be due to using a field collected population of *S. epius* in the laboratory and rearing them under variable environmental conditions as was done by Venod Kumar et al. (2008b). The number of *P. citri* prey stages consumed by *S. epius* in the present study was slightly more than that of *M. hirsutus* (Dinesh and Venkatesha 2011). The variations in the amount of prey stages consumed by *S. epius* on *P. citri* and *M. hirsutus* could be owing to the minor size variations in the prey stages of these two species.

The fully sedentary first and second instar preferred eggs to nymphs and adults of *P. citri* as in the case of *M. hirsutus* (Dinesh and Venkatesha 2011). Dinesh and Venkatesha (2011) reported that this could be due to the constant movement of the young prey nymphs, which generally escape from predation. While feeding on eggs, sometimes the young larvae nibbled the nymphs and adults of *P. citri*, killing them as reported with *P. citri* and *M. hirsutus* (Dinesh et al. 2010; Dinesh and Venkatesha 2011). Whenever *P. citri* eggs were not available for feeding, the young larvae of *S. epius* fed on nymphs and adults and could complete their development as reported by Dinesh et al. (2010) and Dinesh and Venkatesha (2011). From the fifth day onwards (i.e., the third and fourth instar larvae) the well developed and large sized predatory larva (6.6–11.4 mm) consumed all stages of *P. citri* as observed in the case of *M. hirsutus* (Dinesh and Venkatesha 2011).

Successful biological control depends on the ability of a natural enemy to suppress a pest population before it reaches its economic threshold level. Our study showed that a single *S. epius* larva during its 8-day development preyed effectively on large numbers of developing *P. citri*. Therefore *S. epius* should be considered an important biological control agent in India and worldwide, since they have the potential to reduce the population size and subsequent damage caused by developing nymphs and adults of this pest.

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