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Development and Growth Ratio of Predaceous Coccinellid, *Sasajiscymnus quinquepunctatus* (Weise) on Papaya Mealybug, *Paracoccus marginatus* Williams & Granara de Willink

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ABSTRACT

The predaceous coccinellid, *Sasajiscymnus quinquepunctatus* (Weise) (Coleoptera: Coccinellidae) was identified as a potential candidate for biological control of the papaya mealybug, *Paracoccus marginatus* Williams & Granara de Willink (Hemiptera: Pseudococcidae), which is native to the Neotropical region and in 2008 was detected as an invasive alien insect species in Thailand. It was subjected to laboratory investigation on its development, life history and growth ratio for its possible use in augmentative biological control. *P. marginatus* was cultured on Thai pumpkin (*Cucurbita moschata*) as an artificial host and was used as prey for the coccinellid. The development from egg stage to adult was described and their respective sizes measured. The development time (mean ± SD) from egg to adult was 29.96 ± 0.80 d. The incubation period, the duration of four larval instars, and the pupal stage, all reported as (mean ± SD), were 6.37 ± 1.03, 2.62 ± 0.64, 2.58 ± 0.56, 2.70 ± 0.59, 5.06 ± 0.80, and 10.58 ± 1.19 d, respectively. Adult longevity (mean ± SD) was 32.16 ± 10.56 d. The mean geometric growth ratio of 1.65 using the head capsule width in successive larval instars as a parameter was obtained and it conformed well to Dyar's law. It was advocated that *S. quinquepunctatus* could be further evaluated for utilization in augmentative biological control of the papaya mealybug in Thailand.

Keywords: *Sasajiscymnus quinquepunctatus*, *Paracoccus marginatus*, papaya mealybug, invasive alien species

INTRODUCTION

The predaceous coccinellid, *Sasajiscymnus quinquepunctatus* (Weise) (Coleoptera: Coccinellidae), is one of the coccinellids found in a survey of resident natural enemies of the papaya mealybug, *Paracoccus marginatus* s and Granara de Willink (Hemiptera: Pseudococcidae), in Thailand. It was earlier reported as *Pseudoscymnus quinquepunctatus* (Weise) feeding on mealybugs on mango and citrus in Thailand by Chunram and Sasaji (1980) and Chunram (2002). The genus *Pseudoscymnus* was created by Chapin (1962) but Vandenberg (2004) found that the generic name *Pseudoscymnus* was ambiguous, referring to both a coccinellid genus, *Pseudoscymnus* Chopin and a shark genus, *Pseudoscymnus* Herre. As a consequence,

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Vandenberg (2004) proposed to correct this nomenclatural problem by proposing a new name, *Sasajiscymnus*, as a replacement name for the junior homonym *Pseudoscymnus* (Chapin, 1962). This replacement name has since been accepted and widely used (Flowers *et al*., 2006; Deal, 2007) and as a result *Pseudoscymnus quinquepunctatus* (Weise) became *Sasajiscymnus quinquepunctatus* (Weise). It is also worthwhile to note that the coccinellid generic name *Scymnus* has also been used for several shark and shark-like fish of the Family Dalatiidae long before it was used as a genus for coccinellids (Vandenberg, 2004).

The papaya mealybug, *P. marginatus*, was detected as an invasive alien species in Thailand in 2008 (Anonymous, 2008a, 2008b). Currently, it is found attacking and causing significant damage to papaya, cassava and ornamental plants such as plumeria and hibiscus, and to some extent a few other crop plants and weeds in Thailand. It is native to the Neotropical region in Belize, Costa Rica, Guatemala and Mexico (William and Granara de Willink, 1992). It spread to the Caribbean and Florida, USA in 1994 and 1998, respectively (Watson and Kairo, 1998; Walker *et al*., 2006). The occurrence of papaya mealybug was also reported in Guam in April 2002 (Plant Protection Service, 2003; Meyerdirk *et al*., 2004), in the Republic of Palau in March 2003 (Plant Protection Service, 2003; Muniappan *et al*., 2006), and in Hawaii in May 2004 (Heu *et al*., 2007) in the Pacific region. In Asia, it was reported from Indonesia in Bogor, West Java and the Indian subcontinent in south India in 2008 (Muniappan *et al*., 2008), from Sri Lanka in 2008 (Hettiarachchi, 2009; Hettiarachchi and Silva, 2009) and from Malaysia in 2009 (Anonymous, 2009). Muniappan *et al.* (2009) also reported *P. marginatus* on papaya from Benin in early 2010, Ghana in 2009 and Togo in West Africa in 2010. In Réunion, an island off the east coast of Africa, Germain *et al*. (2010) reported *P. marginatus* as a new pest of papaya in 2010. Later it was reported infesting papaya in the Sultanate of Oman on the Arabian Peninsula in 2011 (R. Muniappan and G. Watson, pers. comm.) and was also intercepted by the National Plant Quarantine Service in Busan, South Korea (Park *et al*., 2011).

A preliminary field survey of *P. marginatus* and its associated natural enemies carried out from late 2008 to 2010 in Thailand revealed that *S. quinquepunctatus* was found associated with and substantially predating on papaya mealybug in all locations. Since no biological information of this predaceous coccinellid is currently available, the objective of the current research was to investigate the basic development, life history and growth ratio of the coccinellid, *S. quinquepunctatus*, on the newly invasive papaya mealybug, *P. marginatus*, as a basis for further evaluation and utilization of this predaceous coccinellid as a candidate biological control agent in the management of the invasive papaya mealybug in Thailand.

MATERIALS AND METHODS

A laboratory culture of the papaya mealybug, *P. marginatus,* was established to provide prey for the study on the development and growth ratio of the predaceous coccinellid, *S. quinquepunctatus*. Egg sacs and nymphs of *P. marginatus* found on infested papaya under field condition were collected and used to initiate and establish a laboratory culture. They were reared using medium-sized Thai pumpkin (*Cucurbita moschata* Duchesne) fruits (approximately 20 cm in diameter) as an artificial host. The pumpkin fruits were first thoroughly washed with tap water, soaked in 5% solution of 6% sodium hypochlorite Hygiene bleach for 15 min, rinsed with tap water again and then left to air dry at room temperature. Each pumpkin fruit was placed on two layers of square paper napkin on a disposable plastic plate. After inoculating each pumpkin fruit with a few egg sacs and mealybug nymphs, the fruits were kept on a cabinet shelf comprising five plywood boards measuring 45 × 90 cm and placed 35 cm apart resulting in an overall height of 1.8 m. Each shelf was fitted with two 60 cm 18W Cool White Sylvania fluorescence lamps and a light:darkness cycle of 12 hr light followed by 12 hr darkness was maintained using an electric switch timer (Panasonic TB178 K, Osaka, Japan) under laboratory conditions of 25–30 ºC and 40–60% relative humidity.

The laboratory culture of *S. quinquepunctatus* was also initiated in the laboratory by releasing 10 field collected adults on each pumpkin fruit with moderate (Vandenberg, 2004) mealybug infestation. Each pumpkin fruit was kept in a clear, square plastic container, measuring 40 × 40 × 40 cm with three screen windows, 10 × 10 × 10 cm, on two opposite sides and on the top cover. The coccinellids were allowed to prey on the mealybugs to complete their development and to reproduce. Newly formed pupae were collected and kept separately for the adults to emerge who were then used for further study on the development, life history and growth ratio. The mealybug-infested pumpkin fruit samples were changed whenever necessary. The rearing containers with both the mealybug and the coccinellid were placed on the same shelf cabinet used for rearing the mealybug.

Description of stages of development

Eggs, larvae of all instars, pupae and adult specimens were examined and used to measure the body size under a stereomicroscope. All the immature stages used in the measurement were first preserved in 70% ethyl alcohol and measured using a micrometer calibrated to the nearest millimeter. Specimens of adults of both sexes were used for measuring the body size. For the measurement of the size of each developmental stage, 20 samples were used.

Development and life history

To extract *P. marginatus* as prey for study on the development and life history of *S. quinquepunctatus*, a piece of tissue paper with a grid drawn on it was placed on heavily mealybug-infested pumpkin to allow the mealybug to attach themselves on the paper which was then kept in Petri dishes. The newly emerged adult coccinellids were kept and allowed to mate at random in the Petri dish with the *P. marginatus* that were attached to the tissue paper. The paper was changed daily and observed under the stereomicroscope to evaluate pre-oviposition, oviposition, post-oviposition periods, larval and pupal developmental stages and adult longevity. The incubation period of each egg was recorded until 50 newly hatched larvae were obtained. Each of the 50 newly hatched first instar larvae was then transferred individually into a new Petri dish containing a piece of tissue paper attached with a mealybug mass as food. Each paper was changed daily and examined under the stereomicroscope to detect any evidence of molting such as the presence of an exuvia and/or the head capsule until each larva had either died or continued its development to become an adult. The development time and the mortality of the larvae and pupae were recorded daily according to the number of surviving individuals in that particular stage to calculate the mean duration of successive larval instars and pupal stage until the adults emerged. The experiment was carried out under the same laboratory conditions used for rearing both the papaya mealybug and the predaceous coccinellid.

Growth ratio

The head capsules cast off during each larval instar of all four larval instars and pupa were collected and preserved in 70% ethyl alcohol from different larvae and pupae during the life history study. The head capsules were measured using an ocular micrometer calibrated to the nearest millimeter and the head capsule width was used to determine the growth ratio of *S. quinquepunctatus*. This parameter was employed to determine if the geometric growth ratio of *S. quinquepunctatus* larvae in successive larval instars conformed to Dyar's law (Dyar, 1890; Imms, 1957) using the chi-square test as described by LeClerg *et al.* (1966), Weinberg and Schumaker (1969), and Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

Description of stages of development

S. quinquepunctatus laid its spherical eggs singly or in a cluster of 3–6 eggs inside, underneath or near the mealybug colonies. The larvae were of the oligopod type and slightly fusiform. Each newly hatched larva was yellow in color and without cottony wax before being covered with a light waxy substance when it was about a half to one day old. Pope (1973) reported that many larvae in the tribe Scymnini have waxy coverings that provide protection against carabids and ants but not against larger coccinellids that prey on wax-covered aphids. The color on the thoracic and abdominal regions of the larvae was dark grey; however, the newly molted larvae were yellowish in color. Völkl and Vohland (1996) also reported that the newly emerged larvae of *Sasajiscymnus* sp. were yellow before becoming dark grey in the ventral region and covered with a cottony substance. The larval antennae of *Sasajiscymnus* sp. were two-segmented, one segmented with a thick seta and a few thin, long setae located at the apex. Lynn *et al*. (2002) stated that the identification of the larva to the genus level requires careful microscopic examination as the larval antennae of *S. tsugae*, which initially were described as being one-segmented, were in fact two-segmented after careful examination.

The terminal fourth instar larvae produced short, white, velvety wax over their bodies and became inactive. The larval body became slightly shorter and wider. From the anal segment a dark brown, sticky liquid was produced which changed to a white color over 1–2 d in preparation for pupation and molting. The pupae were of the exarate type and yellowish in color. They attached themselves to a surface by the anal segment and were entirely immobile with a width (mean ± SD) of 1.14 ± 0.18 mm and a length of 1.57 ± 0.09 mm.

The bodies of newly emerged adult beetles were uniformly dull orange without any elytral pattern until after 12 to 24 hr when until the body hardened. The elytra at the dorsal view were bi-colored and an apical third or less of the elytra was yellow-brown to orange, while the remainder of the elytra was black. The epipleura was horizontal or slightly inclined below and the elytra were densely pubescent with uniform length and appearance and with moderately dense semidecumbent grey hairs. The ventral side of the body was brownish black, with sparse almost decumbent grey hairs. The hind wings were twice as long as the elytra. The prosternum was T–shaped with a transverse apical margin. However, the dorsal color patterns on the elytra may not be decisive characteristics for identification of *S. quinquepunctatus*. Sasaji (1971) illustrated at least six color patterns on the elytra of *S. quinquepunctatus* including its subspecies *quinquepunctatus* (Weise) and *okinawanus* (Kamiya).

In the adult stage, the head of *S. quinquepunctatus* was not drawn into the prothorax, therefore the eyes were distinct and the appendages were yellow to orange with sparse almost decumbent grey hairs. The antennae were 11 segmented, fine and short with two dilated basal segments (scape and pedicel) and with a 4-segmented elongate-oval club, which in the resting position was kept under the eyes, folding in two and inserted on the lateral surface of the head between the eyes and the base of the mandible. Moreover, Chapin (1962) reported that *Pseudoscymnus* (= *Sasajiscymnus*) was once grouped in the genus *Scymnus* but the species found in Japan were significantly different and a new genus was designated. The reason for the separation into two genera was that *Pseudoscymnus* (= *Sasajiscymnus*) species have nine-segmented antennae and a trimerous tarsal formula, while *Scymnus* have either 10- or 11-segmented antennae. *Scymnus* and *Sasajiscymnus* (= *Pseudoscymnus*) were similar in body form and behavior, making them difficult to distinguish without using a microscope (Delucchi, 1954; Sasaji and McClure, 1997). In the lateral aspect, the upper side of the body was moderately convex; the ventrum was weakly jutting out from under the elytra. All legs were yellowish-brown to orange. In general, the legs of *Sasajiscymnus* sp. were short and the apices of the femora were hardly exposed from under the outline of the elytra. In the current study, the shape of the adult in the dorsal aspect was elongate-oval, widest at the middle, shoulders prominent; the pronotal base was a little narrower than the width of the elytra at the shoulders.

The size of eggs, larvae of different instars, pupae and adults of *S. quinquepunctatus* measured in the current study are shown in Table 1.

Development and life history

Under laboratory conditions, the incubation period (mean ± SD) of *S. quinquepunctatus* eggs was 6.37 ± 1.03 d. The larvae developed through four instars to reach the pupal stage and then become adults. The duration (mean ± SD) of the first to the fourth larval instars was 2.62 ± 0.64, 2.58 ± 0.56, 2.70 ± 0.59, and 5.06 ± 0.80 d, respectively. The total larval duration (mean ± SD) was 14.31 ± 0.69 d. In development to the pupal stage, the fourth instar larvae produced short, white, velvety wax over the body and became inactive. The body became slightly shorter and wider and produced a dark brown, sticky liquid from the anal segment within 1–2 d of pupation. The yellowish exarate pupae spent on average (mean ± SD) 10.58 ± 1.19 d in the pupal stage. They attached themselves to a surface of any substrate by using the anal segment and were entirely immobile. Thus, the total duration (mean ± SD) of development from egg to adult of *S. quinquepunctatus* averaged 29.96 ± 0.80 d or about one month. The adult beetles were also observed to begin mating repeatedly after eclosion from the pupae and began to oviposit when aged 6–7 d. The reproductive period observed in the current study ranged from 15 to 30 d and the (mean ± SD) adult longevity was 32.16 ± 10.56 d.

Growth ratio

The mean width of the head capsule yielded a consecutive geometric progression during each stage, ranging from 1.43 to 1.83 and

Table 1 Size of eggs, larvae of different instars, pupae and adults of *Sasajiscymnus quinquepunctatus*.

Stage of development (n = 20)	Width (mm)		Length (mm)	
	Mean ± SD	Range	Mean ± SD	Range
Egg	0.04 ± 0.01	0.03–0.05	–	–
Larvae				
1st Instar	0.30 ± 0.04	0.26–0.38	0.84 ± 0.14	0.70–0.99
2nd Instar	0.50 ± 0.06	0.43–0.56	1.49 ± 0.28	1.20–2.98
3rd Instar	0.60 ± 0.07	0.53–0.67	2.24 ± 0.23	2.01–2.47
4th Instar	0.90 ± 0.07	0.83–0.96	3.05 ± 0.09	2.94–3.15
Pupa	1.14 ± 0.18	0.95–1.33	1.57 ± 0.09	1.48–1.66
Adult	1.13 ± 0.12	1.01–1.26	1.56 ± 0.31	1.25–1.87

averaging 1.65. The calculated or theoretical width was thus computed from the mean geometric progression of 1.65. The computed pooled chi-square value of $\chi^2 = 0.12$ (5 degrees of freedom) was highly significant ($P < 0.01$; Table 3). Therefore, the difference or the discrepancy between the observed and theoretical head capsule widths was not significant and the geometric growth ratio of *S. quinquepunctatus* larvae conformed well to Dyar's law (Imms, 1957) and was verified using the chi-square test described by LeClerg *et al.* (1966), Weinberg and Schumaker (1969) and Snedecor and Cochran (1989).

Dyar (1890) observed the head capsule width of 28 species of Lepidoptera and found that it followed a regular geometric progression in successive instars. In the study on the larval growth ratio of the New Guinea Sugarcane Weevil, *Rhabdoscelus obscurus* Boisduval (Coleoptera: Curculionidae), Napompeth *et al*. (1972) found that apart from its larval growth conforming to Dyar's law, a straight line relationship between the logarithmic width of the head capsules and larval instars including the pupal stage was obtained and could be used to determine the number of larval instars. However, Gordh and Headrick (2001)

Table 2 Duration of developmental stages of *Sasajiscymnus quinquepunctatus* reared on Thai pumpkin fruit under laboratory conditions (25–30°C and 40–60% RH).

Stage of development	Number of samples (n)	Duration (d)	
		Mean ± SD	Range
Egg	10	6.37 ± 1.03	4–8
Larvae			
1st Instar	40	2.62 ± 0.64	2–4
2nd Instar	37	2.58 ± 0.56	2–4
3rd Instar	36	2.70 ± 0.59	2–4
4th Instar	35	5.06 ± 0.80	4–8
Total larval period	–	14.31 ± 0.69	14–15
Pupa	33	10.58 ± 1.19	9–14
Egg to adult	–	29.96 ± 0.80	29–30

Table 3 Geometric growth ratio of *Sasajiscymnus quinquepunctatus* larvae using head capsule width as a parameter.

Stage of development	Number of samples (n)	Mean observed head capsule width (mm)	Geometric progression	Theoretical head capsule width (mm)	χ^2
Larvae					
1st Instar	40	0.028	0.042/0.028 = 1.50	0.046	0.007
2nd Instar	37	0.042	0.077/0.042 = 1.83	0.069	0.011
3rd Instar	36	0.077	0.14/0.077 = 1.82	0.127	0.020
4th Instar	35	0.140	0.20/0.14 = 1.43	0.231	0.036
Pupa	33	0.200	0.49	0.330	0.051
		0.097	Mean geometric progression = 1.65	0.16	$\chi^2 = 0.12$ $P < 0.01$

argued and explained that the law could only be used as a predictive tool for estimating the instar number for some species, and while it had been extrapolated to other groups of insects, subsequent investigations have shown that Dyar's law and Prizbram's rule (Przibram and Megušar, 1912) using a doubling of the weight of the larvae, were not accurate predictors of the instar number and were not generally valid as a means of determining allometric growth. It has been advocated that more investigation on the growth ratio of insects, especially of the hemimetabolous insects, be undertaken using other suitable morphological characteristics to support or disprove Dyar's law.

CONCLUSION

As one of the recently detected invasive alien species, the papaya mealybug, *P. marginatus*, deserves immediate and timely pest management action. In the field survey of resident natural enemies associated with the papaya mealybug, the predaceous coccinellid, *S. quinquepunctatus* was observed as one of the dominating predators preying on the mealybug in almost all locations. Therefore, it was selected for further investigation on its development, life history and growth ratio with a view to exploring its amenability as a biological control agent of the papaya mealybug in Thailand. It could be deduced from this study that *S. quinquepunctatus* might play a role as one of the specialist predators of the papaya mealybug and could be a promising augmentative biological control agent of *P. marginatus* in the field. However, it should be realized that its predatory potential and effectiveness require further evaluation together with its amenability to laboratory mass rearing prior to its utilization in field releases. For most agricultural systems, conservation techniques for predaceous coccinellids are lacking, even though the insect is abundant in such systems. At the same time, in the use of resident natural biological controls of certain insect pests, the phenomena of cannibalism and intraguild predation should be investigated and taken into consideration. These remarks and observations remain applicable and relevant for the augmentative use of *S. quinquepunctatus* for biological control of *P. marginatus* in Thailand.

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