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Melally Giddegowda Venkatesha & Anegunda Shankar Dinesh

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RESEARCH ARTICLE

Mass rearing of *Spalgis epius* (Lepidoptera: Lycaenidae), a potential predator of mealybugs (Hemiptera: Pseudococcidae)

Melally Giddegowda Venkatesha* and Anegunda Shankar Dinesh

Insect Science Laboratory, Department of Zoology, Bangalore University, Jnana Bharahti, Bengaluru 560056, India

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Spalgis epius (Lepidoptera: Lycaenidae) has been recorded as a potential predator of various species of mealybug crop pests worldwide. We describe the mass rearing of *S. epius*, as no information on this topic is available. Outdoor nylon tent cages of different dimensions were provided to achieve mating and oviposition as *S. epius* adults did not mate in the laboratory cages. Adults mated only in the tent cage ($6 \times 6 \times 10$ m) placed over a native tree (9 m height). The presence of a tree canopy inside the cage is essential to achieve courtship and mating. Gravid females of *S. epius* deposited eggs on the mealybug-infested pumpkins inside the different sized nylon cages with or without a bush/tree. *Spalgis epius* eggs were maintained on mealybug-infested pumpkins in the laboratory and developmental stages of the predator were reared. Adults fed on various diets laid significantly higher number of eggs than those of starved individuals. *Spalgis epius* with a life cycle completed in 21.2 days and 55.7 larvae, could be reared on a single mealybug-infested pumpkin.

Keywords: *Spalgis epius*; biocontrol agent; mass rearing; Lycaenidae; mealybugs

1. Introduction

Several species of mealybugs (Hemiptera: Pseudococcidae) are major pests of economically important crops in temperate and tropical regions. Some of the important species are *Planococcus citri* (Risso), *P. lilacinus* (Cockerell.), *Phenacoccus solenopsis* Tinsley, *Paracoccus marginatus* Williams and Granara de Willink, *Ferrisia virgata* (Cockerell.), and *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae). These mealybugs are serious pests of various crops, such as coffee *Coffea canephora* Pierre ex A. Froehner, citrus *Citrus* spp., cocoa *Theobroma cacao* L., guava *Psidium* spp., grapes *Vitis vinifera* L., papaya *Carica papaya* L., cotton *Gossypium* spp., mango *Mangifera indica* L., mulberry *Morus alba* L., vegetable crops and ornamental plants worldwide (Bartlett and Lloyd 1958; Le Pelley 1968; Summy, French, and Hart 1986; Browning 1992; Williams and Willink 1992; Franco, Gross, Carvalho, Blumberg, and Mendal 2001).

Satisfactory control of different species of mealybugs has not been achieved with insecticides because of their protective wax body coating and ability to escape exposure inside bark crevices and other inaccessible parts of plants (Krishnamoorthy and Singh 1987; Browning 1992; Joyce, Hoddle, Bellows, and Gonzalez 2001).

*Corresponding author. Email: venkatmelally@gmail.com

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Biological control of mealybugs using parasitoids and predators is the most important control method as chemical control is less effective and environmentally undesirable (Berlinger 1977; Bentley 2002).

The apefly, *Spalgis epius* (Lepidoptera: Lycaenidae: Miletinae) attacks various species of mealybugs i.e., *P. lilacinus, P. citri*, *F. virgata*, *P. marginatus,* and *M. hirsutus*; and scale insects *Dactylopius* sp. (Hemiptera: Dactylopiidae) and *Chloropulvinaria polygonata* (Ckll.) (Hemiptera: Coccidae) (see Dinesh and Venkatesha 2011a). Moreover, *S. epius* has been reported as a potential predator of *P. citri, P. lilacinus,* and *M. hirsutus* in the field (Chacko, Bhat, and Ramanarayan 1977; Mani 1995; Gowda, Manjunath, Datta, and Kumar 1996; Rahiman and Vijayalakshmi 1998).

Spalgis epius occurs in India, Burma, Sri Lanka, Philippines, Java, Bangladesh, Thailand, and Krakatau Island (Indonesia) (see Dinesh and Venkatesha 2011a). Adults of *S. epius* are found in agricultural and wooded areas and are inconspicuous in the field because of their small size and drab colour (Venkatesha, Shashikumar, and Gayathri Devi 2004). The morphology, development, life history, and behaviour of *S. epius* has been studied (De Niceville 1890; Aitken 1894; Bingham 1907; Vinod Kumar, Vasudev, Seetharama, Irulandi, and Sreedharan 2006; Dinesh, Venkatesha, and Ramakrishna 2010). Venkatesha et al. (2004), Venkatesha (2005), and Vinod Kumar, Vasudev, Seetharama, Irulandi, and Sreedharan (2008a) reported the interactions between *S. epius* and ants in the field. The predatory activity and feeding potential of *S. epius* on different species of mealybugs has been studied both in the field and laboratory (Pushpaveni, Rao, and Rao1973; Mani and Krishnamoorthy 1996, Rahiman and Vijayalakshmi 1998; Vinod kumar, Vasudev, Seetharama, Irulandi, and Sreedharan 2008b; Dinesh and Venkatesha 2011a, Dinesh and Venkatesha 2011b). Furthermore, *S. epius* has been reported as an effective predator of mealybugs in the field (Mani 1995; Mani and Krishnamoorthy 1996; Rahiman and Vijayalakshmi 1998; Thangamalar, Subramaian, and Mahalingam 2010; Mahalingam et al. 2010). The Animal and Plant Health Inspection Service (APHIS) of the U.S. Department of Agriculture (USDA) at one time proposed introducing this non-indigenous predator *S. epius* into the continental United States and US territories in the Caribbean Islands for control of mealybugs *Planococcus minor* (USDA-APHIS 2002).

Although there are well developed methods for mass production of several moth species for economic purposes, e.g., sterile control programs and production of biocontrol agents (parasitoids and microbial pathogens), methods for mass rearing of many economically important or endangered butterfly species in captivity have not been thoroughly investigated (Shorey and Hale 1965; Parrella, Heinz, and Nunney 1992; Hassan 1993). Even though many butterflies are amenable to mass rearing, often difficulties are encountered with inducing mating in captivity, and thus large scale production for many butterfly species (Mattoni, Longcore, Krenova, and Lipman 1998). Gowda et al. (1996), Vinod Kumar et al. (2006, 2008b), and Thangamalar et al. (2010) collected the eggs and larvae of *S. epius* from the field and studied its biology and feeding potential in the laboratory as they could not induce mating in captivity. Moreover, Vinod Kumar et al. (2006) reported that artificial rearing of *S. epius* is not possible. Dinesh et al. (2010) reared *S. epius* in captivity and studied its biology, but did not describe a mass rearing method. Furthermore, no mass rearing method has been developed until now for any other hemipterophagous

lycaenid butterflies i.e., *Spalgis lemolea* Druce, *S. substrigata* (Snell), *Taraka mahanetra* Doherty, *Taraka hamada* (Druce), and *Feniseca tarquinius* (Fabricius) (Lamborn 1914; Smith 1914; Hall, Minno, and Butler 2007; Lohman and Samarita 2009). Therefore, we describe in this paper a mass rearing method of *S. epius* in captivity to exploit this predator as a biocontrol agent for mealybugs.

2. Materials and methods

2.1. Laboratory rearing of prey

To rear *S. epius* in the laboratory, *P. citri* was cultured on pumpkins (*Cucurbita maxima* Duchesne) following standard methods for production of natural enemies (Dinesh and Venkatesha 2011a). The mealybug-infested pumpkins were maintained individually in nylon rearing cages ($30 \times 30 \times 30$ cm) similar to rearing of the mealybug predator, *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) (Chacko et al. 1978). For regular availability of prey, fresh pumpkins were infested with *P. citri* whenever needed.

2.2. Field source and laboratory rearing of predator

The field-collected predator larvae were reared on the mealybug-infested pumpkins in the laboratory following the method of Dinesh and Venkatesha (2011b). Fresh *S. epius* adults eclosed in the laboratory were utilized for mass rearing experiments.

2.3. Mating cages

To study the standard setting needed to induce mating in *S. epius* under captive conditions, experiments were conducted using different sized mating cages. As *S. epius* adults did not mate in the laboratory rearing cage ($30 \times 30 \times 30$ cm), outdoor mating cages of two dimensions with two types of environment were provided as follows: (1) nylon tent cage ($2 \times 2 \times 2$ m) (henceforth referred to as the medium mating cage); (2) nylon tent cage ($2 \times 2 \times 2$ m) placed over a small bush of *Codiaeum* sp. (1.0 m height); (3) nylon tent cage ($6 \times 6 \times 10$ m width $\times$ length $\times$ height) (henceforth referred to as the large mating cage); and (4) nylon tent cage ($6 \times 6 \times 10$ m) placed over a tree *Polyalthia longifolia* (Sonn.) (9 m height) in the field (Figure 1a–d). The common and conveniently available *Codiaeum* sp. and *P. longifolia* plants in the field at Bangalore University campus, Bengaluru, India (latitude 12°58′N, longitude 77°35′E, elevation 921 m above sea level) were utilized in the mating experiments to simulate natural conditions within the mating cages. The tent cages were suspended from the top four corners with the help of nylon thread. The bottom edges of the tent cages were closely fixed to the ground to prevent escape of adult butterflies. Five, 1-day-old pairs of virgin *S. epius* adults were released into these cages and observations were made on their courtship and mating behaviour during the active period of adults (i.e., from 08:00 to 17:00 h). For the large mating cage, close observations on the events of courtship and mating activities were made from a plank platform (6 m height), which was set up adjacent to the tree canopy. All individuals of common predators of *S. epius* adults i.e., red ants *Oecophylla smaragdina* L. (Hymenoptera: Formicidae) and different species of spiders

Figure 1. Outdoor mating and oviposition cages for *Spalgis epius*. (a) Medium cage, (b) medium cage with a bush *Codiaeum* sp., (c) large cage, and (d) large cage with a tree *Polyalthia longifolia*.

(Arachnida: Araneae) present inside the outdoor mating cages were removed before releasing the adults. Nests of *O. smaragdina* and cobweb present on the tree were also removed.

2.4. Oviposition

To induce oviposition by *S. epius* gravid females, mealybug-infested pumpkins were kept inside the outdoor mating cages on a stand (60 cm height). The mated females were collected using an aerial insect net, immediately after copulation. Their egg laying behaviour was studied under the above four types of outdoor mating cages and in the laboratory rearing cage as well. The experiment was repeated with 10 gravid females in each type of cage, and we counted the number of eggs deposited on the mealybug-infested pumpkins with the help of a magnifying lens (20 ×). To prevent naturally occurring infestation of sucking pests (mealybugs and aphids) on

the weeds, which attract gravid *S. epius* females for egg deposition, weeds were removed inside the cages.

2.5. Mass rearing method

One-day-old male and female butterflies reared in the laboratory were released into the suitable outdoor mating cage in the early morning for mating and egg laying. The mealybug-infested pumpkins were kept in an outdoor mating cage for 2 days for egg deposition by *S. epius* gravid females. The mealybug-infested pumpkins containing *S. epius* eggs were maintained individually in the rearing cage in the laboratory. Emerged predator larvae were allowed to feed on mealybugs on the pumpkin and complete their development.

The average number of *S. epius* larvae reared on a single mealybug-infested pumpkin was recorded. When mealybug population was exhausted on pumpkins or pumpkins began decaying, the larvae were carefully transferred onto another mealybug-infested pumpkin with the help of camel hair brush. Similarly, if the pumpkin with *S. epius* pupae showed signs of rotting, the pupae were removed using a Cutter Knife© (18 cm length), peeling the epidermis of the pumpkin and transferring them to a blotting paper in another cage for emergence of adults. The predator was reared at $28.0 \pm 1.0^{\circ}$C, $65.0 \pm 5.0\%$ RH and a 12 h L:12 h D photoperiod in an environmental chamber. These environmental conditions were chosen for rearing of *S. epius* as the high incidence of the predator population was observed in the field almost under similar ecological conditions. Moreover, a temperature range of 25–30°C is suitable for rearing of *P. citri* (Hamid and Michelakis 1994).

2.6. Life cycle and mortality of predator

The life cycle of the predator was studied following the method of Dinesh et al. (2010) in an insect environmental chamber at $28 \pm 1^{\circ}$C, $65.0 \pm 5.0\%$ RH and a 12 h L:12 h D photoperiod. The egg, larval, prepupal, and pupal developmental period, number of instars in a life cycle and mortality in each stage were recorded.

2.7. Influence of diet on fecundity

To test the influence of different diets on fecundity, 10 pairs of fresh *S. epius* adults were fed separately in the laboratory on three diets: (1) 10% honey (diluted in water), (2) a cotton swab soaked in tap water, and (3) a ripe banana. Adults without food were maintained as a control. These fed and unfed adults were allowed to mate separately one day after pupal eclosion and deposit eggs on the mealybug-infested pumpkins in the large mating cage. The total number of eggs deposited by these gravid females was recorded.

2.8. Data analysis

The number of eggs deposited in different cages, and the fecundity of females fed on different diets including the unfed individuals were analyzed utilizing a one-way ANOVA–Tukey HSD test ($P < 0.05$) (SPSS Inc. 2008).

3. Results

3.1. Mating

Spalgis epius adults did not mate in the outdoor medium-sized mating cage with or without the small bush. Rarely the adults made short flights and most of the time they were resting at top corners of the cage and sometimes on the bush as well. Similarly, the adults did not mate in the large outdoor mating cage and were resting at top corners of the cage. However, the adults did make courtship flights and subsequently mated in the large outdoor mating cage placed over a *P. longifolia* tree. Adults mated a day after eclosion in the cage. Initially males marked their territory by perching on foliage at different locations in the range of 6–9 m height from the ground. They marked their territory by dragging their abdominal tip on the leaf. Adult males then made spontaneous flights in a limited range within the 2–3 m radius around the perch points on branches in the tree canopy. Males defended their territory if another male intruded. Under such circumstances, the two males undertook a circling flight, and the resident typically chased away the intruder. Within 20–25 minutes of establishing a territory, a female was attracted to a male. As soon as the female entered the territory, the pair engaged in courtship flights. The courtship flight distance between the pair was 12–18 cm. Periodically the pair tried flying beyond the roof of the net. The pair engaged in 1–4 flights at an interval of 4–5 min and then the female alighted on a perch in the territory. Immediately, a male approached a female, and the pair copulated in a tail-to-tail position (Figure 2a). The pair remained in copula for 55.7 ± 1.5 minutes. The courtship and mating activities took place under both bright and diffused sunlight. Observations were made in 150 copulated pairs; both sexes were found to mate only once in their lifetime. The once mated females were unreceptive when new males tried to court them.

3.2. Oviposition

The gravid females never laid eggs on mealybug-infested pumpkins in the laboratory rearing cage. However they oviposited on the mealybug-infested pumpkins, about the same number of eggs, when inside medium and large cages, with or without plants (bush/tree) present: on average 76.6 ± 1.6, 77.2 ± 2.3, 79.5 ± 1.4, and 73.9 ± 1.7 eggs in 2–3 days, respectively ($F = 1.607$; df = 3, 36; $P = 0.205$) (one-way ANOVA). *Spalgis epius* gravid females were attracted to the mealybug-infested pumpkin during oviposition period in the cages. The preoviposition period was 1–4 days after mating. The female rapidly deposited eggs in the mass of mealybugs generally between 11:30 and 15:30 h under bright/diffused sunlight. It laid eggs singly interspersed with many short circling flights around the pumpkin, and in each flight, 3–6 eggs were laid ca. within the 6 cm radius at different spots. The female deposited 37.2 ± 4.3 eggs per day. In the mating cages the gravid females were flying all over the cage area in search of prey infestation.

3.3. Larval rearing

Spalgis epius larvae consumed the eggs, nymphs and adults of mealybugs and successfully completed their development in the laboratory (Figure 2b). The fully developed fourth instar larva stopped feeding and cleared debris from their body

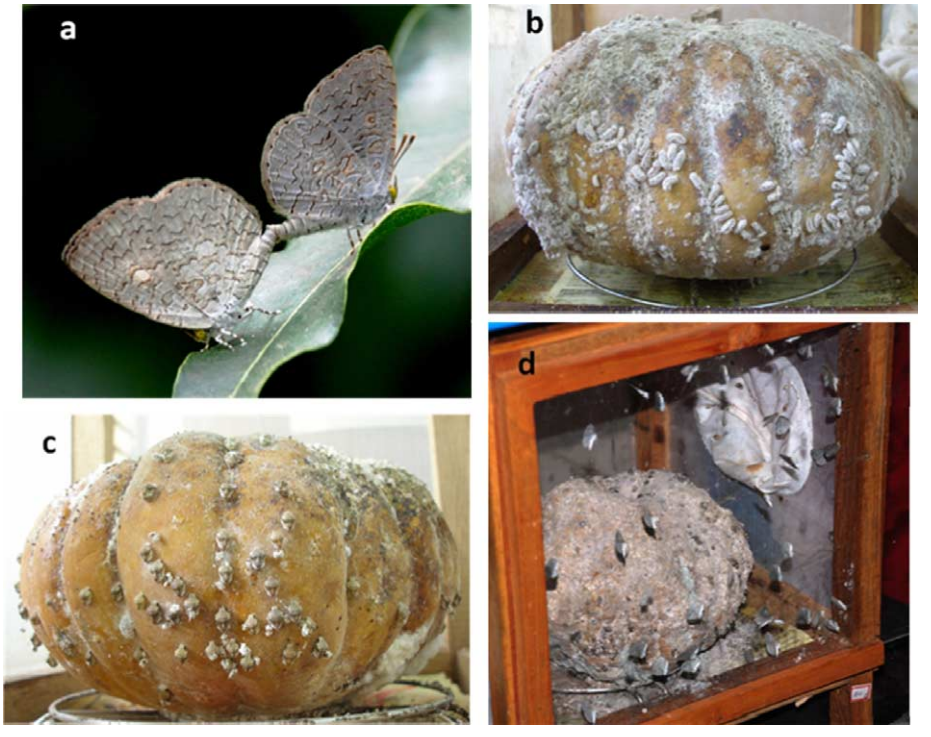

Figure 2. Mass rearing of *Spalgis epius*. (a) Adults in copula, (b) mature larvae on the mealybug-infested pumpkin, (c) pupae on the pumpkin, and (d) adults in the rearing cage.

surface before entering into a prepupal stage. The prepupa generally moved away from the mealybug colony and firmly attached itself to the hard surface on the pumpkin (Figure 2c), and inner walls of the cage.

3.4. Life cycle and mortality

Developmental period of the egg, larval instars, prepupa, and pupa are presented in Table 1. *Spalgis epius* completed its life cycle in 21.2 ± 0.12 days at $28.0 \pm 1.0^{\circ}$C, $65.0 \pm 5.0\%$ RH and a 12 h L:12 h D photoperiod. Mortality was observed only in the egg and the first instar stage (Table 2). A mean of 55.7 ± 6.9 larvae ($n = 20$ pumpkins) could be reared on a single pumpkin. No incidence of any diseases was encountered on any developmental stages during mass rearing.

3.5. Eclosion

The adults eclosed between 11:30 and 15:30 h. Fresh adults rested on the pumpkin as well as on the walls of the cage (Figure 2d). Same aged female and male butterflies eclosed at about the same time. The female-to-male sex ratio of 5000 laboratory-bred individuals was 1.38:1. A few (9.3%) abnormal adults emerged with curled wings.

Table 1. Developmental period for different stages of *Spalgis epius* at 28.0 ± 1.0°C and 65.0 ± 5.0% RH.

Stages	*n*	Duration of development (days)	
		Mean ± SE	Range
Egg	35	3.0 ± 0.05	2.5–3.5
Larva			
I instar	35	1.9 ± 0.06	1.5–2.5
II instar	35	1.7 ± 0.05	1.0–2.0
III instar	35	1.9 ± 0.04	1.5–2.5
IV instar	35	2.6 ± 0.08	2.0–3.5
Total larval period	35	8.3 ± 0.09	7.5–9.0
Prepupa	35	1.0 ± 0.03	1.0–1.5
Pupa	35	8.8 ± 0.08	8.5–9.5
Egg to adult	35	21.2 ± 0.12	20.0–23.0

3.6. Influence of nutrition on fecundity

The fresh male and female butterflies readily accepted liquid food or juicy fruit in the laboratory. Butterflies presented with a liquid or solid food produced about the same number of eggs (Figure 3). However, unfed gravid females laid significantly fewer eggs than those fed artificial diets ($F = 79.03$; df = 3, 36; $P < 0.05$).

4. Discussion

Mass rearing and release of natural enemies (e.g., parasitoids and predators) is often used for biocontrol of mealybugs (Mustu, Kilincer, Ulgenturk, and Kaydan 2008). *Spalgis epius* mating could be induced in captivity, and mass rearing was possible in the laboratory. A tree canopy of 9 m height inside a large mating cage was needed to initiate courtship and mating in *S. epius* adults. *Spalgis epius* appears to need a large space and more height from the ground level for territorial and mating flights to achieve successful copulation. The presence of a tree canopy is needed to achieve mating in *S. epius* adults as it provides a suitable condition to undertake perching activity, territorial and courtship flights. Earlier, many scientists in India were unable to induce mating in *S. epius* as they had provided either very small mating cages or

Table 2. Percent mortality for developmental stages of *S. epius* at 28.0 ± 1.0°C and 65.0 ± 5.0% RH.

Stages	*n*	% Mortality
Egg	40	5.7
Larva		
I instar	40	3.0
II instar	40	0.0
III instar	40	0.0
IV instar	40	0.0
Prepupa	40	0.0
Pupa	40	0.0

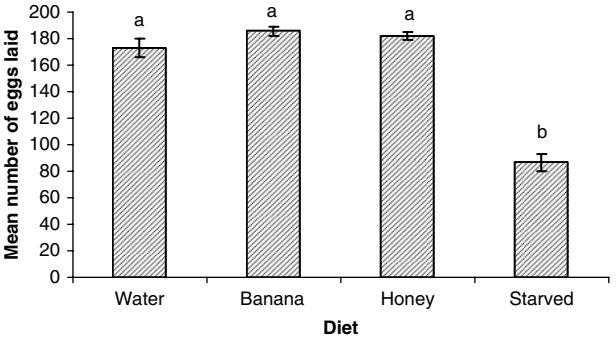

Figure 3. Fecundity of *Spalgis epius* adults fed on different diets. Bars with different letters indicate significant difference between different diet treatments at $P < 0.05$ (one-way ANOVA–Tukey HSD test) (vertical line indicates the SE of the mean total fecundity).

large mating cages without a canopy (H.G. Seetharama, personal communication). Additionally, bright/diffused sunlight is an essential factor for courtship and mating, as observed in other species of lycaenids e.g., *Incisalia iroides* (Boisduval) (Powell 1968), and *Favonius taxila* Bremer (Takeuchi and Imafuku 2005). Oviposition has been induced in small lycaenid butterflies held in tiny containers that restricted movement (Friedrich 1986), and for the lycaenid *Lycaeides melissa samuelis* Nabokov (Lane and Welch 1994; VanLuven 1994). In contrast, large cages are needed by gravid *S. epius* for oviposition, and a tree canopy is needed for mating.

The laboratory culturing method for *S. epius* larvae on the mealybug-infested pumpkin is similar to that of *C. montrouzieri* (Chacko et al. 1978). As observed in *S. epius,* eclosion of abnormal adults was also reported in *Glaucopsyche lygdamus palosverdesensis* Perkins and Emmel (Mattoni et al. 1998). Although lepidopterans are susceptible to a wide variety of infectious diseases during mass rearing (Boucias and Pendland 2001), no occurrence of disease was found while rearing the various life stages of *S. epius.* Temperature and humidity are important variables in determining an insect activity and development (Saul-Gershenz, Arnold, and Scriber 1995). *Spalgis epius* completed its life cycle in 21.2 days under controlled rearing conditions, compared to 23.8 days under variable conditions (Dinesh et al. 2010). The high percent of egg hatching and low percent mortality of developing stages under the tested environmental conditions suggests that these settings are highly suitable for the production of *S. epius* in the laboratory.

The effect of adult diet on fecundity clearly indicated that food enhances the fecundity of *S. epius.* Newly eclosed adults of *S. epius* should be fed on liquid food or banana *Musa paradisiaca* L. before mating to increase egg production. Sucrose is known to enhance the fecundity and longevity of lycaenids *Jalmenus evagoras* (Dovan) (Hill and Pierce 1989) and *Lycaena hippothoe* L. (Fischer and Fiedler 2001).

This study provides the first detailed mass rearing method for *S. epius* in captivity. The ability to mass rear this predator could likely provide another, effective and affordable natural enemy for the control of mealybugs worldwide.

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