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ASPECTS OF BIOLOGY OF *Acerophagus papayae* Noyes & Schauff (HYMENOPTERA: ENCYRTIDAE), PARASITOID OF THE PAPAYA MEALYBUG

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ABSTRACT

***Aspects of biology of* Acerophagus papayae *Noyes & Schauff (Hymenoptera: Encyrtidae), parasitoid of papaya mealybug*.** *Acerophagus papayae* Noyes & Schauff (Hymenoptera: Encyrtidae) is an important parasitoid of the papaya mealybug, *Paracoccus marginatus* Williams & Granara de Willink (Hemiptera: Pseudococcidae). The study was conducted with the objective to determine various aspects of the biology of *A. papayae* which include the effect of diet on adult longevity, fecundity and progeny, host stage susceptibility and preference, the effect of host stages on immature development, body size, and sex ratio of progenies. Effects of diet on adult longevity was done in the absence of hosts. Fecundity was measured by the number of mealybugs parasitized. Host stage susceptibility and preference were carried out by exposing 2nd and 3rd nymphal instars and pre-reproductive adults of mealybugs to parasitoids. Results showed adult parasitoids fed with 10% honey solution lived almost fourfold longer than those provided only water. *A. papayae* parasitized 30.1±4.92 mealybugs, with a range of 13–60 mealybugs, during 5.8 days of adult life. In no-choice (susceptibility) and paired-choice (preference) tests, the percentage of parasitized hosts were significantly greater in 2nd and 3rd instar nymphs than in adults. The mean immature developmental time of *A. papayae* was longer when the parasitoids develop in large host. Developmental time of male parasitoids was shorter than the females. Female wasps which emerged from hosts parasitized at the 3rd instar nymphs and adults were significantly larger than those from the 2nd instar nymphs. Sex ratios of the offspring emerged from hosts that were parasitized as 2nd instars were strongly male-biased, while the later stages yielded more females than males.

Key words: *Acerophagus papayae*, *Paracoccus marginatus*, parasitoid

INTRODUCTION

Papaya mealybug, *Paracoccus marginatus* Williams & Granara de Willink (Hemiptera: Pseodococcidae), is an insect pest native to Mexico and Central America (Miller *et al*., 1999). The insect was reported for the first time in Indonesia in 2008 (Muniappan *et al*., 2008). Recently, the pest is reported to spread widely to some countries in Asia and Africa (CABI, 2018). Papaya mealybug is a polyphagous pest with more than 135 host plants in 49 families (Morales *et al*., 2016). In Indonesia, among host plants severely infested by *P. marginatus* are papaya, cassava, and purging nut (Maharani *et al*., 2016).

In its country of origin, papaya mealybug has never been reported to cause severe damage on cultivated plants because it is naturally controlled by various natural enemies. Parasitoids reported as the natural enemies of papaya mealybug are *Anagyrus loecki* Noyes, *Acerophagus papayae* Noyes & Schauff, and *Pseudleptomastix mexicana* Noyes & Scauff (Hymenoptera: Encyrtidae) (Meyerdirk *et al*., 2004). Papaya mealybug causes serious damage when the insect is accidentally introduced into new areas where its natural enemies is not available. In this regard, introduction of natural enemies from the country of pest origin (classical biological control) is an effective approach for managing invasive insects like papaya mealybug.

Classical biological control for the papaya mealybug was initiated when the three species of parasitoids were introduced from Mexico into Puerto Rico, Dominican Republic, Florida, and Guam (Meyerdirk *et al*., 2004), and the Republic of Palau (Muniappan *et al*., 2006). The introduction and release of the three parasitoids has significantly reduced the population of papaya mealybug (Meyerdirk *et al*., 2004; Muniappan *et al*., 2006). Afterward, the three parasitoids were introduced into Sri Lanka in 2009 and India in 2010 (Mani *et al*., 2012). Field evaluations showed that among the three parasitoids, *A. papayae* was the most effective and most efficient parasitoid of the papaya mealybug

(Amarasekare *et al*., 2009). Therefore, further biological control programs of the papaya mealybug in Ghana in 2011 and in Benin in 2013 were conducted by introducing only parasitoid *A. papayae* (Goergen *et al*., 2014).

In Indonesia, parasitoid *A. papayae* has recently been found in the field. It is supposed that the parasitoid accidentally entered into Indonesia along with the papaya mealybug (Rauf & Sartiami, 2013). Muniappan (2010) assumed that in other countries in Southeast Asia and South Asia where papaya mealybug has invaded, the parasitoid entered into those countries in a similar way. The fortuitous biological control of some other invasive mealybug species has been reported earlier, such as on the cases of *Aenasius bambawalei* Hayat (Hymenoptera: Encyrtidae) against *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) in India (Gautam *et al*., 2009), and *Acerophagus* n. sp. near *coccois* (Hymenoptera: Encyrtidae) against *Phenacoccus peruvianus* Granara de Willink (Hemiptera: Pseodococcidae) in Spain (Beltra *et al*., 2013a).

Use of parasitoids in biological control requires information on varoius aspects of parasitoid biology such as fitness, adult longevity and fecundity, progeny production, sex ratio of the progeny, and host selection behaviour (Roltberg *et al*., 2001). Such information has not been available yet. Hence, research was conducted with the objectives to determine (1) effect of diet on longevity of parasitoid adult, (2) fecundity and progeny production of parasitoid, (3) susceptibility of different host stages to parasitism by *A. papayae*, and (4) effect of host stages on immature development, fitness, and sex ratio of parasitoid progeny. Information obtained from this study will provide basic understanding on the potential of *A. papayae* as a biological control agent of papaya mealybug as well as developing techniques for parasitoid mass-rearing and field release.

MATERIALS AND METHODS

Research Site. The study was conducted in the Laboratory of Insect Bionomy and Ecology, Department of Plant Protection, Faculty of Agriculture, Bogor Agricultural University, from December 2017 until April 2018. The temperature, air relative humidity and light intensity in the laboratory were set at about 27 °C, 60%, and 12 hours dark–light cycle, respectively.

Papaya Mealybug Rearing. Papaya mealybug adults and ovisacs were collected from infested papaya plants in the field in Bogor. They then were infested onto buds of potato tuber that have been prepared and then were placed in a cylindrical plastic cage (10 cm in diameter and 20 cm in height), and were kept in the laboratory. Population of papaya mealybug were maintained continously in the laboratory for parasitoid rearing.

Rearing of Parasitoid. Individuals of parasitoid *A. papayae* were obtained from the field by collecting parasitized papaya mealybug (mummies) from infested papaya plants in Bogor. The mummies then were reared in the laboratory until the emergence of parasitoid adults. Newly emerged parasitoid adults then were kept in a cylindrical plastic cage (10 cm in diameter, 20 cm in height). A number of laboratory-cultured papaya mealybug nymphs (on buds of a potato tuber) were put into the cage for parasitization. The parasitoid adults were fed by providing 10% honey solution inside the cage. Seven days after exposure, the parasitized hosts (mummies) were collected using a fine paintbrush. The mummy was kept individually in a gelatine capsule until it emerged as a parasitoid adult. Some of emerged parasitoid adults were used for experiments, and the remaining were used for parasitoid rearing.

Study on the Effect of Diets on Parasitoid Adult Longevity. The experiment consisted of three treatments of parasitoid adult diet, i.e. 10% honey solution, water, and control (without food). Each treatment used 20 parasitoid adults as replication. A pair of newly-emerged parasitoid adults were kept in a cylindrical plastic cage (3.7 cm in diameter and 5.0 cm in height) covered with organdy cloth. Food of parasitoid adults was provided by merging a piece of cotton into 10% honey solution, pure water, or none (accordance with the diet treatment), and pluging the cotton into a hole that has been made on the side of the plastic cage. The parasitoid food was added on the cotton every day. The number of survived and died parasitoid adults was observed and recorded every day until all parasitoid adults died.

Study on the Fecundity and Progeny Production of Parasitoid. A one-day-old mated parasitoid female was put into a cylindrical plastic cage (20 cm in height and 8 cm in diameter). The top of the cage was covered with organdy cloth as ventilation. As a source of parasitoid adult food, 0.25 mL of 10% honey solution was provided inside the cage. Ten 3rd instar nymphs of papaya mealybug infested on a piece of unrippen papaya rind (7.5 cm long, 2.5 cm wide, and 0.2 cm thick) then were put into the cage and were allowed to be parasitized by the parasitoid. After 24 hours of exposure, mealybug nymphs were removed from the cage, and were replaced with new 10 3rd instar mealybug nymphs. Host replacement was done every day until the parasitoid died. The exposed mealybug nymphs then were maintained in another cage and were observed every day until parasitization could be detected. The parasitized mealybugs (mummies) were collected and the emerged parasitoid adults (progenies) were recorded daily. If *A. papayae* adults did not emerge, then mealybug mummies were dissected. The experiment used 10 adult females of *A. papayae* as replication.

Study on the Host Stage Susceptibility to Parasitization (No-Choice Test). Three different stages of papaya mealybug, i.e. 2nd instar, 3rd instar, and adult, were tested in the study. Ten individuals of each stage of papaya mealybug were infested onto a piece of papaya rind (7.5 cm long, 2.5 cm wide, and 0.2 cm thick) and then were put into a cylindrical plastic cage (20 cm in height and 8 cm in diameter) with a ventilation on the top of the cage covered with organdy cloth. A one-day-old until two-day-old mated female of *A. papayae* then put into every cage for parasitization for 24 hours. As a source of parasitoid adult food, 0.25 mL of 10% honey solution was provided inside the cage. After 24 hours of exposure, the exposed mealybugs were transferred and reared on buds of potato tuber, and were observed every day. Host susceptibility for parasitization was determined by the total number of parasitized hosts (mummies) with parasitoid exit hole. There were 20 replications (20 females of parasitoid) for each host stage.

Study on the Host Stage Preference of Parasitoid (Paired-Choice Test). The experiment consisted of three pairs of mealybug stage combinations, i.e. 2nd instar vs 3rd instar, 2nd instar vs adult, and 3rd instar vs adult. Ten individuals of each stage of papaya mealybug were infested onto a piece of papaya rind (7.5 cm long, 2.5 cm wide, and 0.2 cm thick). A pair of two different stages of mealybug then were put into a cylindrical plastic cage (20 cm in height and 8 cm in diameter) with a one- or two-day-old mated female of *A. papayae* inside for parasitization. The exposure of mealybugs to parasitoids and observation in this experiment were conducted similar to the no-choice test study. The experiment was conducted with 20 replications for each host stage combination.

Study on the Influence of Host Stages on Immature Developmental Time, Body Size and Sex Ratio of Parasitoid. Three different stages of papaya mealybug, i.e. 2nd instar, 3rd instar, and adult, were tested in the study. Ten individuals of each development stage of papaya mealybug were infested onto a piece of papaya rind (7.5 cm long, 2.5 cm wide, and 0.2 cm thick) and then were put into a cylindrical plastic cage (20 cm in height and 8 cm in diameter) with a ventilation on the top of the cage covered with organdy cloth. A one-day-old until two-day-old mated female of *A. papayae* then was put into every cage for parasitization for 24 hours. The cages was provided with 0.25 mL of 10% honey solution as parasitoid adult food. Ten parasitoid adult females were used in the experiment as replication. Mealybugs in this experiment were reared until mummy formation. The formed mummies were transferred individually into a transparent gelatin capsule. The total number of emerged parasitoid adults were counted every day and differentiated based on the sex. The size of ten emerged parasitoid adults, including the length of the body and hind tibia, were measured under stereo microscope.

Data Analysis. Effect of diets on the longevity of parasitoid adults, host stage effect on parasitization, immature developmental time, and size of emerged parasitoid adults were examined by analysis of variance followed by Tukey test at the 5% significance level. Host stage preference was evaluated by t-test. All analyses were done using IBM SPSS Statistics version 22. Diet effect on parasitoid adults survivorship curve was analyzed by Kaplan-Meier method in PAST 2.17 (Hammer *et al*., 2001). Sex ratio of parasitoid progeny were analyzed with chi-square test (http://udel.edu/~mcdonald/ statchigof.html) to determine its fitness to the theoretical sex ratio (1:1).

RESULTS AND DISCUSSION

Effect of Diets on the Longevity of Parasitoid Adult. Provision of 10% honey solution as parasitoid food significantly prolonged the longevity of parasitoid females ($F_{2,57}$=33.17; $P<0.001$) and males ($F_{2,57}$=32.99; $P<0.001$). The longevities of parasitoid adults fed with honey solution of the two sexes were fourfold longer than those of other treatments (water and control) (Figure 1). The average longevity of female adults was 9.80±1.35 days when they were fed with 10% honey solution, and about 1–2 days when they were fed with water or without food. The average longevity of males fed with 10% honey solution reached 8.25±1.03 days, while for the other treatments were only 1 day. Similar result was reported by Divya *et al*. (2011) in which *A.*

papayae females fed with 10% honey solution lived for 9.0 days, and females fed with water lived for only 3.3 days.

The effect of honey and water as parasitoid adult food can also be examined from the survival curves (Figure 2). Survival of parasitoid adult females fed with 10% honey solution descended gradually and was significantly different from adult females fed with water (χ^2=26.95, P<0.001) and control (χ^2 = 31.22, P<0.001). Survival curves of adult females fed with water and parasitoid females without food were similar, both were declined sharply, and were not significantly different (χ^2=1.57, P=0.21). Survival curves of parasitoid adult males showed similar pattern with those of females. Survival curve of adult males fed with 10% honey solution was significantly different from those fed with water (χ^2=39.44, P<0.001) and without food (χ^2=39.09, P<0.001). There was no significant difference (χ^2=0.02; P=0.88) in survival curve between parasitoid adult males fed with water and the ones without food. Different trend of survival curves was also reported when *Acerophagus* n.sp. near *coccois*, a parasitoid of *P. peruvianus*, was fed with honey and water (Beltra *et al*., 2013b).

Previous studies reported longevity of parasitoid adults were longer with the availability of food sources. *Anagyrus kamali* Moursi (Hymenoptera: Encyrtidae), a parasitoid of mealybug *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae), would die without food within 48 hours , but it would live much longer time with honey as a food source (Sagarra *et al*., 2000). da Silva *et al*. (2017) reported that longevity of *Blepyrus clavicornis* (Compere) (Hymenoptera: Encyrtidae) with the addition of honey as its food was 33 days, but it would only be 4 days when only water available. The female adults of *Anagyrus ananatis*

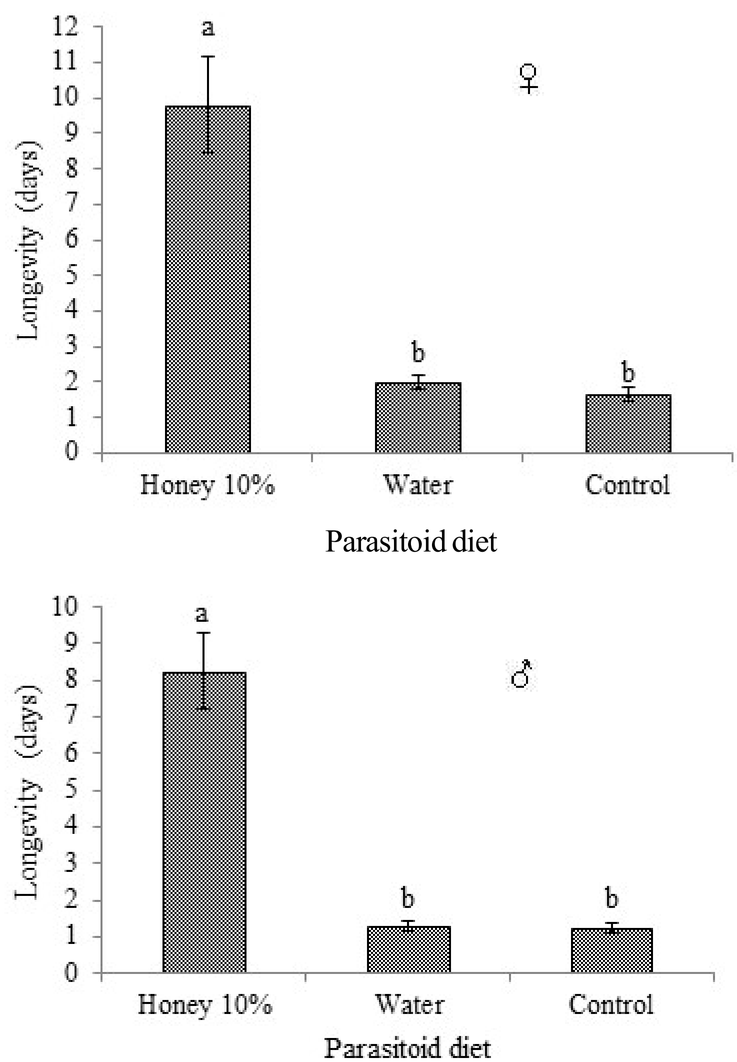

Figure 1. Longevity of *A. papayae* parasitoids fed with three different diets.

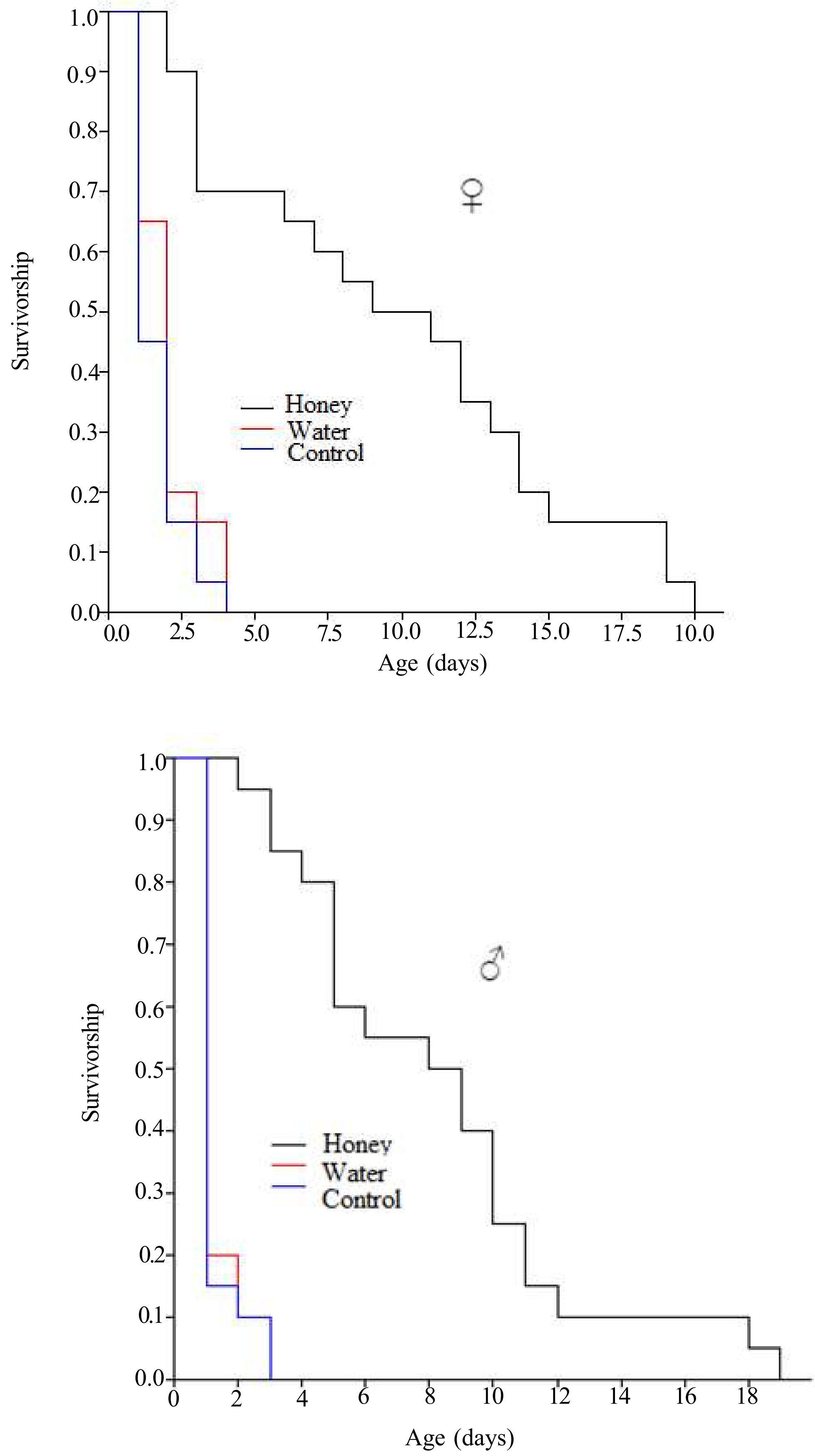

Figure 2. Survivorship curves of adult *A. papayae* parasitoids fed with three different diets based on Kaplan-Meier method.

Gahan (Hymenoptera: Encyrtidae), a parasitoid of *Dysmicoccus brevipes* (Cockerell) (Hemiptera: Pseudococcidae), would be alive for 21–31 days with honey and only 3 days without honey (Gonzales-Hernandez *et al.*, 2005).

Provision of water did not extend the longevity of *A. papayae* adults. It might indicate that energy reserves in parasitoid body were low enough to keep them alive. Some parasitoid adult females belong to order Hymenoptera feed on the host body fluid to get supplementary protein source and to invest into her future reproduction (ovigenesis) (Heimpel & Collier, 1996). However, host-feeding behavior by *A. papayae* females was not observed in this study. Beltra *et al.* (2013b) also reported that *Acerophagus* n. sp. *near coccois* did not show host-feeding behavior. Because of no host-feeding behavior, the primary food sources of *A. papayae* adults in the field are nectar and honey dew. In this regard, management of habitats is needed to support the conservation and augmentation of parasitoids, for example by providing flowering plants in the fields (Landis *et al*., 2000). Honeydew secreted by the papaya mealybug or other sucking insects is also a source of carbohydrates for parasitoid adults. Sandanayaka *et al*. (2009) reported that adult females of *Pseudaphycus maculipennis* Mercet (Hymenoptera: Encyrtidae) lived for 15 days with honeydew of *Pseudococcus viburni* (Signoret) (Hemiptera: Pseudococcidae).

Parasitoid Fecundity and Progeny. Based on the number of mealybugs mummified by *A. papayae*, total number of eggs laid by a parasitoid female over her lifetime (representing her real fecundity) ranged 13–60 eggs, with an average of 30.10 ± 4.92 eggs laid within 5.8 days. Our study resulted in lower fecundity compared to Kanwal *et al.* (2017) who found an average of 40.9 eggs within 6.9 days lifetime. Suma *et al.* (2012) reported that fecundity of *Anagyrus pseudococci* (Girault), a parasitoid of *Planococcus citri* (Risso), was 30.2 egg/female. Total number of mealybugs, *D. brevipes*, parasitized by *A. ananatis* for its lifetime (10.8 days) was 27.7 individuals (Gonzalez-Hernandes *et al*., 2005). Sandanayaka *et al.* (2009) found that the total number of mealybug, *P. viburni*, parasitized by *P. maculipennis* for its lifetime was 45.9 individual. Amarasekare *et al.* (2012) reported a much higher fecundity i.e. 92.8 individuals within its lifetime of 13.9 days. The differences in parasitoid fecundity can be affected by many factors, such as the temperature and air relative humidity of the laboratory, and also time and the way of host exposure to the parasitoids.

Figure 3 shows daily mummy formation that indicates daily oviposition by an adult female of *A. papayae* during its lifetime. It shows that parasitoid females started to produce offsprings when they were 1 day old. This result indicated that the preoviposition period was remarkably short, less than 24 hours. The oviposition peak, 6 eggs, occurred when the adult females were one day old (the 1st day of egg laying) and decreased gradually.

Not all parasitoid adults emerged from the mummified hosts. Mean emerged parasitoid adults was 17.10 ± 3.03. Dissection of mealybug mummies showed that not all pupae developed to the next stage (adult), and some adults failed to emerge from the mummified hosts. During this study, there was a case in which more than one parasitoid adults emerged from a host. Mastoi *et al.* (2014) reported that *A. papayae* can be gregarious when the parasitoid parasitizes adult

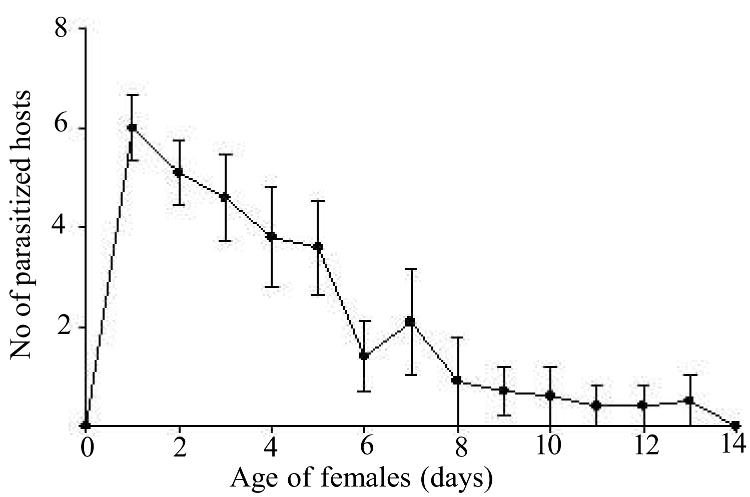

Figure 3. Daily oviposition curve of *A. papayae* based on the number of formed mummies

mealybug. Beltra *et al*. (2013b) argued that parasitoid of *P. peruvianus*, *Acerophagus* n. sp. *near coccois*, was a facultative gregarious parasitoid; she would be a solitary parasitoid on 2nd and 3rd instar nymphs (small hosts), but gregarious on a larger hosts (adults). Some species of *Acerophagus* such as *A. maculipennis*, *A. coccois* Smith, *A. flavidulus* (Brethes), and *A. angelicus* (Howard) (Hymenoptera: Encyrtidae) were facultative gregarious, with the number of parasitoids emerged per host increased as the host get larger (Karamaouna & Copland 2009; Sandanayaka *et al*., 2009).

Host Stage Susceptibility and Preference. In the no-choice test, the proportion of mealybugs parasitized by *A. papayae* was significantly different among host stages ($F_{2,57}$ = 8.7; P=0.001), with parasitization rate on 2nd and 3rd instars was each 76% and on adults 46% (Figure 4A). This result implied that 2nd and 3rd instars were more susceptible to *A. papayae* than adults. In the choice test, the proportion of 2nd instar mealybugs parasitized (89%) was significantly higher (t=13.45; P <0.001) than adults (29%). Similarly, proportion of 3rd instar parasitized by *A. papayae* (78.5 %) was higher (t=13,0; P<0.01) than adults (22%) (Figure 4B). Between the two nymphal stages, parasitization was higher (t=3.29; P=0.002) on 2nd instar (85%) than on 3rd instar (68.5%). Result of susceptibility and preference test revealed that *A. papayae* preferred nymphs for oviposition rather than adults, and smaller nymphs (2nd instar) were most preferred rather than the large ones (3rd instar).

Most parasitoids of the family Encyrtidae that parasitizes mealybugs show host size preference during oviposition (Chong & Oetting, 2006). Our choice and no-choice test showed that females of *A. papayae*

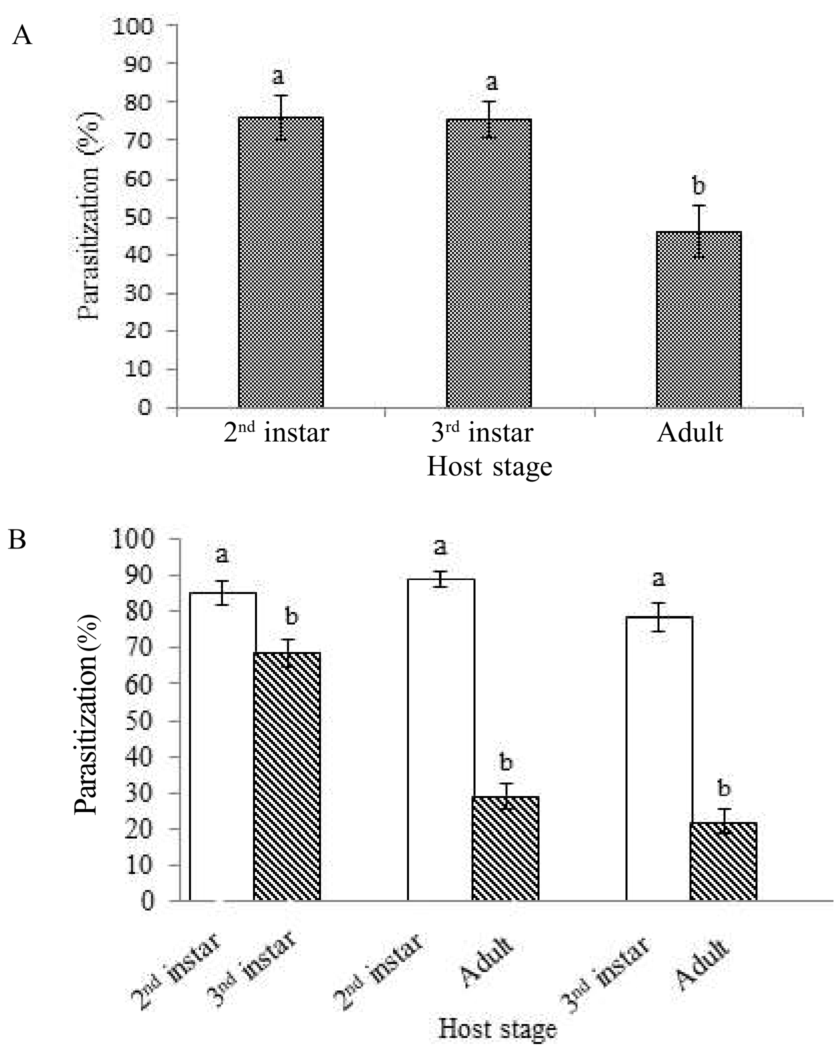

Figure 4. Preference of *A. papayae* on different host stages in no-choice test (A) and paired-choice test (B)

preffered to parasitize small size hosts (2nd instar nymphs) than the large ones (3rd instar nymphs and adults). Amarasekare *et al*. (2010) also reported that parasitization by *A. papayae* was highest on the 2nd instar nymph (82.8%), followed by 3rd instar nymph (71.2%), and adult (60.8%). Host size is an important factor in host selection by parasitoids (Vinson & Iwantsch, 1980). Parasitization rate decreases as the host size increases. Mealybugs being parasitized by a parasitoid adult show defense response by flipping their abdomen, walking away, and reflex bleeding (Bugila *et al*., 2014). The defensive capability of mealybugs is better for bigger size individuals than ther smaller ones. Therefore, the higher parasitization rate on the 2nd instar nymphs might be caused by the low defense ability of the small size hosts. Furthermore, host handling time by parasitoids is longer for larger hosts than the smaller ones (Bertschy *et al*., 2000).

Another possibility that causes low parasitization rate on the mealybug adults is the failure of parasitism due to encapsulation (Blumberg, 1997). Increase of encapsulation rate with the increasing host age or size was reported in various species of mealybugs (Karamaouna & Copland, 2009; Beltra *et al*., 2013b). Hence, many parasitoids tend to parasitize young instar hosts (Blumberg, 1997).

Immature Developmental Time. Immature developmental times of parasitoid males was significantly different for each host stages ($F_{2,19}$=22.27; P<0.001), and so of those females ($F_{2,26}$= 7.09; P=0.003) (Table 2). Immature developmental time increased with the increasing host age. The immature developmental time of parasitoids was 14.33 days (male) and 16.08 days (female) when the parasitoids parasitize mealybug adults, and those are 3–4 days longer than those parasitizing 2nd instar nymphs. Mastoi *et al*. (2014) also reported similar result in which immature developmental time of *A. papayae* was longer on papaya mealybug adults.

Longer immature developmental time of *A. papayae* on more advanced host stage might be caused by low quality of nutrition. In addition, advanced hosts have a better defense capability against parasitoids that can delay parasitoid development. This is in accordance with our previous finding in which parasitoid *A. papayae* preferred 2nd and 3rd instars than adults. Mastoi *et al*. (2014) suggested that a longer immature developmental time of parasitoid on mealybug adults was due to competitive inhibition, since more than one larva can live in a single host. For most species of mealybug parasitoids, immature developmental time generally is shorter in larger hosts (Karamaouna & Copland, 2009; Chong & Oetting, 2006).

In general, for each different host stage, immature developmental time of male was one day shorter than female (Table 2), as reported previously by Amarasekare *et al*. (2012). The immature developmental time of parasitoids for all host stages averaged 12.77 ± 0.28 days for males and 15.03 ± 0.33 days for females (t=4.97; P<0.001). The short developmental time for male parasitoids was also reported in the previous studies, such as *Acerophagus pseudococci* (Girault) on *P. citri* (Islam & Copland, 1997), and *Allotropa suasaadi* Sarkar & Polaszek (Hymenoptera: Platygasteridae) on the cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae) (Sarkar *et al*., 2015).

Body Size. The length of the left hind tibia of *A. papayae* developed in different host stages were not significantly different for both males ($F_{2,19}$=3.77; P=0.042) and females ($F_{2,26}$=1.25; P=0.30). Body length of parasitoids developed from the 2nd instar nymph was significanty different ($F_{2,26}$=5.74; P=0.009) from the 3rd instars nymph and adult for the females, but was not significanty different for the males ($F_{2,19}$=2.71; P=0.092) (Table 3). The length of hind tibia is often used as an indicator of body length and fitness of parasitoids (Chong & Oetting, 2006; Sagarra *et al*., 2001).

In general, size of emerged parasitoids was influenced by the size of hosts at time of oviposition. Parasitoids emerged from 3rd instar nymph and adult

Table 2. Immature developmental time of males and females of parasitoid *A. papayae* on different host stages

Host stages	Immature developmental time (days)	
	Male	Female
2nd instar nymph	11.72 ± 0.19a	12.50 ± 0.19a
3rd instar nymph	13.62 ± 0.32b	14.42 ± 0.20b
Adult	14.33 ± 0.33b	16.07 ± 0.59b

Values in the same column followed by the same letters are not significantly different (Tukey Test α= 5%).

mealybugs were larger in size than those emerged from the 2nd instar nymphs. Bertschy *et al*. (2000) reported that, *Aenasius vexans* Kerrich (Hymenoptera: Encyrtidae), a parasitoid of *Phenacoccus herreni* Cox (Hemiptera: Pseudococcidae), emerged from 3rd instar mealybugs, was larger than that emerged from 2nd instars. Similarly, *Leptomastix epona* (Walker) and *Pseudaphycus flavidulus* (Brethes) (Hymenoptera: Encyrtidae), parasitoids of *P. viburni*, which emerged from large host showed a wider head capsule than those emerged from small hosts (Karamaouna & Copland, 2009). Body size of parasitoid is correlated with its fitness, especially for the female. The female adults with large body size can lay more eggs (van Dijken & van Alphen, 1991). Therefore, 3rd instars nymphs and adults of papaya mealybug were the most suitable host stages for *A. papayae*.

Sex Ratio. The sex ratio of progenies produced by a single mated female of *A. papayae* varied and was influenced the host stages. The parasitoids emerged from the 2nd instar mealybugs were mostly males, with proportion of female was 15% significantly different from the theoretical sex ratio 1:1 (χ^2=6.23; P=0.01) (Figure 5). The parasitoid progenies emerged from the 3rd instar mealybugs comprised of 64% males and 36% females, and it was not significantly different from the theoretical sex ratio (χ^2=1.64; P=0.20). Adults emerged from adult mealybugs were dominated by females (81%), and its sex ratio was significantly different from the theoretical sex ratio (χ^2=6.25; P=0.01).

The above result indicated that host stages influenced the sex ratio of *A. papayae* progenies, in which proportion of females increased with the increasing host size. The higher proportion of females from larger host size is also reported in other studies. More *L. epona* males emerged from small hosts than the large ones (Karamaouna & Copland, 2009). Daane *et al*. (2004) reported that, more *A. pseudococci* females emerged from the large hosts. *A. vexans*, a parasitoid of *P. herreni*, which parasitized 2nd instar nymphs produced many male offspring than instars with

Table 3. Effect of host stages on body size of parasitoid *Acerophagus papayae*

Host stages	Body length (mm)		Left tibia length (mm)	
	Male	Female	Male	Female
2nd instar nymph	0.56 ± 0.02a	0.55 ± 0.08a	0.18 ± 0.01a	0.20 ± 0.03a
3rd instar nymph	0.59 ± 0.02a	0.68 ± 0.01b	0.23 ± 0.01b	0.22 ± 0.01a
Adult	0.68 ± 0.01a	0.68 ± 0.01b	0.21 ± 0.01ab	0.22 ± 0.01a

Values in the same column followed by the same letters are not significantly different (Tukey α = 5%).

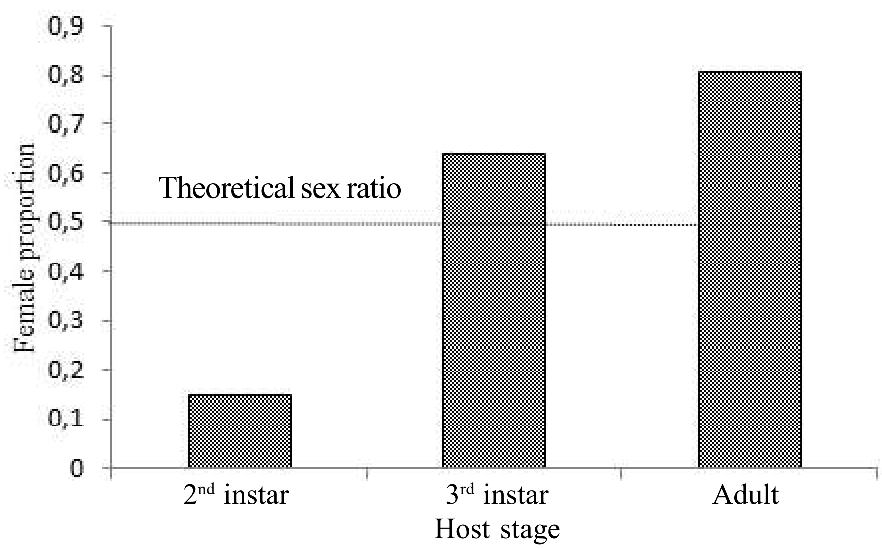

Figure 5. Sex ratio of parasitoid progenies emerged from three different host stages (* = significantly different)

the large size (Bertschy *et al*., 2000). Host selection behavior is most important in determining the sex ratio of arrhenotokous parasitoids (King, 1987). A female parasitoid can adjust the sex ratio of her offspring by controlling fertilization during oviposition (King, 1987). This follows prediction of King (1987) that proportion of parasitoid males emerged from large hosts was lower than those from small hosts. Parasitoid females tend to lay only fertilized eggs (develop into females) on larger hosts, and unfertilized eggs (develop into males) on the smaller hosts.

Implications for Biological Control Programs. Good quality parasitoids can be indicated by high parasitization rate, rapid immature developmental time, female-biased sex ratio, and large body size (Roitberg *et al*., 2001). Based on the rate of paratisization, the 2nd and 3rd instar nymphs of papaya mealybug were deemed to be the most suitable stages for parasitoid mass-rearing. However, 2nd instar nymphs produced parasitoid progeny with male-biased sex ratio and mealybug adults resulted in lower rate of parasitization and longer immature developmental time. Therefore, it is sugested to use 3rd instar nymphs of the papaya mealybug for mass-rearing of parasitoid *A. papayae*. The 3rd instar nymphs offers several advantages for *A. papayae*: a high rate of parasitization, short immature developmental time, female-biased sex ratio, and large body size. According to Ellers *et al.* (1998) size of parasitoid females is positively correlated with the fitness (longevity and reproductive rate) and dispersal capacity. Releasing high fitness parasitoids would increase the effectiveness of biological control program (Chong & Oetting, 2006). In term of time, the release of parasitoid in the field is suggested when the population of the papaya mealybug is dominated by the 2nd and 3rd instar nymphs. Releasing parasitoid *A. papayae* in a proper time will increase the effectiveness and sustainability of the biological control program of the papaya mealybug.

CONCLUSION

Best quality of *A. papayae* can be obtained by rearing the parasitoid using 3rd instar nymphs of papaya mealybug as the host, and provided with 10% honey solution as a food source. Field release of parasitoids should be made when most of the papaya mealybug population consisted of 2nd and 3rd instar nymphs.

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