INFLUENCE OF HOST STAGE ON OVIPOSITION, DEVELOPMENT, AND SEX RATIO OF *Anagyrus lopezi* (DE SANTIS) (HYMENOPTERA: ENCYRTIDAE), A PARASITOID OF THE CASSAVA MEALYBUG, *Phenacoccus manihoti* MATILE-FERRERO (HEMIPTERA: PSEUDOCOCCIDAE)

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ABSTRACT

Influence of host stage on oviposition, development, and sex ratio of* Anagyrus lopezi *(De Santis) (Hymenoptera: Encyrtidae), a parasitoid of the cassava mealybug,* Phenacoccus manihoti *Matile-Ferrero (Hemiptera: Pseudococcidae). The parasitoid *Anagyrus lopezi* (De Santis) (Hymenoptera: Encyrtidae) was introduced from Thailand into Indonesia in early 2014 to control the invasive cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae). Because of the need to produce large numbers of high-quality females, research was conducted in the laboratory to determine host stage preference for *A. lopezi* on different instars of *P. manihoti*. Individual female wasps were exposed to first, second, third instar nymphs, and pre-reproductive adult mealybugs. In the no-choice test, the frequency of parasitized hosts and the number of eggs laid per host was significantly higher in second and third instar nymphs as well as adult mealybugs compared to first instar nymphs. In the two-choice test, third instars nymphs and adult mealybugs were the most preferred host for oviposition. Immature development of parasitoids was faster and the ratio of female to male parasitoids was higher following oviposition in second and third instar nymphs and pre-reproductive adult hosts, compared to the first instar nymphs. Our findings indicate that the use of pre-reproductive adults as hosts in a mass-rearing program would be the most productive and fastest way to produce *A. lopezi* populations with a female-biased sex ratio. Field release of parasitoids should be conducted when the host's third instar nymph is the most abundant because the period during which preferred and suitable host stages are available would be the longest.

Key words: *Anagyrus lopezi,* cassava, mealybug, parasitoid, *Phenacoccus manihoti*

INTRODUCTION

The cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae), was an invasive pest native to South America. The pest was accidentally introduced into Africa in the early 1970s (Bellotti *et al*., 2012) and Asia in 2008 (Winotai *et al*., 2010; Parsa *et al*., 2012; Graziosi *et al.,* 2016). In Indonesia, the pest was first detected in Bogor in 2010 (Muniappan *et al*.,2009), and since then has spread throughout the regions of Java and Lampung (Abduchalek *et al*., 2017) and eastern Indonesia (Wyckhuys *et al*., 2018a; Fanani *et al*., 2019). Severe attacks had caused yield losses of up to 80% in Africa (Bellotti *et al.*, 2012) and 40–50% in Asia (Wyckhuys *et al*., 2018b). The presence of *P. manihoti* threatened

cassava production and food security in affected areas (Yonow *et al*., 2017). To control the pest, a solitary endoparasitoid, *Anagyrus lopezi* (De Santis) (Hymenoptera: Encyrtidae), was introduced from Brazil to Africa in 1981 (Bellotti *et al*., 2012). The parasitoid was introduced from Benin to Thailand in 2008 (Winotai *et al*., 2010), and subsequently from Thailand to Indonesia in 2014 (Wyckhuys *et al*., 2014).

The degree to which parasitoids exploit host insect populations depends largely on the availability of the host stages that were suitable for the parasitoid development (Stacconi *et al*., 2015; Khakasa *et al*., 2016; Li *et al*., 2017; Schoeller & Redak, 2018). Because not all stages of the host life cycle were equally suitable and the relative abundance of the most suitable stages varies both spatially and temporally, parasitoids had evolved

an array of behavioral, ecological, and physiological adaptations to discriminate and utilize their hosts (Vinson & Iwantsch, 1980; Harvey & Malcicka, 2016). Solitary parasitoids generally determine the host quality by the size of the host. Large hosts were supposed to be better quality, as they were believed to contain more resources than small ones. However, host size might not always be equated to host quality at the time of oviposition by a parasitoid. The influence of host size on parasitoid development might differ between idiobiont and koinobiont parasitoids (Waage, 1986; Harvey & Malcicka, 2016). Idiobiont parasitoids paralyzed their hosts before oviposition, thereby fixing the host larval resources. On the other hand, koinobiont parasitoids, which did not paralyze their hosts at the time of parasitism, allowed hosts to grow, so host size was not directly representative of larval resources.

Knowledge of host selection was indispensable for the efficient use of parasitoids, both for mass rearing and biological control of pests (Zhang *et al*., 2016). The parasitoid's biology were greatly influenced by the quality of the host (Monticelli *et al*., 2019). Host stage was an important ecological variable, which may influence a parasitoid's rate of attack, survival of its immature stages, and sex ratio of its offspring (Waage, 1986). Host selection behavior was most important in determining the sex ratio of arrhenotokous parasitoids, which showed a haplodiploid sex determination mechanism (King, 1987). A female parasitoid could manipulate the offspring sex ratio at oviposition by regulating fertilization. A particular host size may be more suitable for the development of one sex. In general, a female-biased off spring sex ratio was produced from the largest hosts and a male-biased one from the smaller hosts (King, 1987). Host stage often had a significant impact on the development, survival, and reproduction of the foraging parasitoid (Zhang *et al*., 2016). The choice of host stage was an important determinant for progeny fitness in parasitoids (Ueno, 2015; Harvey & Malcicka, 2016; Mayhew, 2016). Generally, parasitoid fitness was positively correlated with host size especially for females (Charnov *et al*., 1981).

To gain a better understanding of the parasitoid-host interaction between *A. lopezi* and *P. manihoti,* examination of various aspects of the parasitoids host selection behavior were needed. Among host-parasitoid interactions that need to be understood were those concerning host selection and host suitability. Therefore, the present study was conducted to determine which host stages were susceptible to parasitism (oviposition), preferred for oviposition by female parasitoids, and suitable for development, production, and sex ratio of the parasitoid.

MATERIALS AND METHODS

Research Site. The study were conducted at the Insect Bionomy and Ecology Laboratory, Department of Plant Protection, Faculty of Agriculture, IPB University, Bogor, Indonesia from August 2014 to August 2015. The temperature, relative humidity, and light intensity in the laboratory were maintained at 27 °C, 60%, and 12 hours dark-light cycle, respectively.

Rearing of Mealybug and Parasitoid. First instars of *P. manihoti* were collected from an insectary culture and placed into 3-week old cassava cuttings. After two weeks, the mealybugs had molted to third instar. Some of the third instar mealybugs were allowed to become adults and reproduce, while the rest were used for rearing parasitoids and experiments. Each cassava cutting with third instars was placed in a 12.5 × 9 cm plastic container filled with water. The container was propped up with styrofoam to make the cassava cutting stand upright. The containers then placed inside a wooden cage measuring 50 × 45 × 45 cm, with a door to put cassava cuttings inside. About 50 pairs of *A. lopezi* wasps were introduced into the cage and a cotton ball soaked with 10% honey solution was hung on the roof of the cage, as a food source for adult parasitoids. After about two weeks, parasitized mealybugs (mummies) on cassava leaves were collected and put into plastic containers for adult emergence. The emerged parasitoids were used for experiments and mass rearing. A pair of newly emerged male and female wasps were kept in a glass tube for two days and fed with a drop of 10% honey deposited on the wall. The mated females were used for host stage susceptibility and suitability experiments.

Preparing the Mealybug Instars. The research was done by transfering the first instar nymphs of *P. manihoti* from the insectary culture into a 3-weeks old cassava cutting which then placed into a transparent plastic cylinder cage (h= 47 cm; d= 14 cm) with the top covered with organdy cloth. After five days, the first instar nymphs had molted to the second instars and were ready to be used for experiments. Similarly, third instar nymphs and pre-reproductive adults were obtained by allowing the first instar nymphs to developed for 10 and 15 days, respectively.

Host Stage Susceptibility and Preference. In the host stage susceptibility test (no-choice test), a batch of each stage of *P. manihoti* was separately exposed to a female parasitoid. For that purpose, ten mealybugs of each stage (first instar, second instar, third instar, pre-reproductive adult) were infested separately on a cassava leaf. The leaf was then placed into a cage made from a transparent plastic cylinder (h= 7 cm; d= 10 cm) with the top covered with organdy cloth. A 2-days old mated female *A. lopezi* then was introduced into the cage and fed with a drop of 10% honey deposited on the wall. Parasitoids were allowed to forage and oviposit for 24 h. After 24 h, the adult parasitoids were removed. Each mealybug was immediately dissected in a drop of Scott's saline solution (1% NaCl), and the number of eggs in each host was counted and recorded. After dissections, mealybugs were mounted on microscope slides and examined under a compound microscope to ensure that all of the eggs were counted. The number of hosts parasitized per parasitoid, the total number of eggs laid per replicate, and the number of eggs per host parasitized were used as the criteria for determining host stage susceptibility. Experiments were carried out using 20 batches of each mealybug stage as replicates.

In the host stage preference test (two-choice test), the experimental procedures were similar to those in the host stage susceptibility test, except that two combinations of host stages were exposed to a female parasitoid. The following combinations of cassava mealybugs were used in the two-choice tests: first instar vs second instar, first instar vs third instar, first instar vs adult, second instar vs third instar, second instar vs adult, and third instar vs adult. For that purpose, 10 mealybugs of each stage (first instar, second instar, third instar, pre-reproductive adult) were infested separately on a cassava leaf. The leaf was then placed into a cage made from a transparent plastic cylinder (h= 7 cm; d= 10 cm) with the top covered with organdy cloth. A 2-days old mated female *A. lopezi* then was introduced into the cage and fed with a drop of 10% honey deposited on the wall. The parasitoids were allowed to forage and oviposit for 24 h. After 24 h, the adult parasitoids were removed. Each mealybug immediately dissected in a drop of Scott's saline solution, and the number of eggs in each host was recorded. After dissections, mealybugs were mounted on microscope slides and examined under a compound microscope to ensure that all of the eggs were counted. The number of hosts parasitized per parasitoid, the total number of eggs laid per replicate, and the number of eggs per host parasitized were used

as the criteria for determining host stage preference. Experiments were carried out on 20 batches of each mealybug stage as replicates.

Host Stage Suitability. Twenty mealybugs of each stage (first, second, and third instar, as well as pre-reproductive adult) were transferred onto a sprouted potato and placed into a cage made from a transparent plastic cylinder (h= 20 cm; d= 12 cm) with the top covered with organdy cloth. Two days old mated parasitoid females were introduced into the cage for 24 h. Mealybugs were then allowed to develop on sprouted potatoes. Mummified mealybugs were collected and stored in petri dishes for parasitoid emergence. Parasitoids were collected and sexed and the size was estimated based on the length of the body (base of the head to the tip of the abdomen) and length of left hind tibia. The criteria used to determine suitability were the number of emerged parasitoids per replicate, the sex ratio, the immature developmental time, and the size of emerged parasitoids.

Data Analysis. Analysis of variance (ANOVA) was carried out to examine the effects of different host stages on the number of hosts parasitized, the number of eggs laid per wasp, and the number of eggs laid per host in the no-choice test. ANOVA was also performed to compare immature developmental time, number, and size of emerged parasitoids in the host suitability test. The means were then separated using the Tukey test at a 5% of significance level. In the two-choice test, differences in the average number of hosts parasitized, the number of eggs laid per wasp, and the number of eggs laid per host was examined by t-test. The sex ratio of parasitoid progeny was analyzed with the chi-square test to determine its fitness to the theoretical sex ratio of 1:1. All analyses were done using SPSS 16.0.

RESULTS AND DISCUSSION

Host Stage Susceptibility and Preference. In the no-choice test (host stage susceptibility test), when each stage was exposed separately, all four of the *P. manihoti* stages were susceptible to parasitism by *A. lopezi* (Table 1). However, it was significantly higher on second and third instar nymph and adult female hosts ($F_{3,79}$= 50.63; P< 0.001) than the first instar nymph. Parasitization of second and third instar nymph and pre-reproductive adult female hosts was not significantly different from each other, with the mean number of parasitized hosts were 7.15, 7.05, and 8.15, respectively. The least parasitized

stage was first instar nymph, 3.25 hosts parasitized, and that stage was significantly different from all other stages.

The number of eggs oviposited was also significantly affected by host stages ($F_{3,79}= 18.79$; $P< 0.001$). In the first instar, the total number of oviposited eggs per wasp was 3.70, it was lower than those in other stages (9.95–10.60). The number of eggs deposited per parasitized hosts was also significantly ($F_{3,84}= 3.20$; $P= 0.028$) lower in the first instar (1.09) than those in the third instar (1.57) and the adult female (1.55).

In the two-choice test (preference test), when offered a choice between two stages of *P. manihoti*, parasitoid females showed a significant preference for second and third instar nymphs and adult females compared to the first instar nymphs (Table 2). The number of hosts parasitized on first instar nymphs were significantly lower than those on second instar ($t= 9.98$; $P< 0.001$), third instar ($t= 10.29$; $P< 0.001$), and adult ($t= 14.11$; $P< 0.001$).

Likewise, the average number of eggs laid in the first instar nymphs was lower compared to second instar ($t= 8.13$; $P< 0.001$), third instar ($t= 9.12$; $P< 0.001$), and adult ($t= 10.14$; $P< 0.001$). In the first instar nymphs, the average number of eggs oviposited per parasitized host was also significantly lower compared to second instar ($t= 3.37$; $P= 0.002$) and adult ($t= 4.43$; $P< 0.001$), but barely significant compared to third instar ($t= 1.78$; $P= 0.083$). Third instar nymph was more preferred than second instar by the parasitoid, in term of number of hosts parasitized ($t= 5.19$; $P< 0.001$), number of parasitoid eggs laid ($t= 5.11$; $P< 0.001$), and number of eggs oviposited per parasitized host ($t= 2.75$; $P= 0.009$). Meanwhile, the average number of parasitized hosts, the total number of parasitoid eggs laid, and the number of parasitoid eggs per parasitized host did not differ significantly ($P> 0.05$) between the adult host with each second instar and third instar nymphs, with the exception of the number of parasitoid eggs oviposited between adult and second instar ($t= 3.07$; $P= 0.004$).

Overall, the results of no-choice and two-choice tests indicated that the first instar nymph was the least preferred host by the parasitoids for oviposition. This might due to (1) the smaller host stages were being less frequently encountered by the parasitoids, (2) the small

Table 1. Oviposition of *Anagyrus lopezi* (De Santis) on four stages of *Phenacoccus manihoti* Matile-Ferrero

Mealybug stages	Mean ($\bar{x} \pm$ SE) number of hosts parasitized	Mean ($\bar{x} \pm$ SE) number of parasitoid eggs	Mean ($\bar{x} \pm$ SE) number of parasitoid eggs per parasitized host
First instar	3.25 ± 0.43 a*	3.70 ± 0.51 a	1.09 ± 0.08 a
Second instar	7.15 ± 0.23 b	9.95 ± 0.63 b	1.39 ± 0.07 ab
Third instar	7.05 ± 0.25 b	10.60 ± 0.89 b	1.57 ± 0.22 b
Adult female	8.15 ± 0.25 b	9.95 ± 0.63 b	1.55 ± 0.09 b

*Mean in a column with the same letters are not significantly different at $p \leq 0.05$.

Table 2. Oviposition of *Anagyrus lopezi* (De Santis) on pairs of two stages of *Phenacoccus manihoti* (Matile Ferrero)

Pair of mealybug stages	Mean ($\bar{x} \pm$ SE) number of hosts parasitized	Mean ($\bar{x} \pm$ SE) number of parasitoid eggs	Mean ($\bar{x} \pm$ SE) number of parasitoid eggs per parasitized host
First instar	2.30 ± 0.42 a*	2.45 ± 0.47 a	0.83 ± 0.09 a
Second instar	7.55 ± 0.31 b	9.40 ± 0.72 b	1.23 ± 0.06 b
First instar	3.45 ± 0.39 a	3.70 ± 0.41 a	0.99 ± 0.11 a
Third instar	8.05 ± 0.22 b	9.70 ± 0.51 b	1.20 ± 0.05 a
First instar	0.90 ± 0.22 a	0.95 ± 0.22 a	0.75 ± 0.12 a
Adult	7.40 ± 0.41 b	10.40 ± 0.90 b	1.39 ± 0.22 b
Second instar	4.85 ± 0.39 a	5.20 ± 0.47 a	1.05 ± 0.02 a
Third instar	7.45 ± 0.32 b	9.10 ± 0.60 b	1.21 ± 0.05 b
Second instar	6.35 ± 0.35 a	7.60 ± 0.59 a	1.19 ± 0.05 a
Adult	7.05 ± 0.40 a	10.95 ± 0.92 b	1.64 ± 0.27 a
Third instar	6.30 ± 0.37 a	7.40 ± 0.43 a	1.15 ± 0.04 a
Adult	6.90 ± 0.42 a	8.40 ± 0.82 a	1.18 ± 0.06 a

*Pairs of means in a column with the same letters are not significantly different at $p \leq 0.05$.

body size could be difficult for oviposition, and (3) the small body size was being identified by the wasps as being insufficient to support the development of parasitoid larvae (Islam & Copland, 1997; Pacheco da Silva *et al.*, 2017). On the other hand, the large host stages, especially third instar nymphs and adult females, had more eggs. This was in accordance with Chong & Oetting (2006) who found that the parasitoid *Anagyrus* spec. nov near *sinope* Noyes & Menezes preferred third instar nymph and adult female of *P. madeirensis* (Green) for oviposition. Bertschy *et al.* (2000) also reported that the parasitization of *Aenasius vexans* (Kerrich) attacking *P. herreni* (Cox & Williams) was higher in the third instar nymphs and adult female hosts. Similar results were reported form *A. kamali* (Moursi) that were attacking *Maconellicoccus hirsutus* (Green) (Sagarra & Vincent, 1999), *A. bambawalei* (Hayat) that were attacking *P. solenopsis* (Tinsley) (Fand *et al.*, 2011; Zain-ul-Abdin *et al.*, 2012; Vijaya & Ram, 2013; Badshah *et al.*, 2016; Shahzad *et al.*, 2016), and *Blepyrus clavicornis* (Compere) that were attacking *Pseudococcus viburni* (Signoret) (Pacheco da Silva *et al.*, 2017). By choosing a large host, the parasitoids gained access to more food resources so that they could improve the progeny fitness (Sarkar *et al.*, 2015; Ueno, 2015).

Our observations of the number of parasitoid eggs laid per parasitized host, both in the no-choice test and two-choice test, indicated the occurrence of superparasitism. This could be examined from the average number of eggs laid per parasitized host with a value of > 1.0. In the two-choice test, superparasitism only occured in second and third instar nymphs and adults. Superparasitism was common in Encyrtidae parasitizing mealybugs (Noyes & Hayat, 1994). It had been suggested that superparasitism might be used by the parasitoids as a strategy for overcoming host immune response (encapsulation) (Blumberg, 1997; Luna *et al.*, 2016). The presence of more eggs per host increased the probability that one of these will survive and emerge from the host (Godfray, 1994).

Host Stage Suitability. The parasitoid *A. lopezi* developed and emerged successfully from all parasitized host stages. However, host stages significantly affected immature development time (egg to adult) of males ($F_{3, 107}= 17.49$; $P< 0.001$) and females ($F_{3, 94}= 41.66$; $P< 0.001$). The immature developmental time of *A. lopezi* was longer when oviposition occurred in the first instar nymphs (Table 3). Immature developmental time of males and females emerging from third instars was not significantly different from those of the second instars. Immature developmental time was the shortest when oviposition occurred in the adult host stages. The older the host that was attacked, the faster a wasp would develop. Vankosky & Hoddle (2019) found that larval development and adult longevity of *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae), a parasitoid of *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), were positively correlated to female oviposition preference.

The results of our research were similar to those of Sagarra & Vincent (1999) who reported that *A. kamali*, parasitoid of *M. hirsutus*, required a shorter time to develop from egg to adult eclosion from parasitized adult and third instar than parasitized first and second instar nymphs. Conversely, the immature developmental time of parasitoid took a longer time to develop in a smaller host (first instar). Similar results were reported for other encyrtids that parasitize mealybugs; such as *A. indicus* attacking *Nipaecoccus vastator* (Mask.) (Nechols & Kikuchi, 1985), *A. vexans* attacking *P. herreni* (Bertschy *et al.*, 2000), *Leptomastix epona* (Walker) and *Pseudaphycus flavidulus* (Brethes) De Santis attacking *P. viburni* (Karamaouna & Copland, 2009). Prolonging the development of the parasitoid *A. lopezi* in its earlier host instars was also reported by other researchers

Table 3. Development period of *Anagyrus lopezi* (De Santis) in various host stages

Mealybug stages	Mean ($\bar{x} \pm$ SE) number of days for parasitoid development (egg to adult emergence)	
	Male	Female
First instar	24.50 ± 0.96 a*	32.00 ± 1.00 a
Second instar	19.09 ± 0.65 b	21.27 ± 0.57 b
Third instar	18.08 ± 0.64 bc	19.50 ± 0.85 b
Adult	16.19 ± 0.16 ac	15.90 ± 0.13 c

*Mean in a column with the same letters are not significantly different at p≤0.05.

(Kraaijeveld & van Alphen, 1986). The delayed development of the parasitoid in the first instar *P. manihoti* may be due to a lack of food or a delayed development in a koinobiont life cycle as also reported in *A. pseudococci* (Girault) attacking *Planococcus citri* (Risso) (Islam & Copland, 1997). The developing of *A. lopezi* slowed the development of its hosts. The earlier stages of hosts had less nutrient supply for the parasitoid at the time of parasitization and a slowing down of the host development after parasitization might delay the development of the parasitoid in early instar hosts (Islam & Copland, 1997). The pooled immature developmental time of male and female were 19.5 and 22.2 days, respectively. The shorter developmental times of males than females was also reported to occur in other species, such as *A. kamali* in *M. hirsutus* (Sagarra & Vincent, 1999) and *A. indicus* in *N. vastator* (Nechols & Kikuchi, 1985).

The parasitoid emergence was significantly ($F_{3,1} = 12.13$; $P < 0.001$) higher for second instar (12.8), third instar (12.4), and adults (11.4) compared to first instar nymphs (4.0) (Table 4). This might be related to the behavior of parasitoid female which prefer to lay eggs in second and third instar nymphs and adults than in the first instar as mentioned previously. Besides, the mortality of parasitoid larvae was reported to be higher in the first instar nymph (Kraaijeveld & van Alphen, 1986). Sarkar *et al*. (2015) also reported that a much lower emergence of *Allotropa suasaardi* (Sarkar & Polaszek) when parasitizing first instar nymphs of *P. manihoti* compared to other instars.

The host stages also affected the sex ratio of the parasitoid progenies. Mealybugs in the first instar nymph yielded a considerably higher proportion (0.90) of *A. lopezi* males, significantly different ($\chi^2 = 12.8$; $P = 0.0003$) from a theoretical sex ratio of 1:1. The proportion of male parasitoids decreased with the increasing host size (stages). *P. manihoti* parasitized as second or third instar nymphs produced almost equal proportion (0.53 and 0.65), but adult females produced-female-biased off spring with a proportion of male of

0.29. The decreasing proportion of males with increasing host stage had been found in other mealybug parasitoids. Kraaijeveld & van Alphen (1986) reported that second instar *P. manihoti* produced no female *A. lopezi* parasitoids. Islam & Copland (1997) reported that *A. pseudococci* parasitizing second instar nymphs of *P. citri* yielded 91% males, whereas parasitized adults yielded 33% males. Sagarra & Vincent (1999) found that 97% of males in *A. kamali* when oviposited on the first instar of *M. hirsutus* and 39% of those oviposited on the third instar. Nechols & Kikuchi (1985) also reported that the first and second instar nymphs of *N. vastator* yielded mostly *A. indicus* males, while the third instar nymph and adult yielded mostly females. A similar result was reported for *A. suasaadi* attacking *P. manihoti* (Sarkar *et al*., 2015), and *A. arizonensis* (Girault) attacking *P. solenopsis* (Karmakar & Shera, 2018b).

The varied sex ratio of *A. lopezi* with different host stages reflects a similar preference for host size and active sex allocation (Islam & Copland, 1997). Host selection behavior was most important in determining the sex ratio of arrhenotokous parasitoids. A female parasitoid can adjust the sex ratio of her offspring by controlling fertilization during oviposition (King, 1987). Parasitoid females tended to lay only unfertilized eggs (which develop into a male) in smaller sized hosts, and fertilized eggs (develop into a female) in larger sized hosts (Godfray, 1994; King, 1987). This host-stage dependent sex ratio corroborates Charnov's model (Charnov *et al*., 1981), which postulated that parasitoid preferentially lay female eggs in the most suitable hosts. Consequently, the parasitoid sex ratio was influenced by the availability of hosts of different sizes (Bertschy *et al*., 2000). Our results were based on the sex ratio at emergence, which might not correspond to the sex allocation at oviposition (Bertschy *et al*., 2000). Such sex ratio might be influenced by differential mortality of the sexes during larval development. One cause of mortality was the encapsulation of the parasitoid inside the host. It had been reported that hosts have a better

Table 4. Number and sex ratio of parasitoid *Anagyrus lopezi* (De Santis) emerging from four stages of *Phenacoccus manihoti* Matile-Ferrero

Mealybug stages	Mean ($\bar{x} \pm$ SE) number of adult parasitoids emerged	Sex ratio (males/total progeny)
First instar	4.00 ± 1.22 a*	0.90
Second instar	12.80 ± 1.20 b	0.53
Third instar	12.40 ± 0.60 b	0.65
Adult female	11.40 ± 1.54 b	0.29

*Mean in a column with the same letters are not significantly different at $p \leq 0.05$.

encapsulation ability at a later stage in their development (Blumberg, 1997). The encapsulation rate of *A. lopezi* by adult hosts was quite low (8.4%) (Adriani *et al.*, 2016), and was unlikely to have a significant effect on sex ratio. Differential mortality for parasitoid males and females also occured in cases of superparasitism (King, 1987). Odebiyi & Bokonon-Ganta (1986) suggested that a high proportion of females in *A. lopezi* populations was a result of competition between parasitoid larvae in the host, with the female larvae as the winners.

The host stages affected significantly the length of body ($F_{3, 94}$= 6.00; P= 0.001) and left tibia ($F_{3, 94}$= 7.73; P< 0.001) of the parasitoids. The body length of female parasitoids emerging from hosts of the first instar was 1.30 mm, much shorter than those parasitoids emerging from hosts of third instar nymph (1.89 mm) and adult (1.79 mm) (Table 5). Similarly, the length of the tibia of female parasitoids emerging from hosts of the first instar (0.35 mm) was shorter than parasitoids emerging from third instar hosts (0.52 mm). Female parasitoids emerging from hosts of the third instar were significantly larger than parasitoids emerging from other host stages, including adult hosts as also reported for *Leptomastidea abnormis* (Girault) attacking *P. citri* (Cadée & van Alphen, 1997) and *A. vexans* (Hymenoptera: Encyrtidae) attacking *P. herreni* (Bertschy *et al.*, 2000). Karmakar & Shera (2018a) reported that parasitism of third instar and adult *P. solenopsis* yielded larger sized *A. arizonensis* adults in the progeny. In general, large parasitoids emerge from large hosts (Ueno, 2015).

Cadée & van Alphen (1997) suggested that parasitoids that emerged from adult mealybugs were lighter because resources that the adults used for egg production were not available to the parasitoid larvae. The third instar nymphs provided the parasitoids with superior resources, resulting in larger size wasps. The size of adult parasitoids was usually correlated with fitness, especially in females; larger females lay more eggs over their lifetime (Charnov *et al.*, 1981; van Dijken & van Alphen, 1991). Therefore, third instar nymphs of

mealybugs were likely to be the most suitable for *A. lopezi*. However, developmental time was most favorable in adult hosts; males and females both developed fastest in adult hosts. Similar results were reported for *A. vexans* attacking *P. hereni* (Bertschy *et al.*, 2000) and *A. bambawalei* attacking *P. solenopsis* (Vijaya & Ram, 2013).

Implication for Biological Control. Although the population of *P. manihoti* had successfully been controlled by the parasitoid *A. lopezi* (Le *et al.*, 2018; Thancharoen *et al.*, 2018; Wyckhuys *et al.*, 2018a; Wyckhuys *et al.*, 2018b) their presence in the newly infested region posed a potential threat to food security (Yonow *et al.*, 2017). For example, *P. manihoti* had been spreading into the islands of Timor and Flores, but *A. lopezi* had not yet been found in these islands (Wyckhuys *et al.*, 2018a; Fanani *et al.*, 2019). Therefore, it would be necessary to introduce the parasitoids into the newly infested regions. In this context, the results of this study provided direct applications for mass rearing and field release of the parasitoids. *Anagyrus lopezi* prefered to oviposit in the third instar nymphs and pre-reproductive adults of *P. manihoti*, and these two host stages yielded more progeny with a relatively higher proportion of females. Therefore, third instar nymphs and pre-reproductive adults would be the most suitable host stages for parasitoid mass rearing. In addition, parasitoids emerging from third instar nymphs and pre-reproductive adults were larger in size. The size of female parasitoids was positively correlated with the level of fitness (longevity and reproduction rate) and dispersal capacity (Charnov *et al.*, 1981; van Dijken & van Alphen, 1991). Releasing high fitness parasitoids would increase the effectiveness of the biological control program.

CONCLUSION

Anagyrus lopezi prefers to parasitize third instar nymphs and pre-reproductive adults of *P. manihoti*. In

Table 5. Size of female parasitoid *Anagyrus lopezi* (De Santis) emerged from various mealybug stages

Mealybug stages	Mean ($\bar{x} \pm$ SE) length (mm)		n
	Body	Left tibia	
First instar	1.30 ± 0.00a*	0.35 ± 0.05a	2
Second instar	1.65 ± 0.05ab	0.47 ± 0.01ab	30
Third instar	1.89 ± 0.07c	0.52 ± 0.01c	22
Adult	1.79 ± 0.03b	0.45 ± 0.01ab	41

*Mean in a column with the same letters are not significantly different at p ≤0.05.

the context of a mass rearing program, the use of pre-reproductive adults as hosts would be the most productive and fastest way to produce *A. lopezi* populations with a female-biased sex ratio. Field release of parasitoids should be made when the host's third instar nymph was the most abundant because the period during which preferred and suitable host stages were available would be the longest.

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REFERENCES

Abduchalek B, Rauf A, & Pudjianto. 2017. Kutu putih singkong, *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae): persebaran geografi di Pulau Jawa dan rintisan pengendalian hayati. *J. HPT Tropika*. 17(1): 1–8.

Adriani E, Rauf A, & Pudjianto. 2016. Laju enkapsulasi parasitoid *Anagyrus lopezi* (De Santis) (Hymenoptera: Encyrtidae) oleh kutu putih singkong *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae). *JEI.* 13(3): 147–155.

Badshah H, Ullah F, Calatayud PA, & Crickmore N. 2016. Host stage preference and parasitism behaviour of *Aenasius bambawalei* an encyrtid parasitoid of *Phenacoccus solenopsis*. *Biocontrol Sci. Technol*. 26(12): 1605–1616.

Bellotti A, Campo BVH, & Hyman G. 2012. Cassava production and pest management: Present and potential threats in a changing environment. *Trop. Plant Biol*. 5(1): 39–72.

Bertschy C, Turlings TCJ, Bellotti A, & Dorn S. 2000. Host stage preference and sex allocation in *Aenasius vexans*, an encyrtid parasitoid of the cassava mealybug. *Entomol. Exp. Appl*. 95(3): 283–291.

Blumberg D. 1997. Parasitoid encapsulation as a defense mechanism in the Coccoidea (Homoptera) and its importance in biological control. *Biol. Control*. 8(3): 225–236.

Cadée N & van Alphen JJM. 1997. Host selection and sex allocation in *Leptomastidea abnormis,* a parasitoid of the citrus mealybug *Planococcus citri*. *Entomol. Exp. Appl*. 83(3): 277–284.

Charnov EL, Los-den-Hartogh RL, Jones WT, & van den Assem J. 1981. Sex ratio evolution in a variable environment. *Nature*. 289(5793): 27–33.

Chong JH & Oetting RD. 2006. Host stage selection of the mealybug parasitoid *Anagyrus* spec. nov near *sinope*. *Entomol. Exp. Appl*. 121(1): 39–50.

Fanani MZ, Rauf A, Maryana N, Nurmansyah A, & Hindayana D. 2019. Geographic distribution of the invasive mealybug *Phenacoccus manihoti* and its introduced parasitoid *Anagyrus lopezi* in parts of Indonesia. *Biodiversitas*. 20(12): 3751–3757.

Fand BB, Gautam RD, & Suroshe SS. 2011. Suitability of various stages of mealybug, *Phenacoccus solenopsis* (Homoptera: Pseudococcidae) for development and survival of the solitary endoparasitoid, *Aenasius bambawalei* (Hymenoptera: Encyrtidae). *Biocontrol Sci. Technol*. 21(1): 51–55.

Godfray HCJ. 1994. *Parasitoids: Behavioral and Evolutionary Ecology*. Princeton University Press, New Jersey.

Graziosi I, Minato N, Alvarez E, Ngo DT, Hoat TX, Aye TM, Pardo JM, Wongtiem P, & Wyckhuys KAG. 2016. Emerging pests and diseases of South-east Asia cassava: a comprehensive evaluation of geographic priorities, management options and research needs. *Pest. Manag. Sci.* 72(6): 1071–1089.

Harvey JA & Malcicka M. 2016. Nutritional integration between insect hosts and koinobiont parasitoids in an evolutionary framework. *Entomol. Exp. Appl.* 159(2): 181–188.

Islam KS & Copland MJW. 1997. Host preference and progeny sex ratio in a solitary koinobiont mealybug endoparasitoid, *Anagyrus pseudococci* (Girault), in response to its host stage. *Biocontrol Sci. Technol.* 7(3): 449–456.

Karamaouna F & Copland M. 2009. Fitness and life history parameters of *Leptomastix epona* and *Pseudaphycus flavidulus*, two parasitoids of the obscure mealybug *Pseudococcus viburni*. *BioControl*. 54(1): 65–76.

Karmakar P & Shera PS. 2018a. Does host stage affect the morphometry of *Aenasius arizonensis* (Girault), a solitary endoparasitoid of *Phenacoccus solenopsis* Tinsley. *J. Entomol. Zool. Stud*. 6(6): 537–543.

Karmakar P & Shera PS. 2018b. Seasonal and biological interactions between the parasitoid, *Aenasius arizonensis* (Girault) and its hosts, *Phenacoccus solenopsis* Tinsley on cotton. *Phytoparasitica*. 46(5): 661–670.

Khakasa SWR, Mohamed SA, Lagat ZO, Khamis FM, & Tanga CM. 2016. Host stage preference and performance of the aphid parasitoid *Diaeretiella rapae* (Hymenoptera: Braconidae) on *Brevicoryne brassicae* and *Lipaphis pseudobrassicae* (Hemiptera: Aphididae). *Int. J. Trop. Insect Sci*. 6(1): 10–21.

King BH. 1987. Offspring sex ratios in parasitoid wasps. *Q. Rev. Biol*. 62(4): 367–396.

Kraaijeveld AR & van Alphen JJM. 1986. Host-stage selection and sex allocation by *Epidinocarsis lopezi* (Hymenoptera; Encyrtidae), a parasitoid of the cassava mealybug, *Phenacoccus manihoti* (Homoptera: Pseudococcidae). *Meded. Fac. Landbouwwet. Rijksuniv. Gent*. 51: 1067–1078.

Le TTN, Graziosi I, Cira TM, Gates MW, Parker L, & Wyckhuys KAG. 2018. Landscape context does not constrain biological control of *Phenacoccus manihoti* in intensified cassava systems of southern Vietnam. *Biol. Control*. 121: 129–139.

Li X, Zhu L, Meng L, & Li B. 2017. Brood size and sex ratio in response to host quality and wasp traits in the gregarious parasitoid *Oomyzus sokolowskii* (Hymenoptera: Eulophidae). *PeerJ*. 5: e2919.

Luna MG, Desneux N, & Schneider MI. 2016. Encapsulation and self-superparasitism of *Pseudapanteles dignus* (Muesebeck) (Hymenoptera: Braconidae), a parasitoid of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *PLoS ONE*. 11(10): e0163196.

Mayhew PJ. 2016. Comparing parasitoid life histories. *Entomol. Exp. Appl*. 159(2): 147–162.

Monticelli LS, Nguyen LTH, Amiens-Desneux E, Luo C, Lavoir AV, Gatti JL, & Desneux N. 2019. The preference-performance relationship as a means of classifying parasitoids according to their specialization degree. *Evol. Appl*. 12(8): 1626–1640.

Muniappan R, Shepard BM, Watson GW, Carner GR, Rauf A, Sartiami D, Hidayat P, Afun JVK, Goergen G, & Rahman AKMZ. 2009. New records of invasive insects (Hemiptera: Sternorrhyncha) in Southern Asia and West Africa. *J. Agric. Urban Entomol*. 26(4): 167–174.

Nechols JR & Kikuchi RS. 1985. Host selection of the spherical mealybug (Homoptera: Pseudococcidae) by *Anagyrus indicus* (Hymenoptera: Encyrtidae): Influence of host stage on parasitoid oviposition, development, sex ratio, and survival. *Environ. Entomol*. 14(1): 32–37.

Noyes JS & Hayat M. 1994. *Oriental Mealybug Parasitoids of the Anagyrini (Hymenoptera: Encyrtidae)*. CABI, London.

Pacheco da Silva VC, Garcia M, & Botton M. 2017. Biology of *Blepyrus clavicornis* (Compere) (Hymenoptera: Encyrtidae), a parasitoid of *Pseudococcus viburni* (Signoret) (Hemiptera: Pseudococcidae). *Rev. Bras. Entomol*. 61(3): 257–261.

Parsa S, Kondo T, & Winotai A. 2012. The cassava mealybug (*Phenacoccus manihoti*) in Asia: first records, potential distribution, and an identification key. *PLoS ONE* 7(10): e47675.

Odebiyi JA & Bokonon-Ganta AH. 1986. Biology of *Epidinocarsis* [=Apoanagyrus] *lopezi* [Hymenoptera : Encyrtidae] an exotic parasite cassava mealybug, *Phenacoccus manihoti* [Homoptera : Pseudococcidae] in Nigeria. *Entomophaga*. 31(3): 251–260.

Sagarra LA & Vincent C. 1999. Influence of host stage on oviposition, development, sex ratio, and survival of *Anagyrus kamali* Moursi (Hymenoptera: Encyrtidae), a parasitoid of the Hibiscus mealybug, *Maconellicoccus hirsutus* Green (Homoptera: Pseudococcidae). *Biol. Control*. 15(1): 51–56.

Sarkar MA, Suasa-ard W, & Uraichuen S. 2015. Host stage preference and suitability of *Allotropa suasaardi* Sarkar & Polaszek (Hymenoptera: Platygasteridae), a newly identified parasitoid of pink cassava mealybug, *Phenacoccus manihoti* (Homoptera: Pseudococcidae). *Songklanakarin J. Sci. Techno*. 37(4): 381–387.

Schoeller EN & Redak RA. 2018. Host stage preference of *Encarsia noyesi, Idioporus affinis*, and *Entedononecremnus krauteri*: parasitoids of the giant whitefly *Aleurodicus dugesii* (Hemiptera: Aleyrodidae). *Environ. Entomol.* 47(6): 1493–1500.

Shahzad MQ, Abdin ZU, Abbas SK, Tahir M, & Hussain F. 2016. Parasitic effects of solitary endoparasitoid, *Aenasius bambawalei* Hayat (Hymenoptera: Encyrtidae) on cotton mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae). *Adv. Entomol.* 4(2): 90–96.

Stacconi MVR, Buffington M, Daane KM, Dalton DT, Grassi A, Kaçarc G, Miller B, Miller JC, Baser N, Ioriatti C, Walton VM, Wiman NG, Wang X, & Anfora G. 2015. Host stage preference, efficacy and fecundity of parasitoids attacking *Drosophila suzukii* in newly invaded areas. *Biol. Control* 84: 28–35.

Thancharoen A, Lankaew S, Moonjuntha P, Wongphanuwat T, Sangtongpraow B, Ngoenklan R, Kittipadakul P, & Wykhuys KAG. 2018. Effective biological control of an invasive mealybug pest enhances root yield in cassava. *J. Pest Sci.* 91(4): 1199–1211.

Ueno T. 2015. Effects of host size and laboratory rearing on offspring development and sex ratio in the solitary parasitoid *Agrothereutes lanceolatus* (Hymanoptera: Ichneumonidae). *Eur. J. Entomol.* 112(2): 281–287.

van Dijken JJ & van Alphen JJM. 1991. Sex allocation in *Epidinocarsis lopezi*: the influence host-size distribution and its effect on the population sex ratio in cassava fields in Africa. *Redia.* 74(3, Appendix): 195–201.

Vankosky MA & Hoddle MS. 2019. Two parasitoids of *Diaphorina citri* (Hemiptera: Liviidae) have shared, stage-specific preference for host nymphs that does not impact pest mortality rates. *Fla. Entomol.* 102(1): 49–58.

Winotai A, Goergen G, Tamò M, & Neuenschwander P. 2010. Cassava mealybug has reached Asia. *Biocontrol news and information.* 31(2): 10N–11N.

Wyckhuys KAG, Rauf A, & Ketelaar J. 2014. Parasitoid introduced into Indonesia: part of a region-wide campaign to tackle emerging cassava pests and diseases. *Biocontrol news and information.* 35(4): 35–37.

Wyckhuys KAG, Wongtiem P, Rauf, A, Thancharoen A, Heimpel GE, Le NTT, Fanani MZ, Gurr GM, Lundgren JG, Burra DD, Palao LK, Hyman G, Graziosi I, Le VX, Cock MJW, Tscharntke T, Wratten SD, Nguyen LV, You M, Lu Y, Ketelaar JW, Goergen G, & Neuenschwander P. 2018a. Continental-scale suppression of an invasive pest by a host-specific parasitoid underlines both environmental and economic benefits of arthropod biological control. *PeerJ.* 6: e5796.

Wyckhuys KAG, Zhang W, Prager SD, Kramer DB, Delaquis E, Gonzalez CE, & van der Werf W. 2018b. Biological control of an invasive pest eases pressures on global commodity markets. *Environ. Res. Lett.* 13(9): 094005.

Yonow T, Kriticos DJ, & Ota N. 2017. The potential distribution of cassava mealybug (*Phenacoccus manihoti*), a threat to food security for the poor. *PLoS ONE.* 12(3): e0173265.

Zhang J, Huang J, Lu Y, & Xia T. 2016. Effects of temperature and host stage on the parasitization rate and offspring sex ratio of *Aenasius bambawalei* Hayat in *Phenacoccus solenopsis* Tinsley. *PeerJ.* 4(1): e1586.

Vijaya & Ram P. 2013. Effect of host stage on parasitization and biological characteristics of *Aenasius bambawalei* Hayat (Hymenoptera: Encyrtidae), a parasitoid of *Phenacoccus solenopsis* Tinsley. *Journal of Biological Control.* 27(2): 126–129.

Vinson SB & Iwantsch GF. 1980. Host suitability for insect parasitoids. *Ann. Rev. Entomol.* 25: 397-419.

Waage JK. 1986. Family planning of parasitoids: adaptive patterns of progeny and sex allocation. In: Waage JK & Greathead D (Eds.). *Insect Parasitoids.* pp. 63–89. Academic Press, London.

Zain-ul-Abdin, Arif MJ, Gogi MD, Arshad M, Hussain F, Abbas SK, Shaina H, & Manzoor A. 2012. Biological characteristics and host stage preference of mealybug parasitoid *Aenasius bambawalei* Hayat (Hymenoptera: Encyrtidae). *Pak. Entomol.* 34(1): 47–50.