Chapter 1
Print ISBN: 978-93-5547-428-5, eBook ISBN: 978-93-5547-435-3

Some Biological Parameters of *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae) and Its Natural Enemy *Acerophagus papayae* Noyes et Schauff (Hymenoptera: Encyrtidae)

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DOI: 10.9734/bpi/rabs/v3/15958D

ABSTRACT

The papaya mealybug *Paracoccus marginatus* Williams and Granara De Willink (Hemiptera: Pseudococcidae) is an invasive insect species attacking diverse host plants causing enormous damage to crops including those of very high economic importance. Biological control of this mealybug is achieved through the field release of its natural enemy, *Acerophagus papayae* Noyes and Schauff (Hymenoptera: Encyrtidae). The study aims to determine the developmental time and mortality rate of the different stages of *P. marginatus*; daily fecundity and pre- oviposition, oviposition and post-oviposition times of a female *P. marginatus* and the total average number of emerging individuals of the hosts parasitized in 24 hours by a female and the total average development time of *A. papayae*.

This study, which was conducted in the laboratory under conditions of 28 ± 2°C, 75 ± 5% RH, 12: 12 LD, determined the average daily fecundity of a female *P. marginatus* and the average number of offspring of a female *A. papayae* per day. The results obtained showed that a female *P. marginatus* has three periods of reproductive activity, namely the pre-oviposition, oviposition and post-oviposition periods, which are on average last 7.74 ± 1.26, 6.13 ± 3.02 and 7.45 ± 4.27 days respectively. During oviposition, a female *P. marginatus* lays an average of 25.262 ± 11.16 eggs per day, and an average total of 224.32 ± 29.99 eggs during her lifetime, which averages 18.44 ± 3.31 days. Also, during the development of the pre-imaginal stages of *P. marginatus*, significant mortality rates were noted in the first and second larval stages, which are 61.40 ± 0.05 and 52.8 ± 0.025 respectively, whereas in the third stage, this rate is 35.02 ± 0.03 for the female and zero for the male. The average total development time of the pest was 25.98 ± 4.47 days for the female and 29.70 ± 5.58 days for the male. In contrast, the progeny of its natural enemy *A. papayae* averaged 8.22 ± 4.676 males and 10.53 ± 4.43 females per day, giving an average total of 18.75 ± 9.087 offspring per day. During its lifetime (13.02 ± 0.08 days), a female *A. papayae* produced an average of 205.96 ± 9.87 offspring in an average development time of 13.82 ± 0.013 days. These results obtained on the progeny of a female and the development time of *A. papayae* species in relation to that of its host can be used to evaluate the effectiveness of the parasitoid in the management of the pest populations in Togo.

Keywords: Biological control; daily oviposition; mortality rate; papaya mealybug; Acerophagus papaya; Paracoccus marginatus; Togo.

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1. INTRODUCTION

Global fruit production has been booming over the past 30 years [1]. Unfortunately, this production is threatened by pest attacks including insects. Among the insect pests of fruit are mealybugs, including the papaya mealybug (*Paracoccus marginatus* Williams and Granara de Willink). Native to Mexico and/or Central America [2, 3] it has been accidentally introduced to the Caribbean islands and the Pacific [2]. On the African continent, it was first detected in Ghana in late 2009 [4]. Subsequently, it has spread to Togo, Benin, Nigeria, Cameroon and most recently Gabon [5]. According to the work of Cham et al. [3] *P. marginatus* attacks a large number of tropical and subtropical fruits of great economic importance such as pawpaw (*Carica papaya* L., Caricaceae), citrus, mangoes (*Mangifera indica* L., Anacardiaceae), guavas (*Psidium guajava* L., Myrtaceae). This causes severe economic losses from damage such as yellowing, leaf crypts, development of powdery mildew (fumaginia), early leaf and fruit dropping, stunted plant growth leading to subsequent plant death [6-9]. Heavy infestations can lead to yield losses of up to 65%, which in Ghana, for example, led to the reduction of papaya orchards from 2,500 ha to 380 ha in 2010. As a result, the export earnings of the papaya industry have decreased significantly and 1700 people in the sector have lost their jobs [5]. Globally, data indicate that papaya mealybug can develop on more than 86 plant species in 35 plant families [2, 10-15]. Faced with this situation, control of this pest by small and large-scale farmers involves several management strategies. Most farmers rely mainly on synthetic chemicals such as dimethoate, malathion, carbaryl, chlorpyrifos, diazinone, acephate [16,17] neo-necotinoid insecticides (acetamiprid, clothianidin, dinotefuran, imidacloprid, thiamethoxam) and insect growth regulators (IGRs) such as pyriproxyfen to control insects in general and mealybugs in particular [18]. Indiscriminate and frequent applications of these synthetic chemical insecticides result in negative impacts on natural enemies, environmental contamination, health risks, pesticide residues and the development of resistance [19]. In addition, the effectiveness of chemicals in managing mealybugs is often problematic because of the thick waxy secretion covering their bodies and their ability to rapidly create dense populations. Also, the high degree of polyphagia of *P. marginatus* is another factor limiting the effectiveness of chemical control. Hence the need to develop non- chemical methods such as biological control, an effective and environmentally friendly management strategy [20]. Of the widely accepted general approaches to biological control, classical biological control is the most common [21]. This approach has been identified as an important option in the management of *P. marginatus* since it is an exotic pest that poses a threat to many agricultural crops. With this in mind, the search for specific natural enemies of *P. marginatus* was undertaken and in 1999, APHIS (Animal and Plant Health Inspection and Research Service) and the USDA Agricultural Research Service collected and reared four species of wasp from Mexico. They introduced them into Puerto Rico and the Dominican Republic and reduced the populations of *P. marginatus* in both countries by more than 95% [22]. All four wasp species have been observed parasitising second and third instar larvae of *P. marginatus* [10]. Of these four parasitoids, *A. papayae* was found to be a dominant parasitoid, effective in controlling papaya mealybug in its country of origin and has the ability to establish itself when introduced into new localities [23, 24]. Thus, given the magnitude of the problem posed by the introduction of *P. marginatus* in the African continent, immediate and sustained action to prevent the pest from spreading and causing serious damage was initiated. Nigeria, the third largest papaya producer in Africa, is at risk. Furthermore, given the success of the biological control method with the introduction of *A. papayae* in Asia and the Pacific, a classical biological control programme was launched as a joint-venture by the Plant Protection and Regulatory Services Directorate of Ghana (PPRSD), the Food and Agriculture Organization of the United Nations (FAO) and the International Institute of Tropical Agriculture (IITA) in 2010 to address the problem at the regional level. This programme, known as the TCP emergency project (technical cooperation programme project), financed by the Swiss Agency for Development and Cooperation (SDC) in Togo, allowed the release of the *A. papayae* microgae in early 2016 throughout the country. To this end, this study was done to obtain data on the biology of the pest *P. marginatus* and its natural enemy *A. papayae*. Specifically, the study aimed to determine the development time and mortality rate of the different stages of *P. marginatus*; the daily fecundity and the pre-oviposition, oviposition and post-oviposition times of a female *P. marginatus* and the total average number of emerging individuals of the hosts parasitized in 24 hours by a female and the total average development time of *A. papayae*.

2. MATERIALS AND METHODS

2.1 Study Site

This study was conducted in the Biodiversity Centre (or Biodiversity Museum) which measures approximately 200 m^2. This centre is an integral entity of the International Institute of Tropical Agriculture in the commune of Abomey-Calavi, Benin (IITA-Benin), where colonies of *A. papayae* are mass produced.

2.2 Production of Host Plant for Experiments

A nursery was planted with papaya seeds (*C. papaya* L.). Two weeks after the first seedlings emerged, they are transplanted into plastic pots at a rate of three per pot. Two weeks after this transplantation, the seedlings are pruned to obtain sturdy stems. The seedlings are watered as needed with clean water.

After the regrowth of new stems and leaves, pots containing seedlings with sturdy stems are selected. These stems are washed and placed in breeding cages in the laboratory.

2.3 Pest Breeding: *Paracoccus marginatus*

Individuals of the papaya mealybug, *P. marginatus*, were initially collected from papaya plants (*C. papaya* L) located within the IITA-Benin station. The ovisacs were collected and put in transparent jars covered with very fine mesh netting. The breeding was carried out in a breeding room under conditions of 28 ± 2°C, 75 ± 5% RH (Relative Humidity) and photoperiod of 12:12 (L:D). The ovisac jar was then placed in a breeding cage measuring 58 × 50 × 45,5 cm covered laterally with very fine mesh nets, at the top with plexiglass glass and at the bottom with plywood and longitudinally surmounted by light bulbs supported by iron bars containing pots of previously cultivated papaya plants. When the eggs hatch, the neonate larvae invade the papaya plants in the cages.

2.4 Study of Reproductive Activity and Survival of Females *P. marginatus*

In ten breeding cages each containing three pots of papaya, *P. marginatus* ovisacs were introduced in order to permanently have individuals at different stages of the pest for different experiments.

To assess the daily egg laying potential of an adult female *P. marginatus*, a 24-hour egg ovisac was collected from the reserve colony (where pre-oviposition females are selected and marked with an indelible marker). These eggs were then transferred to three healthy papaya plants potted in another cage and followed until hatching. As soon as the eggs hatched, the neonate larvae were observed daily until adult females appeared and were marked with an indelible marker. In each pot, the number of females was reduced to 10 and unevenly spread on the three plants according to their size (large plants carried 4 individuals and small plants, 2 individuals) by removing the excess. These 10 females were monitored daily before, during and after the laying period. The eggs laid by each female were collected daily (using a small brush and hand magnifier) and counted using a hand tally counter with a binocular magnifier and a punch needle to open the ovisac.

This procedure was repeated daily until the last eggs laid by the females; their pre-oviposition, oviposition and post-oviposition times were recorded. A female was considered dead when no reaction was observed after contact with the hair of the small brush.

This experiment was replicated 5 times, which corresponds to a total follow-up of 50 females.

The number of days between emergence of the females and laying of the first eggs, referred to as the pre-oviposition period, was noted for each female. Knowing the length of this period in all monitored females, the average pre-oviposition duration was determined.

Then the period during which a female lays eggs (oviposition period) was recorded and the average was calculated from the oviposition time of all females monitored. The number of days between the laying of the last eggs and the death of the females, constituting the post- oviposition period of each female, was noted. Knowing this duration for all females, the average post-oviposition duration was determined as well as the average post-oviposition age.

The mortality rate (Rm) of each stage of development of *P. marginatus* was then evaluated by collecting and counting exuvia from all stages until the appearance of adult females in the pre-oviposition period. This rate (Rm) was calculated using the formula below:

$$R_m = \frac{N_1 - N_2}{N_1} \times 100$$

With N_1: Total number of individuals of the stage of development of *P. marginatus* considered, N_2: Total number of individuals in the next stage of development of *P. marginatus*.

2.5 Study of Reproductive Activity and Survival of *Acerophagus papayae* Females

Mummies of *P. marginatus* were collected from papaya leaves brought from the field and placed in transparent capsules serving as mummy banks. These mummy banks were monitored daily for 30 days while the parasitoid adults emerged. Each day, the emerged adults were collected and their sex noted using a magnifying glass. The presence of an ovipositor made it possible to distinguish between female and male individuals. The emerged adults were then placed in collection tubes to allow mating. For this purpose, ten males and five females were placed together in these tubes for a period of 24 hours and fed with a 1:1 dilute honey solution (1 ml of concentrated honey and 1 ml of clean water). These tubes were covered from above with a very fine mesh net. Twenty-four hours later, the tubes were transferred to petri dishes; the parasitoid individuals were put back into new transparent capsules in order to record their sex again and to recover the females for different tests.

In 5 other petri dishes, each containing Whatman paper N°1 soaked in water (to avoid drying of the papaya leaf), a papaya leaf carrying 30 mealybug individuals consisting of larval stages and adult females was glued to the What man paper. In each of these petri dishes, a female of *A. papayae* was introduced.

After 24 hours of contact with the mealybugs, the *A. papayae* females were removed and placed in new petri dishes prepared as before; this procedure was repeated until the parasitoid females died. The parasitized hosts were then monitored daily and as soon as mummies were obtained, they were collected and placed in transparent capsules. When the adults of the parasitoid emerged, they were counted and recorded according to sex.

This experiment was repeated 10 times for a total of 50 females monitored.

2.6 Statistical Analysis

XLSTAT 2008.6.03 software was used to analyze the results of the experiments. Comparisons of the means were made by analysis of variance (ANOVA) followed by the SNK (Student Newman Keul) test at the 5% threshold.

3. RESULTS

3.1 Determination of Some Biological Parameters of *P. marginatus*

3.1.1 Development time of *P. marginatus* on papaya

The average development time varies according to the stage and sex of the insect. It is 10.71 ± 2.49 days for eggs and 5.71 ± 0.75 days for L_1. For L_2, it is 7.28 ± 0.75 days for females and 7.14 ± 0.37 days for males. For L_3, it is 2.28 ± 0.48 days for females and 3.00 ± 0.53 days for males. The mean total development time of the male is relatively longer (29.70 ± 5.58 days) than that of the female (25.98 ± 4.47 days) (Table 1).

Table 1. Average development time of different stages of *P. marginatus* reared on papaya(n = 25)

Duration of development stages							**Total average duration**	
Eggs	**L_1**	**L_2**		**L_3**		**L_4**		
		Female	**Male**	**Female**	**Male**	**Male**	**Female**	**Male**
10.71 ± 2.49	5.71 ± 0.75	7.28 ± 0.75	7.14 ±0.37	2.28 ± 0.48	3.00 ± 0.53	3.14 ± 0.69	25.98 ± 4.47	29.70 ± 5.58
-	-	F = 27.8 P = 0.006		F = 2.64 P = 0.086		-	F = 7.2 P = 0.043	

3.1.2 Mortality rates of the different pre-imaginal stages of *P. marginatus*

Mortality rates for stages L1 and L2 are higher than for stages L3 and L4. The highest L1 mortality (61.40%) was offset by low L3 female mortality. There was no mortality in L3 and L4 stage males (Table 2).

Table 2. Mortality rate (%) of each larval stage of *P. marginatus* (n = 25)

Mortality rates (%) at different stages				
L1	L2	L3		L4
		Female	Male	
61.40 ± 0.05	52.8 ± 0.025	35.02 ± 0.03	0	0

3.1.3 Periods of reproductive activity and daily fertility of a Female *P. marginatus*

The different periods of *P. marginatus* reproductive activity, i.e. pre-oviposition, oviposition and post-oviposition periods are on average long 7.74 ± 1.26 ; 6.13 ± 3.02 and 7.45 ± 4.27 days respectively (Table 3). The oviposition period is from the 7th to the 24th day after the emergence of females. The number of eggs laid by females of *P. marginatus* fluctuated with their age. Females laid a maximum number of eggs (23 to 38) between the 8th and 11th days after emergence. Peak egg-laying is obtained on the 10th day after emergence (Fig. 1).

The average fertility of a female *P. marginatus* is 224.32 ± 29.99 eggs during her lifetime with a daily egg laying of 25.262 ± 11.16 eggs.

Table 3. Average duration of different periods of reproductive activity of *P. marginatus* females (n = 50)

Periods of reproductive activity	Average Duration (Days)
Pre-oviposition	7.74 ± 1.26
Oviposition	6.13 ± 3.02
Post-oviposition	7.45 ± 4.27

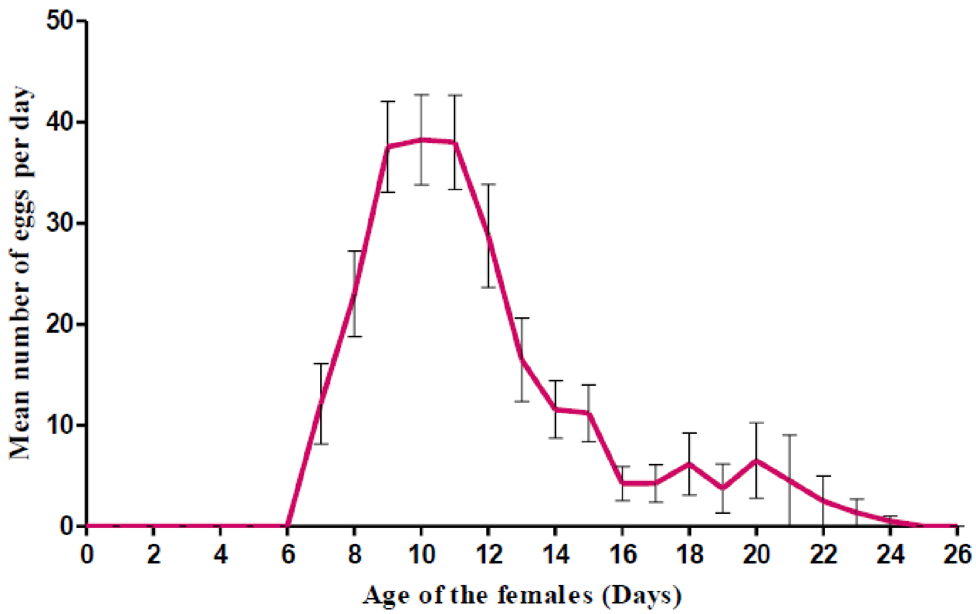

Fig. 1. Daily egg-laying capacity of the females of *P. marginatus* at 28 ± 2°C; 75 ± 5% HR; 12:12h LD
(F = 61.32; ddl = 49; P < 0.0001)

3.1.4 Survival of *P. marginatus* females

The average life span of females of *P. marginatus* is 18.44 ± 3.31 days. The survival rate of females of *P. marginatus* is highest when the age of the latter is between 1 and 11 days. From day 12 onwards, this rate decreases gradually until day 27 when no more females survive (Fig. 2).

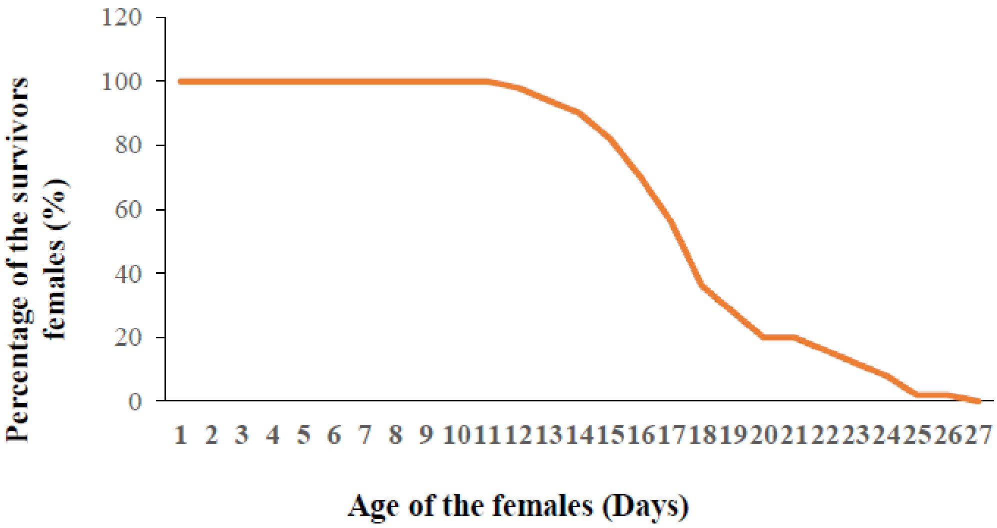

Fig. 2. Percentage of surviving *P. marginatus* females by age

3.2 Determination of Some Biological Parameters of *Acerophagus papaya*

3.2.1 Daily and total fertility of *A. papayae* females

A female *A. papayae* gives an average of 205.96 ± 9.08 offspring during her lifetime and 18.75 ± 9.087 individuals per day (Table 4). Its progeny are large during the first few days of female oviposition and then begin to decline to a single female individual on the last day of oviposition (day 13) (Fig. 3). At the end of the emergence period, the average number of female offspring (115.84 ± 4.43, i.e. 56.24% female) is greater than that of males (90.26 ± 4.67, i.e. 43.82% male) (Table 4).

3.2.2 Development time of *A. papayae* and life span of *A. papayae* females

The average life span of a female *A. papayae* is 13.02 ± 0.08 days (Fig. 4). The survival rate of *A. papayae* females is highest between 1 and 11 days old. From day 12 onwards, this rate decreases progressively until day 14 when no females survive (Fig. 4). Furthermore, the average development time of this species is 13.82 ± 0.013 days. The sex ratio (number of emerged females/number of males) of a female *A. papayae* is 1.28 ± 0.05 (Table 4).

Table 4. Some biological parameters of female *A. papayae* (n = 50) at 28 ± 2°C; 75 ± 5%RH; 12: 12h LD

Biological parameters	Male	Female	Cumulative
Total number of offspring	90.26 ± 4.676 (43.82%)	115.84 ± 4.433 (56.24 %)	205.95 ± 9.087
Number of offspring/day	8.22 ± 4.676	10.53 ± 4.433	18.75 ± 9.087
Sex-ratio (Proportion of females)	-	-	1.28 ± 0.05
Development time (Days)	-	-	13.82 ± 0.013
Longevity of a female (Days)	-	-	13.02 ± 0.080

(F = -2.009, ddl = 49; P < 0.0001)

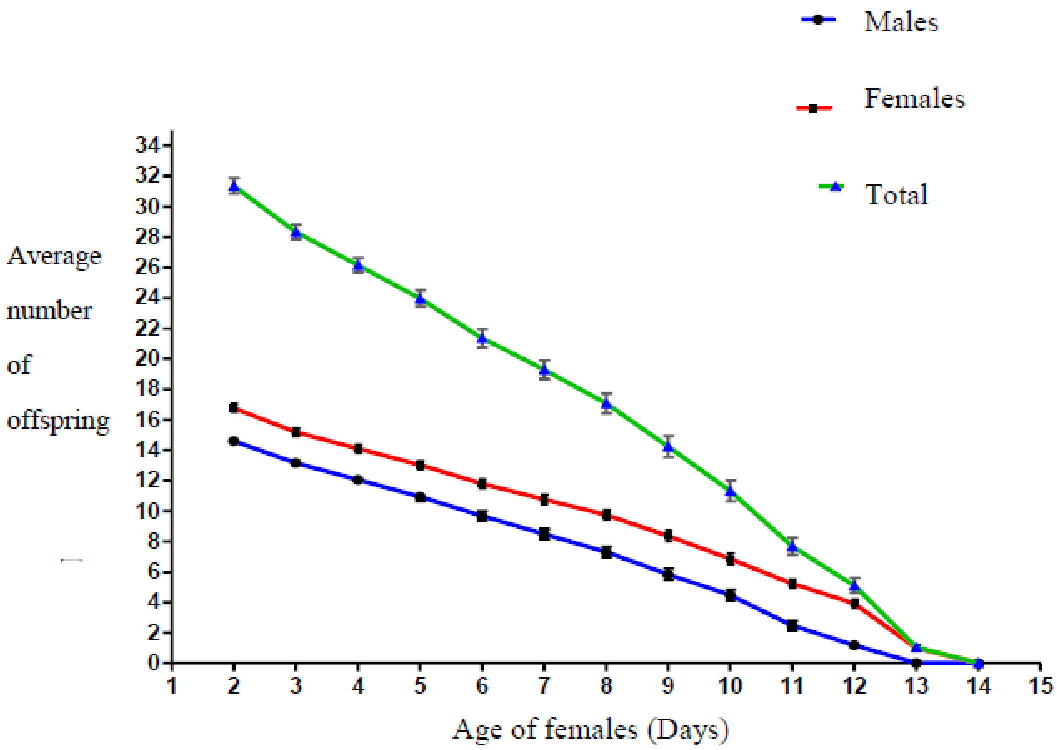

Fig. 3. Daily evolution of the emergence of the offspring of a female *A. papayae* at 28 ±2°C; 75 ± 5% RH; 12: 12 h LD
(F = -2.009, ddl = 49; P < 0.0001)

Fig. 4. Percentage of surviving *A. papayae* females according to age

4. DISCUSSION

The average development time of males of *P. marginatus* at 28 ± 2°C, 75 ± 5% RH (29.70 ± 5.58 days) is relatively longer than that of females (25.98 ± 4.47 days). Indeed, males have an additional stage of development (fourth larval stage which represents the nymph) that females do not have. This explains the relatively longer development time of males compared to females. These results are consistent with Tanwar et al [6], Walker et al [17], Amarasekare et al [25], Amarasekare [26], Mani et al [27] and Munwar et al [28].

In the adult stage, all females do not start laying at the same time. They spend a period called pre-oviposition which corresponds to a period of physiological maturity (oocyte formation and maturation). After this period, the oviposition period corresponding to the time between the first and last egg laying occurs. In this study, it extends to the 24th day of emergence of females. But the average laying period is 6.13 ± 3.02 days. These results are in agreement with those obtained by Hintenou [29] where just after laying, all females of *P. marginatus* do not die at the same time but live longer before dying. This post-oviposition period does not exceed 10 days and averages 7.45 ± 4.27 days. The longevity of a female *P. marginatus* is between 13 and 27 days after emergence and averages 18.44 ± 3.31 days.

A female *P. marginatus* laid an average of 25.26 ± 11.16 eggs per day for an average of 8.88 ± 3 days, which corresponds to 224.46 eggs likely to be laid during the lifetime of a female *P. marginatus*. Indeed, Walker *et al* [17] showed that a female *P. marginatus* usually lays between 100 and 600 eggs over a period of one to two weeks. However, the number of eggs laid daily is not uniform during the laying period. This number fluctuated from the first to the last day of egg laying. This fluctuation would probably be related on the one hand to the depletion of the oocyte stock in the female's ovary and on the other hand to the ageing of the female. Furthermore, the variability in the number of eggs laid per day per female would probably be related to factors intrinsic to each female. This is the first time that such a result has been obtained on the average daily fertility of *P. marginatus*. Furthermore, the high mortality rate in the early larval stages of *P. marginatus* could be explained by their high susceptibility to slight variations in environmental conditions; this may also be due to the movement of the early stages away from leaf tissue and their fall from plants as these stages are highly mobile. Stages intended to produce females are more vulnerable than those intended to give males. Indeed, no mortality was noted during the third and fourth larval stages of males unlike the third larval stage of females where a mortality rate of 35.02% was recorded. This difference would relate to the physiology of both sexes. The male appears progressively by passing through a pupal stage whereas the female is obtained at the end of the deep modifications of the larva of the third stage. These spontaneous changes probably have an impact on the survival of individuals. However, in nature, females play more important roles than males. This difference is probably related to the longevity of these different individuals. Indeed, males have a very short life span (2.5 days) compared to females (19.6 days).

According to Hemerik and Harvey [30] knowledge of the biology and history of a parasitoid is very important in assessing its efficiency and understanding its long-term impacts on the host-parasitoid system. Generally, the best biological control agents are those with a shorter developmental time than their host [31]. Also, the parasitoid must be able to multiply its population more rapidly than its host. According to the work of Amarasekare et al [25] the female of *P. marginatus* has a developmental time on hibiscus of about 25.9 days. Our present results show that the average developmental time of *A. papayae* is 13.82 ± 0.013 days while that of *P. marginatus* is 25.98 ± 4.47 days for the female and 29.70 ± 5.58 days for the male when the pest develops on papaya. Thus, the development time of the pest is almost double that of the parasitoid. This characteristic of the parasitoid shows that it can control its host. This short developmental duration of *A. papayae* obtained confirms that observed by Amarasekare et al [23].

Furthermore according to King [32], the production of progeny of the parasitoid after its introduction is important in its long term establishment as a biological control agent. According to the work of Amarasekare et al [23], a female *A. papayae* gives about 44.5 male and 48.3 female individuals after mating, in 24 hours. Our present results show that a female *A. papayae* after mating, in 24 hours, gives an average of 205.95 ± 9.087 offspring (115.84 ± 4.433 females and 90.26 ± 4.676 males) during her lifetime when the pest is reared on papaya. The preferred host plant of the pest probably influenced the reproductive function of *A. papayae*; indeed Amarasekare et al [23] reared the pest used in their experiments on red potato (*Solanum tuberosum* L.). In line with this, the work of Nisha and Kennedy [33] proves that the host plant on which the pest develops has an impact on the biology of *A. papayae*. Beside the host plant, increase in temperature negatively affected the performance of *A. papayae* in terms of developmental duration, parasitism efficiency and adult emergence [34, 35]. The longevity of a female *A. papayae* is on average 13.02 ± 0.08 days which is consistent with the work of Amarasekare et al [23].

5. CONCLUSION

This work focused on the evaluation of some biological parameters of *P. marginatus* such as: the duration of development of the different stages, the mortality rate of the pre-imaginal stages, the duration of pre-oviposition, the daily fecundity and average life span of a *P. marginatus* female and those of the parasitoid *A. papayae* such as: the daily and total fertility of the females, the average duration of development of the species and the average life span of the *A. papayae* females. The results show that the daily oviposition of a *P. marginatus* female is not uniform during the oviposition period, but fluctuates according to the age of the female. *P. marginatus* females show a pre-oviposition, oviposition and post-oviposition period of reproductive activity. During the oviposition period, oviposition peaks between days 9 and 11 after female emergence and then begins to decrease until day 25 when no oviposition occurs. Also during the development of the pest, the high mortality rates of the first and second female larval instar noted are related to the abiotic conditions of its environment. The average total development time of *P. margiantus* is about 29.70 days for the male and 25.98 days for the female. For *A. papayae*, the offspring are numerous during the first days of female emergence. A female can produce an average of about 205 individuals in her lifetime and 18.75 individuals per day dominated by females. A female *A. papayae* has an average lifespan of 13.02 ± 0.08 days, and the average development time of this species is 13.82 ± 0.013 days.

ACKNOWLEDGMENTS

We would like to thank IDRC/CORAF-WECARD and IITA for their support and for all facilities they provided us for the realization of this research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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This chapter is an extended version of the article published by the same author(s) in the following journal.
Journal of Agricultural Studies, 8(3), 2020.