PARASITISM, SEX RATIO, DEVELOPMENTAL TIME AND GREGARIOUSNESS OF *ACEROPHAGUS PAPAYAE* (HYMENOPTERA: ENCYRTIDAE) ON MALE AND FEMALE HOST STAGES OF *PARACOCCUS MARGINATUS* IN NO-CHOICE SITUATIONS

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Abstract

Acerophagus papayae, accidentally introduced parasitoid of *Paracoccus marginatus* in Malaysia was studied for parasitism, sex ratio, developmental time and gregariousness behaviour. The parasitoid was able to develop in all stages of *P. marginatus* except first instars, prepupa and pupa. The maximum parasitism (59.0 ± 5.66%) was observed in second instar female nymph and minimum (39.0 ± 3.48%) in adult female *P. marginatus*. In adult female, the *A. papayae* showed gregariousness behaviour (2.87 individuals per host). Male biased sex ratio (69.14 ± 3.37%) was observed in second instar male and female biased sex ratio (71.76 ± 4.21% and 71.53 ± 4.33%) was observed in third instar female nymph and adult female *P. marginatus* respectively. The minimum and maximum developmental time for male *A. papayae* (13.00 and 13.89 days) was found in second instar female nymph and adult female *P. marginatus* respectively. However, minimum and maximum developmental time for female *A. papayae* (13.85 and 14.42 days) was found in second instar male nymph and third instar female nymph of *P. marginatus*. The parasitoid showed gregarious behaviour (2.87 individuals per host) only in adult female *P. marginatus*. This vital information can be used in evaluating the efficiency of parasitoid.

Introduction

Papaya mealybug parasitoid, *Acerophagus papayae* Noyes and Schauff, 2003 (Hymenoptera: Chalcidoidea: Encyrtidae) is an introduced solitary koinobiont endoparasitoid of papaya mealybug, *Paracoccus marginatus*. The parasitoid is native of Mexico (Noyes and Schauff, 2003) and from last two decades it is widely used in classical biological control of *P. marginatus* in Puerto Rico, Dominican Republic, Guam, Palau, Sri Lanka and India (Galanihe *et al*., 2010; Meyerdirk *et al*., 2004; Muniappan *et al*., 2006; Shylesha *et al*., 2010; Walker *et al*., 2003). Augmentative biological control requires mass rearing of a large number of high-quality biological control agents in controlled conditions. For rapid multiplication and production of efficient parasitoids, the knowledge of host stages is imperative. Host stage is a vital ecological factor influencing the parasitism rate and progeny sex ratio (Rehman and Powell, 2010). A female parasitoid can manipulate the offspring sex ratio at oviposition by regulating fertilization. A particular host size may be more suitable for the development of one sex, so that, in general, a female-biased offspring sex ratio is produced from the larger hosts and a male biased one from the smaller hosts (King, 1987). Solitary parasitoids generally determine the host quality by the size of the host. Large hosts are supposed to be better quality, as they are believed to contain more resources than small ones. In solitary insect parasitoids, generally only one offspring survives in a host (Vinson, 1976).

An understanding of the susceptibility of host stages selection by a parasitoid allows the manipulation of host stage composition in the insectary to yield biological control agents of suitable quality and quantity. Release of the parasitoids could also be synchronized with the phenology of the pests to ensure that the most suitable host stages are available for parasitism at the time of release. Host selection behavior is most important in determining the sex ratio of arrhenotokous parasitoids, which has haplodiploid sex determination mechanism (King, 1987). The choice of host stage is most important in determination of parasitoid's progeny fitness (Hagvar and Hofsvang, 1991).

There is no information available relevant to parasitism on male instar nymphs and gregariousness behaviour of parasitoid. The previous studies conducted by Amarasekare *et al*. in 2009, 2010 and 2012, used the host stages of *P. marginatus* without differentiating the male instar nymphs. Sex differentiation in *P. marginatus* nymphal stages determined in second instar nymphs when male nymph change its colour from yellow to pink

and according to Miller and Miller (2002), the average size of second instar male nymph was 0.6 mm and for second instar female nymph it was 0.7 mm. The male *P. marginatus* nymphs are equally important because *P. marginatus* females can't reproduce without mating with males. Knowledge of host stages for parasitism, sex ratio, developmental time and gregariousness of parasitoids lead to better understanding of the population dynamics of the host and the parasitoid. Hence, they are important in evaluating and understanding the success of biological control in integrated pest management programs. Therefore the present study was carried out on both male and female instar nymphs of *P. marginatus* to investigate the host stage susceptibility to *A. papayae*.

Materials and Methods

Rearing of *P. marginatus:* *P. marginatus* culture was maintained on un-ripe green papaya fruits in Entomology Laboratory, Faculty of Agriculture, Universiti Putra Malaysia at an ambient environment of 26 ± 2 °C, 60±5% relative humidity with 12:12 (LD) photoperiod. Plastic containers with 10 Litre capacities were used as rearing chambers. 2-3 medium sized un-ripen papaya fruits were placed in each rearing chamber and 10-12 adult gravid females (with or without ovisacs) per papaya fruit were introduced for multiplication of culture.

Rearing of *A. papaya:* The first source of *A. papayae* was collection of mealybug mummies from infested papaya plants in Ladang-10 and Ladang-2 in Universiti Putra Malaysia. The only highly infested papaya leaves were brought to Entomology Laboratory, Faculty of Agriculture, Universiti Putra Malaysia and placed in a plastic aquarium covered with muslin cloth for emergence of adult parasitoids. The only *A. papayae* adults were separated with the help of insect aspirator for further rearing. An up-ripen papaya fruit infested with second instar and third instar *P. marginatus* were offered to *A. papayae* for further rearing of pure culture in laboratory. Four to five streaks of 80% honey solution was given to parasitoid to increase the longevity and fecundity of *A. papayae*. After one week of introduction of *A. papayae*, mummified mealybugs were collected from culture and placed individually in glass vials for eclosion of adults.

Studies on *A. papayae* was conducted on different host stages of *P. marginatus* viz. First instar nymph, second instar male nymph, second instar female nymph, pre-pupa (male), pupa (male), third instar female nymph and adult female.

The experimental arena for the study was consisted on 9 cm diameter plastic petri dishes. About 0.5 cm diameter hole was made in the bottom of the petri dish using a heated cork borer. The cover of petri dish was cut in the centre in square shape about 5 cm diameters to attach muslin cloth with glue to allow the air circulation inside the Petri dish. A tender hibiscus leaf with a 3-4 cm long petiole was placed in each Petri dish with the petiole inserted through the hole at the bottom of the Petri dish. Each petri dish with a hibiscus leaf was placed on a cup of water so that the petiole below immersed in water to keep the leaf fresh. Ten mealybug individuals of each host stages were released on each hibiscus leaf to settle. A one day old single mated female of *A. papayae* was released for 24 hours to each petri dish. A streak of 80% honey solution was also offered to a parasitoid. The sides of petri dish were sealed by using parafilm to avoid the escape of parasitoid or mealybug. The experiment was conducted at an ambient environment of 26 ± 2°C, 60±5% relative humidity and 12:12 (L:D) h photoperiod. The parasitoid was removed after 24 hours and the mealybug cohort retained in same petri dish to continue its development. The mealybug cohort was examined daily and upon mummification, the mummies were collected and isolated in separate vials until eclosion of adult parasitoids. The parasitism rate, sex ratio, developmental time and gregariousness of *A. papayae* on each host stage of *P. marginatus* were recorded.

Data analysis: The experiment was set in complete randomized design. The analysis of variance was performed for parasitism rate, sex ratio, developmental time and gregariousness of parasitoid. The means were separated using Tukey's test. All the statistical analysis was done using SAS 6.2.

Results and Discussion

Parasitism rate, sex ratio, developmental time and gregariousness are important parameters for evaluating the efficiency of parasitoid used in biological control programmes. The results on different host stages are given as under:

Parasitism: The parasitism rate showed significant differences among the host stages. The maximum parasitism rate (59.0 ± 5.66%) was found in second instar female *P. marginatus* followed by, 53.0 ±3.35% and 53.0 ± 4.48 in second instar male and third instar female *P. marginatus*. Both nymphal instars viz. second instar male *P. marginatus* and third instar female *P. marginatus* were statistically non-significant. However, minimum parasitism (39.0 ± 3.48%) was observed in adult female *P. marginatus* (Table 1).

Sex ratio: The higher number of male *A. papayae* (69.14 ± 3.37%) emerged in second instar male *P. marginatus* followed by 42.15 ± 2.04% in second instar female *P. marginatus*. The lower number of male *A. papayae* (28.24 ± 4.21% and 28.47 ± 4.33%) was emerged in third instar female and adult female *P. marginatus* respectively (Table 2). The female *A. papayae* in this study were found maximum (71.76 ± 4.21% and 71.53 ± 4.33%) in third instar female and adult female *P. marginatus* respectively. However, emergence of female *A. papayae* decreased significantly in second instar female and second instars male *P. marginatus* by recording 57.85 ± 2.04% and 30.86 ± 3.37%, respectively (Table 2).

Developmental time: The maximum developmental time for male *A. papayae* (13.89 days) was found in adult female *P. marginatus*. However, developmental time of male *A. papayae* in second instar male *P. marginatus*, second instar female *P. marginatus* and third instar female *P. marginatus* were statistically non-significant by recording 13.01, 13.00 and 13.11 days respectively. The maximum developmental time for female *A. papayae* (14.42 days) was reported in third instar female *P. marginatus*. However, developmental time of female *A. papayae* in second instar male *P. marginatus*, second instar female *P. marginatus* and adult female *P. marginatus* were 13.85, 14.07 and 14.22 days respectively (Table 3).

Gregariousness behavior: Although *A. papayae* is known as solitary but in this study gregariousness behaviour is observed in bigger host stages of *P. marginatus*. In adult female *P. marginatus*, the *A. papayae* showed gregariousness behaviour and 2.87 individuals per host emerged. However, the parasitoid shows solitary behaviour in second instar male, second instar female and third instar female *P. marginatus* (Table 4). The mummies of adult female *P. marginatus* with two and three parasitoids (*A. papayae*) are shown in figure 2.

Discussion

The selection of any parasitoid for biological control programmes depend on efficiency of parasitizing various host stages while maintaining their sex ratio. In this study, higher parasitism was observed in second instar female *P. marginatus* while lowest in adult female. Amarasekare *et al*. (2010) in their studies also found highest percent parasitism in second instar *P. marginatus* but without sex differentiation. They further reported less parasitism in adult female *P. marginatus* than third instar female. This may be due to their defensive behavior and similarly parasitoid needs more time to handle the bigger hosts (Bertschy *et al*., 2000).

The sex ratio of *A. papayae* depends on the size of host stage for depositing of either male or female egg. The male-biased sex ratio was found in second instar male *P. marginatus*, while, female biased sex-ratio was recorded in all female instars of *P. marginatus* viz. second instar female, third instar female and adult female. These results confirms from previous findings of Amarasekare *et al*. (2010); Karamaouna and Copland (2000) that second instar host stages of mealy bugs yielded more male parasitoids. King (1987) also noted a female-biased sex ratio of parasitoid produced from the larger hosts and a male biased from the smaller hosts. Similarly, Amarasekare *et al*. (2010) also recorded higher proportion of progeny females in third instar female and adult female *P. marginatus*. Fand *et al*. (2010) also reported higher number of female parasitoid *A. bambawalei* emerged from third instar mealybug nymphs *P. solenopsis*, whereas, the higher proportion of male parasitoids emerged from second instar nymphs.

Table 1. Parasitism of *A. papayae* in different host stages of *P. marginatus*

Host stages *P. marginatus*	Percent Parasitism
Second instar male nymph	53.00±3.35 ab
Second instar female nymph	59.00±5.66 a
Third instar female nymph	53.00±4.48 ab
Adult female	39.00±3.48 b
F-value	3.81
DF	3,36
Sig-P	.018

Table 2. Sex ratio of *A. papayae* in different host stages of *P. marginatus*

Host stages *P. marginatus*	Male *A. papayae* (%)	Female *A. papayae* (%)
Second instar male nymph	69.14±3.37 a	30.86±3.37 c
Second instar female nymph	42.15±2.04 b	57.85±2.04 b
Third instar female nymph	28.24±4.21 c	71.76±4.21 a
Adult female	28.47±4.33 c	71.53±4.33 a
F-value	28.41	28.41
DF	3,36	3,36
Sig-P	0.00	0.00

Table 3. Developmental time (days) for male and female *A. papayae* in different host stages of *P. marginatus*

Host stages *P. marginatus*	Male *A. papayae*	Female *A. papayae*
Second instar male nymph	13.01 b	13.85 b
Second instar female nymph	13.00 b	14.07 ab
Third instar female nymph	13.11 b	14.42 a
Adult female	13.89 a	14.22 ab
F-value	6.13	3.53
DF	3,36	3,36
Sig-P	0.002	0.024

Table 4. Gregariousness of *A. papayae* in different host stages of *P. marginatus*

Host stages *P. marginatus*	Gregariousness
Second instar male nymph	1.00 b
Second instar female nymph	1.00 b
Third instar female nymph	1.105 b
Adult female	2.87 a
F-value	48.12
DF	3,36
Sig-P	0.00

In this study, the longer developmental time for male and female parasitoid *A. papayae* was found in adult female *P. marginatus*. This could be possibly that more than one parasitoids emerged from this particular stage and ultimately there was competition for food to complete the life cycle. Averagely male *P. marginatus* completed their lifecycle one day earlier than females in all host stages. Similar results were found by Fand *et al*. (2010) that the developmental time of female *A. bambawalei* took longer period than male.

Although, less parasitism was observed in adult female *P. marginatus*, but eclosion of more than two parasitoids were found from single adult female *P. marginatus*. This is the first record of gregarious behaviour of *A. papayae* on *P. marginatus*. Thus, it could be predicted that *A. papayae* is more efficient in controlling *P. marginatus* not only in nymphal stages as solitary parasitoid but, also behave as gregariousness on adult female *P. marginatus*.

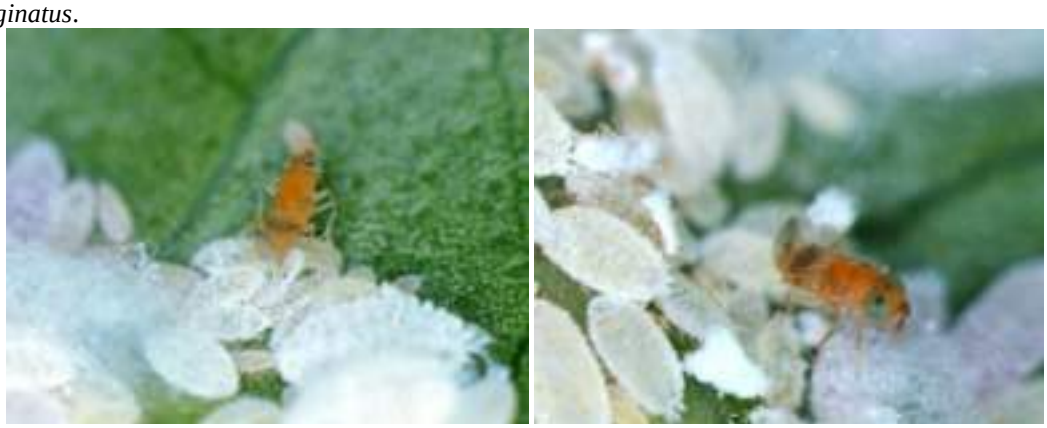

Fig. 1. Rearing of *A. papayae* on *P. marginatus*

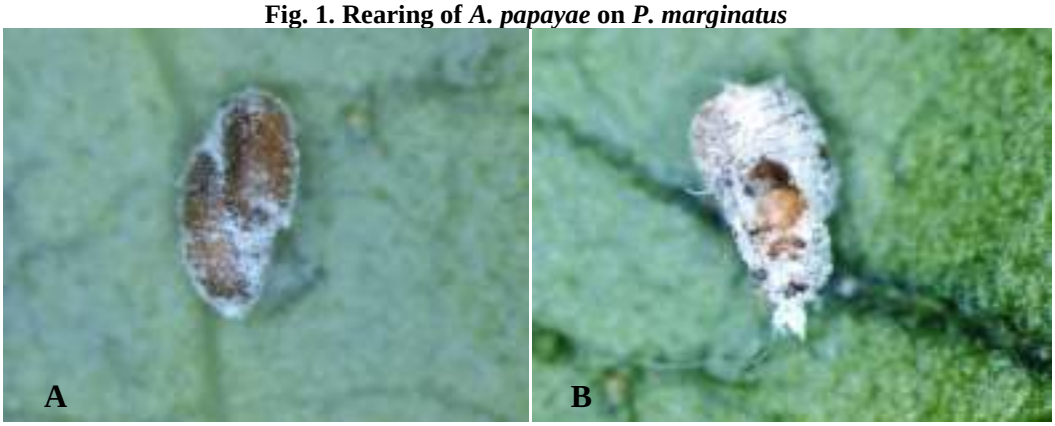

Fig. 2. Mummies of Adult Female *P. marginatus* (A) two parasitoids inside, (B) three parasitoids inside

Conclusion

The *Acerophagus papayae* parasitizes all stages of *P. marginatus* except first instar nymphs, prepupa and pupa. The highest parasitism percentage was recorded in second instar female and lowest in adult female *P. marginatus*. Male-biased sex ratio was observed in second instar male nymphs while, female biased sex ratios were found in second instar female, third instar female and adult female *P. marginatus*. Longer developmental time for male and female *A. papayae* was recorded in adult female *P. marginatus*. *A. papayae* showed gregarious behavior in adult female while, solitary in second instar male, second instar female and third instar female *P. marginatus.*

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