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HOST PLANT SUITABILITY OF THE PAPAYA MEALYBUG, *PARACOCCUS MARGINATUS* WILLIAMS AND GRANARA DE WILLINK (HEMIPTERA: PSEUDOCOCCIDAE), AND THE HOST SPECIFICITY OF ITS NATURAL ENEMY, *ACEROPHAGUS PAPAYAE* NOYES (HYMENOPTERA: ENCYRTIDAE) INTRODUCED INTO AFRICA.

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DECLARATION AND APPROVAL

This is to certify that except for the reference to other people's works which have duly been acknowledged, this research work, which is submitted for the award of MPhil in Agricultural Entomology Degree under the African Regional Postgraduate Programme in Insect Science (ARPPIS) of the University of Ghana, Legon is entirely my own original research and has not been submitted for a degree in any other University.

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DEDICATION

This work is dedicated to the Almighty God for His blessings, to my beloved parents **Adolphe H. P. HINTENOU** and **Georgette L. DOSSOUMOU** for their everlasting love, constant sacrifices and unconditional supports, and to my lovely brothers and sisters, **Thierry**, **Freeda**, **Ulrich** and **Dolgette HINTENOU** for their love, encouragement, and support.

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LIST OF ACRONYMS

ANOVA:	Analysis of variance
APHIS:	Animal and Plant Health Inspection Service
ARPPIS:	African Regional Postgraduate Programme in Insect Science
ARS:	Agricultural Research Service
D/N:	Day / Night
EPPO:	European and Mediterranean Plant Protection Organization
FAO:	Food and Agriculture Organization of the United Nations
FAOSTAT:	Food and Agricultural Organization Corporate Statistical Database
GDP:	Gross Domestic Product
h:	Hour
HSD:	Honestly Significant Difference
IITA:	International Institute of Tropical Agriculture
IDH:	Indice de Développement Humain
PNUD :	Programme des Nations Unies pour le Développement
PPO:	Preferred Provider Organization

PPQ: Plant Protection and Quarantine

PPRSD: Plant Protection and Regulatory Services Directorate

RH: Relative Humidity

TCP: Technical Cooperation Programme project

USDA: United States Department of Agriculture

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ABSTRACT

The papaya mealybug *Paracoccus marginatus* Williams and Granara De Willink (Hemiptera: Pseudococcidae) is an invasive pest insect that can damage a wide range of agricultural crops. Successful biological control of this pest strongly relies on its mass rearing in order to produce its introduced natural enemy, *Acerophagus papayae*. Host susceptibility studies of *P. marginatus* were done on four different host plants: *Manihot esculenta* (cassava), S*olanum macrocarpon* (African egg-plant), *Vernonia amygdalina* (bitter leaf) and the primary host, *Carica papaya* (papaya) at 28° C, 75% RH and a photoperiod of 12:12 (D/N) h. *P. marginatus* failed to complete its development on *V. amygdalina*. The development and survival of host instars, the cumulative development time (first instar to adult), adult fecundity and longevity were significantly affected by host plants species. *C. papaya* has permitted the complete development of both male and female of *P. marginatus*. Females had the shortest developmental time on *C. papaya* (15 days) with low mortality registered during the late instars and the highest fecundity (277.40 eggs). The longest developmental time (19 days) and the longest longevity period (31.50 days) of the adult female were on *M. esculenta*. On this host plant, females laid 227.75 eggs within 10 days. *P. marginatus* developmental time on *S. macrocarpon* was about 18 days. Adult females live longer on *S. macrocarpon* but had laid the lowest number of eggs (141.23 eggs). The pre-oviposition periods were not affected by the host plants while the oviposition and the post-oviposition were significantly different. On the other hand, the potential non-target effects of its natural enemy, *Acerophagus papayae* were assessed. Host specificity tests were conducted on three associated mealybugs (*Phenacoccus manihoti*, *P. solenopsis* and *Ferrisia virgata*) sharing at least one host plant with the target insect pest, *P. marginatus*. The suitability of the four selected mealybug species was evaluated under choice, *P. marginatus* paired with one of the non-target mealybug

and no-choice test conditions. Foraging behavior of the parasitoid recorded in one-minute time segments over a full hour shows that in both choice and no-choice condition, *A. papayae* parasitized all stages of *P. marginatus* except for the ovisacs. First instars and female plus ovisacs being the least parasitized, however, *A. papayae* preferred the third instars and the young females (pre-reproductive females). The presence of non-target organisms does not affect the behavior of *A. papayae* in host selection for oviposition.

RESUME

La cochenille du papayer *Paracoccus marginatus* Williams and Granara De Willink (Hemiptera: Pseudococcidae) est une espèce invasive qui attaque une gamme de plantes hôtes causant de sévère dommage. La lutte biologique contre cette espèce passe par sa production de masse au laboratoire pour la multiplication de son ennemie naturel, *Acerophagus papayae*. La susceptibilité de *P. marginatus* à quatre plantes hôtes : *Manihot esculenta* (manioc), S*olanum macrocarpon* (grande morelle), *Vernonia amygdalina* (vernonia) et l'hôte principal, *Carica papaya* (papaye), a été étudiée au laboratoire à 28° C, 75% RH et une photopériode de 12 :12 (lumière/obscurité). *V. amygdalina* n'a pas permis le développement complet de *P. marginatus*. Le temps de développement et la survie des différents stades, la fécondité et la survie de la femelle adulte sont significativement influencés par les plantes hôtes. *P. marginatus* se développe plus rapidement sur *C. papaya* (15 jours) avec un faible taux de mortalité enregistré au niveau des stades matures. La femelle a une courte durée de vie sur *C. papaya* (22 jours) et pond en moyenne 277, 4 œufs. Par contre la plus longue durée de développement (19 jours) et la plus longue durée de vie (31,50 jours) de la femelle adulte étaient sur *M. esculenta*. Cependant, le plus petit nombre d'œuf est pondu sur *S. macrocarpon* (141,23 jours). La durée pré-ponte n'est pas affectée par les différents plantes hôtes alors que la durée de ponte et post-ponte étaient significativement affectées par les différents plantes hôtes. Le potentiel effet de trois cochenille non-cible (*Phenacoccus manihoti*, *P. solenopsis* et *Ferrisia virgata*) associés à *P. marginatus* a été évalué en condition de choix et de non choix. Par minute, le comportement du parasitoïde a été noté pendant une heure d'observation. En condition de choix et de non choix, *A. papayae* parasite tous les stades de développement de *P. marginatus* excepté les ovisacs. Le premier stade et la femelle adulte plus ovisacs étaient moins parasités. Cependant, *A. papayae* préfère le troisième et la jeune femelle. La

présence d'une cochenille non-cible n'influence pas le comportement de *A. papayae* en matière de choix d'hôtes pour la ponte.

CHAPTER ONE

1.0 General Introduction

1.1 Background

Agriculture for many people of the world remains a subsistence activity. About 1.3 billion of people of the worldwide active population rely on agriculture to live (FAOSTAT, 2011). It contributes to growth as a supplier of raw materials for the industry, to a market for indigenous industrial products, and as a substantial source of foreign export earnings (Raisbeck, 2003). Agriculture forms a significant portion of the economies of all African countries and as a sector, it can contribute towards major continental priorities, such as eradicating poverty and hunger, boosting intra-Africa trade and investments, sustainable resource and environmental management, and creating jobs. In Benin, it is the largest economic sector where it accounts for 38% of the Gross Domestic Products (GDP) and generates 70% of employment (PNUD-IDH, 2011). Agricultural products include grains and oilseeds, livestock, dairy, forest products and others. A large array of agricultural commodities such as fruits and vegetables contribute to economic and social development and play a key role in food security and public health (Aho and Kossou, 1997).

Worldwide, over 73.02 million (M) metric tonnes (t) of tropical fruits were produced in 2010 (FAOSTAT, 2012a). The production of Papaya, *Carica papaya* L. is considered among others one of the most important fruits in the tropical and sub-tropical zones. Papaya is a rich source of antioxidant nutrients, B vitamins, minerals, fibre and of the digestive enzyme papain. The latter is used as an industrial ingredient in brewing, meat tenderizing, pharmaceuticals, beauty products, and cosmetics (Evans and Ballen, 2012). About 60 countries in the world produce papaya with the bulk of production occurring in developing economies. Papaya rankes third with 15.4% of the total tropical fruit production behind mango with 52.9% and pineapple with 26.6%. The African continent has a share of 13.2% of the global papaya production region between 2008 and 2010 (FAOSTAT, 2012a).

In Benin, commercial papaya production is concentrated in Southern Benin including Atlantique, Mono, and Ouémé where agro-ecological conditions are more favourable for all-season development of this plant. The main variety produced in this region is “Sunrise solo 7212”. This variety is small and appreciated because of its flavour. About 100 to 150 tons are produced per hectare within 24 months. Papaya production constitutes a significant source of income (Montcho, 2013).

1.2 Problem statement

Papaya is attacked by a number of insect pests including small, soft-bodied insects called mealybugs that belong to the family Pseudococcidae (Hemiptera: Sternorrhyncha). Mealybugs occur worldwide and are most abundant in the tropics and subtropics (Ben-Dov, 1994). More than 20% of mealybug’s pest species may be polyphagous (Miller *et al.*, 2002). Among the 2000 mealybug species already described, many are important insect pests of various agricultural crops. They cause considerable economic damage by sucking sap from plants (Williams and Granara de Willink, 1992; Miller *et al.*, 2002). Damage results from phloem sap removal, the injection of toxins, honeydew contamination and associated sooty mould growth, and occasionally also from the effects of transmitted plant viruses (McKenzie, 1967; Miller and Miller, 2002; Walker *et al.*, 2003; Heu *et al.*, 2007).

Generally, mealybugs are not pest problems in their country of origin where they are usually controlled by predators and specific parasitoids, however, they may become pests when accidentally introduced in new environments without their natural enemies. In Africa, a rich diversity of indigenous plant species coupled with conducive climatic conditions facilitated their introduction into the continent. A good example of mealybug invasion is the case of the cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero (Homoptera: Pseudococcidae) which devastated cassava crops in Africa, leading to a food shortage in several countries (Herren and Neuenschwander, 1991). In the early 1980's, *Rastrococcus invadens* Williams (Homoptera: Pseudococcidae) was also accidentally introduced into Africa from South-East Asia where it originates (Williams, 1986). It has been observed for the first time in Togo and Ghana before spreading into most countries of West Africa where it causes damage to mango trees and other fruit trees (Agounké and Bokonon-Ganta, 1988; Ivbijaro *et al.*, 1992). More recently, the cotton mealybug, *P. solenopsis* Tinsley (Hemiptera: Pseudococcidae) has spread through Asia and become a key pest of cotton in Nigeria and Benin (Akintola and Ande, 2008; Hodgson *et al.*, 2008). Currently, a new species of mealybug, *Paracoccus marginatus* Williams and Granara de Willink originating from Mexico and Central America was accidentally introduced into the Caribbean, Central America and the Pacific islands (Miller *et al.*, 1999). On the African continent, it was first detected in Ghana in late 2009 (Cham *et al.*, 2011). Subsequently, it spread to Togo, Benin, Nigeria, Cameroon, and lately to Gabon. In Benin, it was reported in early 2010 and rapidly expanded throughout the country (Goergen *et al.*, 2011).

Paracoccus marginatus attacks a large number of tropical and subtropical fruits, vegetables, ornamental, greenhouse plants, wild plants and weeds but papaya is the prime target (Miller and Miller, 2002). It causes severe economic losses due to damages such as yellowing, crinkling distortion of leaves, sooty mould development, early leaves and fruits drop, plant stunting and death. Heavy infestations can cause yield losses of up to 65% which led in Ghana to the shrinking of papaya orchards to 380 ha in 2010 (Cham *et al.*, 2011). As a result, export earnings of the papaya industry dropped significantly and 1700 people in the sector lost their jobs (Goergen *et al.*, 2011). On a world scale, data indicate that the papaya mealybug can develop on over 86 plants species in 35 plant families (Miller *et al.*, 1999; Meyerdirk and Kauffman, 2001; Meyerdirk *et al.*, 2004; Muniappan *et al.*, 2008; ManiChellappan, 2010; Tanwar *et al.*, 2010; Cham *et al.*, 2011; Selvaraju and Sakthivel, 2011).

1.3 Justification

The control of pest mealybugs involves several management strategies by both smallholder and large-scale producers. Most farmers mainly rely on synthetic chemicals such as dimethoate, Malathion, carbaryl, chlorpyrifos, diazinone, acephate (Walker *et al.*, 2003). Currently, neo-necotinoid insecticides (acetamiprid, clothianidin, dinotefuran, imidacloprid, thiamethoxam) and insect growth regulators (IGR) such as pyriproxyfen are commonly in use to control scale insects and mealybugs (Buss and Turner, 2006). Indiscriminate and frequent applications of these synthetic chemical insecticides have resulted in negative impacts on natural enemies, environmental contamination, health risks, pesticide residues and development of resistance (Losenge, 2005). The persistence of these chemicals in the environment can be relatively long, ranging from days to years. Also, the efficiency of chemicals in the management of mealybugs is often problematic because of the thick waxy secretion covering their body and their ability to rapidly build up dense populations. The high degree of polyphagy of some species of mealybugs such as *P. marginatus* is again a limiting factor in the use of insecticides. This has prompted the development of non-chemical methods such as biological control, an effective and environmentally friendly management strategy (Migiro *et al.*, 2010).

Biological control involves the use of natural enemies such as parasitoids, predators, pathogens, antagonists, or competitors to suppress pest populations making them less abundant and thus less damaging (Van Driesche and Bellows, 1996). Among general approaches widely accepted in biological control, classical biological control is the commonest (Orr and Suh, 1998). It consists in the management of an invasive pest population by the introduction of the pest's natural enemy from its native range into the newly invaded area. This approach was identified as an important option in the management of *P. marginatus* since it is an exotic pest that potentially poses a threat to numerous agricultural crops (Walker *et al.*, 2003).

In the search for natural enemies of *P. marginatus*, a team of scientists from the United States Department of Agriculture (USDA), Agricultural Research Service (ARS) went on a foreign exploration in Mexico in 1999. Subsequently, three solitary endoparasitoids previously unknown to science were discovered and described as *Anagyrus loecki* Noyes, *Acerophagous papayae* Noyes and Schauff and *Pseudleptomastrix mexicana* Noyes and Schauff (Hymenoptera: Encyrtidae) (Noyes and Schauff, 2003). These parasitoids were screened under quarantine at the USDA ARS Beneficial Insects Laboratory in Newark, Delaware. Environmental assessments were carried out by Meyerdirk (1999) and following clearance, parasitoids were shipped to San Juan, Puerto Rico. They were then mass produced at the Puerto Rico Department of Agriculture and USDA, Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ). In the framework of several biological control attempts, these parasitoids were released in the Dominican Republic, Puerto Rico, Florida and Guam (Meyerdirk *et al.*, 2004). Ever since, further programmes have been successfully implemented in the Caribbean, South America and Hawaii (Muniappan *et al.*, 2006; Heu *et al.*, 2007).

The magnitude of the problem posed by introduction of *P. marginatus* into the African continent has called for an immediate and sustainable action to prevent the pest from further spreading and causing severe damage. Nigeria, the third biggest producer of papaya in the world, is particularly at risk. Given the success of its bio-control in Asia and the Pacific, a classical biological control program was initiated as a joint effort between the Plant Protection and Regulatory Services Directorate Ghana (PPRSD), Food and Agriculture Organization of the United Nations (FAO) and International Institute of Tropical Agriculture (IITA) in 2010. Under the framework of an emergency TCP (Technical Cooperation Programme project), USDA-APHIS was contacted to provide *P. marginatus* specific parasitoids from their mass-rearing facilities in Puerto Rico. Arrangements for the first consignments to Ghana of the encyrtid endoparasitoids *A. papayae* and *A. loecki* were made in order to establish stock colonies at the PPRSD's Biocontrol Insectary. Whereas first field releases took place in 2011, PPRSD was committed to serve as a parasitoid source for Benin or any other African country affected by *P. marginatus* invasions (Goergen *et al.*, 2011).

In 2012, with regard to the rapid expansion of the pest in West and Central Africa, IITA initiated a new project, funded by the Swiss Agency for Development and Cooperation, to tackle the problem at a regional level. In close collaboration with quarantine authorities of Ghana and Benin, natural enemies were transferred to the IITA facilities of the Biological Control Center for Africa, Cotonou, Benin, where they are now being mass-reared to similarly serve as a source for project partners and other African countries.

The success of the biological control project however strongly relies on the mass rearing of parasitoids which in turn implies a reliable mass production of its natural host (mealybug) under laboratory conditions. The present research thus focused on studying the host plant susceptibility of *P. marginatus* to optimize the production of its parasitoids under laboratory conditions and also assessing the effectiveness of the newly introduced parasitoid. Therefore laboratory tests were carried out to determine the host specificity of *A. papayae* and to verify potential non-target effects on other mealybug species commonly encountered in West Africa.

1.4 Research objectives

1.4.1 General objective

The overall objective of this study was to identify the best adapted host plant of *Paracoccus marginatus* for the continuous production of its natural enemy, *Acerophagus papayae* and the evaluation of its potential as a biological control agent.

1.4.2 Specific Objectives

The following specific objectives are to:

- Assess the reproductive performance of *P. marginatus* on four common field crops;
- Identify the most suitable alternative host for an optimal production of *P. marginatus* and its parasitoid, *A. papayae* under laboratory conditions;
- Assess the host specificity of *A. papayae* on three widespread mealybug species *Phenacoccus manihoti*, *P. solenopsis* and *Ferrisia virgata* commonly occurring together with *P. marginatus* in West Africa and

- Evaluate the host preference of *P. marginatus* developmental stages for the development and reproduction of *A. papayae*

1.5 Research hypothesis

- The reproductive performance of *P. marginatus* is specific to each of the tested host plants;
- All four field crops can be used for the mass production of *P. marginatus* under laboratory conditions.
- *A. papayae* is host specific to *P. marginatus* either in association with non-target mealybugs or not.
- *A. papayae* preferred all development stages of *P. marginatus* for ovipoition

CHAPTER TWO

2.0 Literature Review

2.1 Mealybugs

Mealybugs are small soft-bodied, sap sucking insects that belong to the order Hemiptera, suborder Homoptera and the infra-order Sternorrhyncha. They are members of the superfamily Coccoidea that includes the family Pseudococcidae (Borror *et al.*, 1992). The family comprises approximately 2000 species in 300 genera (Ben-Dov, 1994). Their appellation is due to the mealy or waxy secretions that cover the bodies of these insects (Borror *et al.*, 1992). The layer of fine and powder-like wax often extends laterally to form a series of short filaments (Williams and Granara de Willink, 1992).

Mealybugs have four female instars and five in the male. The body of the adult female is usually elongate to oval, and membranous (Williams and Granara de Willink, 1992). They possess 6 to 9 segmented antennae. The legs have one-segmented tarsi with a single claw (Williams and Granara de Willink, 1992). The mouthparts of the Hemiptera are extensively adapted for piercing and sucking and are generally active throughout their life (Ben-Dov, 1994). They have modified mandibles and maxillae forming a slender bristle-like stylet, which rests in the grooved labium. The stylet is a hollow seta-like structure being capable of limited protrusion and retraction by means of muscular action. Stylets can be sometimes extremely long and in some cases greatly exceed the total body length.

Mealybugs have a diverse array of life history strategies from occurring in grass blade sheaths, feeding on rootlets, to occurring exposed on leaves. Many mealybugs overwinter as second instars, although adults, first instars, and eggs also can behave alike. Eggs or first instars are laid by the adult female. Eggs are normally laid in an ovisac that can enclose all or part of the body of the female. Most species that lay first instars rather than eggs lack any substantial ovisac. Most species have 1 or 2 generations a year, although some are reported to have as many as 8 generations in greenhouses. Both parthenogenetic and sexual species are common. In the tropics, their life cycle may be reduced to less than one month. They often attain high numbers, killing their host plant (Ben-Dov, 1994).

2.1.1 Economic importance of mealybugs

Many of the 2000 mealybug species yet described are important insect pests of a number of agricultural crops (Williams and Granara de Willink, 1992). They are mostly phloem-sucking plant parasites, some of which are very important agricultural pests and about 158 species are recognized as pest worldwide (Miller *et al.*, 2002). They are found throughout the world except in Polar Regions, but are most abundant in the tropics and subtropics (Ben-Dov, 1994). Although some are host specific, mealybugs are mostly polyphagous insects. Their host preference ranges from grasses (Poaceae) to woody plants (Asteraceae) (Ben-Dov, 1994; Ben-Dov and German, 2003), They cause damage by depleting the sap from all plant parts such as feeders on roots, root crowns, stems, twigs, leaves, flowers, and fruits. They can occasionally inject toxins, transmit viruses or excrete large amounts of honeydew stimulating the growth of sooty mould (Ben-Dov, 1994). Injured plants have discolored, wilted, and deformed leaves (Eileen and Turner, 2001).

In their countries of origin mealybugs do not cause serious pest problems due to the presence of specific parasitoids and/or predators. Serious outbreaks occur when mealybugs are accidentally introduced into new countries without their natural enemies. Polyphagous mealybugs pose a serious threat because of their tendency to adopt new host plants easily. Invasive insects such as the cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae) is a serious pest of cassava throughout the cassava belt in Africa (Herren and Neuenschwander, 1991). A newly particularly invasive species is the pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae), serious pest of many plants in tropical and subtropical regions, including Africa, Southeast Asia, and northern Australia (Hoy *et al.*, 2006). *M. hirsutus* invaded several islands in the Caribbean and the U.S. Virgin Islands (Hoy *et al.*, 2006). Most recently, the papaya mealybug, *P. marginatus* Williams and Granara de Willink, has received more attention as a pest of papaya and other economically important fruits, vegetables and ornamentals since its accidental introduced into the Caribbean, the US and the Pacific islands and Africa, from Central America.

2.2 A new invading species of mealybugs in West Africa

2.2.1 *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae)

Paracoccus marginatus was described by Williams and Granara de Willink in 1992. It belongs to the Kingdom: Animalia; Class, Insecta; Order: Hemiptera and the Family: Pseudococcidae. It is commonly named Papaya mealybug. In the genus *Paracoccus*, 79 species are recorded among which two species are recognized as major economic pests, *P. burnerae* (Brain) and *P. marginatus* Willink (Ben-Dov, 1994; Miller *et al.*, 1999).

2.2.1.1 Description of *Paracoccus marginatus*

The adult female of *P. marginatus* is yellow in colour, wingless and measures approximately 2.2 mm in length and 1.4 mm in width (Miller *et al.*, 1999). The body is covered with a white waxy coating with a series of short waxy caudal filaments less than a quarter of the length of the body around the margins (Plate 1) (Miller *et al.*, 1999; Walker *et al.*, 2003). Female adults lay greenish-yellow eggs in an ovisac, positioned caudally beneath the body and can be as much as twice as long as the body (Miller *et al.*, 1999). The newly emerged crawler pass through three immature instars before becoming adult females. Crawlers move actively and search for feeding sites. At the third instar, they are no longer capable of removing their stylet from the plant because its length is long as their body length. They then remain sedentary all along the rest of their life cycle. Two characteristics that are important in distinguishing adult females of *P. marginatus* from all other *Paracoccus* species are: a) the presence of oral-rim tubular ducts of one size aggregated in conspicuous clusters dorsally and restricted to marginal areas of the body, and b) the absence of pores on the hind tibiae (Fig. 1) (Walker *et al.*, 2003).

Plate 1. Adult female of *Paracoccus marginatus*

Source. Chen *et al.*, 2011

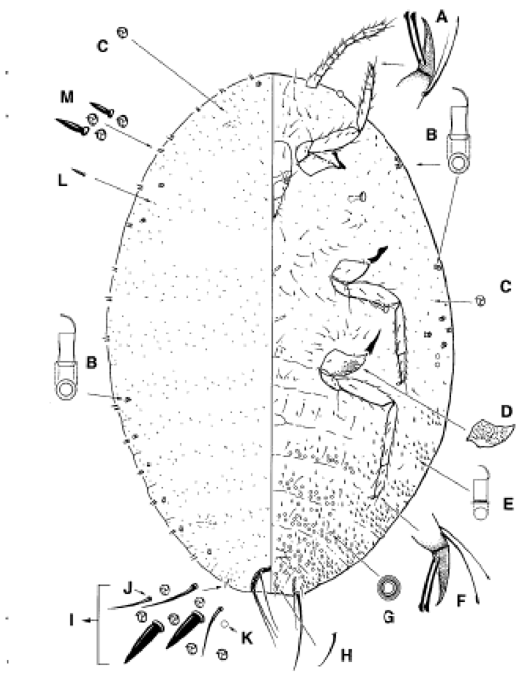


Figure 1. External morphological characters of *Paracoccus marginatus* adult female

Source. Miller and Miller, 2002

During the first and second instar stage, adult color is yellow but turns to pink in the latter part of the second instar throughout the pre-pupal and pupal stages (Miller and Miller, 2002; Sharma *et al.*, 2013). Later, it develops a cottony sack around itself. Adult males are approximately 1.0 mm in length with an elongate-oval body that is widest (0.3 mm) at the thorax (Miller and Miller, 2002; Sharma *et al.*, 2013) (Plate 2). Male goes through four larval stages to reach adulthood unlike the female. Male third instar is termed as "prepupa". The final larval stage of the male (fourth instar) is completed in a cocoon as a so-called pupa (Miller *et al.*, 1999). They have ten-segmented antennae, a distinct aedeagus, lateral pore clusters, a heavily sclerotized thorax and head and well-developed wings. They may be distinguished from other related species by the presence of stout fleshy setae on the antennae and the absence of fleshy setae on the legs (Fig. 2) (Miller *et al.*, 1999; Walker *et al.*, 2003). There is no distinguishable difference between male and female crawlers, and male and female early second instars.

Plate 2. Adult male of *Paracoccus marginatus*

Source. Chen *et al.*, 2011

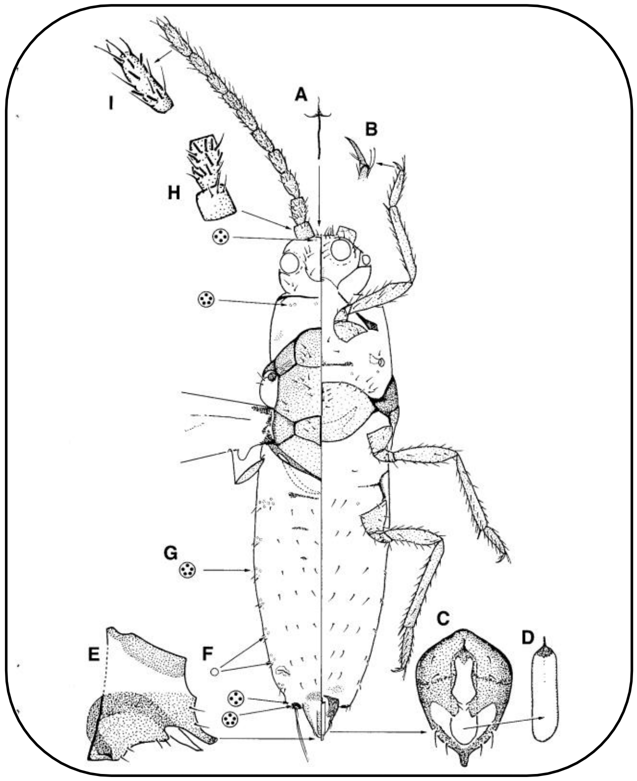


Figure 2. External morphological characters of *Paracoccus marginatus* adult male

Source. Miller and Miller, 2002

2.2.1.2 Origin and Global distribution of *Paracoccus marginatus*

P. marginatus is native to Mexico and Central America where it is not a serious pest since it is controlled there by natural enemies (Miller *et al.*, 1999). It was first described from specimens collected from the neo-tropical region namely in Belize, Costa Rica, Guatemala and Mexico (Williams and Granara de Willink, 1992). It was re-described by Miller and Miller in 2002. In the early 1990s, *P. marginatus* become an invasive species and was found to occur outside its native range and. It was introduced into the Caribbean Islands in 1995 where it spread rapidly and is considered a pest of papaya (Miller *et al.*, 1999). In 1998, *P. marginatus* was recorded on hibiscus for the first time in Florida in Palm Beach County and subsequently it spread into several other counties of that state (Miller *et al.*, 1999). It was recorded on papaya in several Pacific Islands such as Guam in 2002 (Meyerdirk *et al.*, 2004), the Republic of Palau in 2003 (Muniappan *et al.*, 2006), and Hawaii in 2006 (Heu *et al.*, 2007). In early 2008, it has expanded to South-East Asia where it was first discovered in the western provincial districts Colombo and Gampaha in Sri Lanka, infesting a large number of plant species (Galanihe *et al.,* 2011). *P. marginatus* has spread to the Indian subcontinent before reaching the African continent.

In late 2009, *P. marginatus* was discovered for the first time on the African continent in the Accra region in Ghana. Soon it has been reported to severely threat papaya in orchards at Nsawam in the Eastern Region and Bawjiase in the Central Region, about 30 km North and 60 km East of Accra, respectively (Cham *et al.*, 2011). In early 2010, it had been observed in Benin to occur mainly on cassava and Jatropha (Goergen *et al.*, 2011). The pest then expanded along the West African coast and invaded Togo, Nigeria, Cameroon, and lately also Gabon. It was found in Senegal in 2013 and Seychelles in 2014 (Muniappan, 2014).

2.2.1.3 Biology and life cycle of *Paracoccus marginatus*

Paracoccus marginatus is more active in warm, dry weather. The full life cycle requires between one to two months depending on the season (Mahalingam *et al.*, 2010). *P. marginatus* has a rapid rate of development, high survival rate, and enormous reproductive capacity that allows the population to reach high levels in a short time. An individual female usually lays 100 to 600 in an ovisac over a period of one to two weeks (Walker *et al.,* 2003) with the highest fecundity at around 25˚ C (Amarasekare *et al.*, 2008). Eggs hatch in about 10 days and newly emerged crawlers begin to actively search for feeding sites. Female crawlers have four instars, with the life cycle taking 24-26 days to complete at 25˚ C (Tanwar *et al.,* 2010). The development is temperature dependent, with females requiring 294 degree-days to complete the process (Amarasekare *et al.*, 2008). Adult males do not feed and live for a very short time. The adult male life cycle requires 303 degree-days (Amarasekare *et al.*, 2008) and consists of five instars, whereby the fourth is produced in a cocoon (pupal stage). The fifth instar of the male is the only winged form of the species capable of flight (Walker *et al.*, 2003). Males have a longer developmental time (27-30 days) than females (24 - 26 days) at 25±1° C, 65 ± 2% RH and 12:12 (D/N) photoperiod (Tanwar *et al.*, 2010) (Fig. 3).

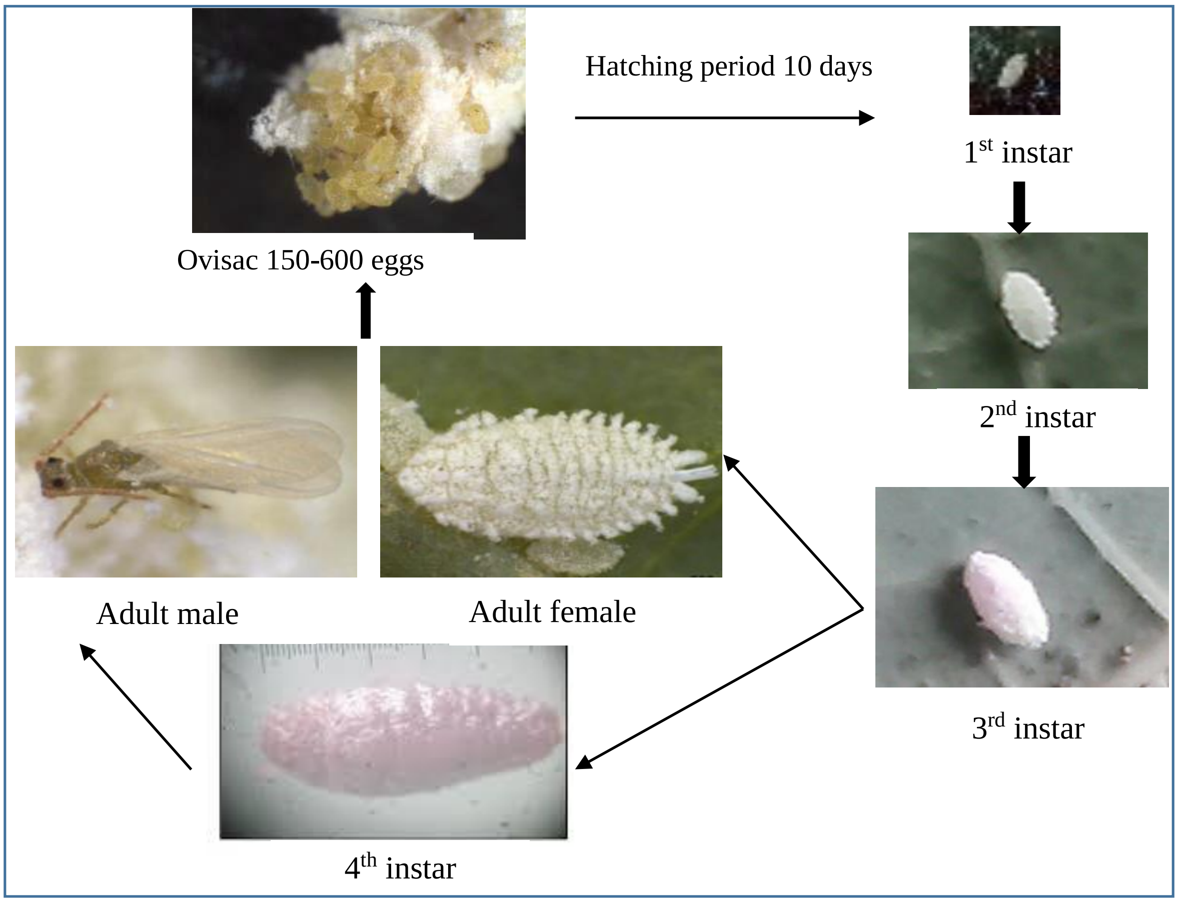


Figure 3. Life cycle of *Paracoccus marginatus*.

Source. Tanwar *et al.*, 2010.

2.2.1.4 Host plant range of *Paracoccus marginatus*

Papaya mealybug is a polyphagous insect even though its main host is papaya (Williams and Granara de Willink, 1992). It has great potential to cause economic damage to a wide range of host plants including crops, fruits, vegetables, and ornamental plants. A total of 86 host-plant species belonging to 35 families were recorded from several part of world to be infested by *P. marginatus* (Miller *et al.,* 1999; Walker *et al.,* 2003; Meyerdirk *et al.,* 2004; Muniappan *et al.,* 2008, Tanwar *et al.,* 2010; Galanihe *et al.,* 2011; Cham *et al.,* 2011; Selvaraju and Sakthivel, 2011). The economically important hosts include papaya, cassava, Jatropha, avocado, citrus, cotton, tomato, eggplant, pepper, beans and peas, sweet potato, mango, hibiscus, and pomegranate (Miller and Miller, 2002; Heu *et al.,* 2007; Cham *et al.,* 2011; Saengyot and Burikam, 2011).

2.2.1.5 Damage symptoms of *Paracoccus marginatus*

Papaya mealybug infestation appears on above ground parts on leaves, stem and fruits as clusters of cotton-like masses (Tanwar *et al.,* 2010). The adults and nymphs of *P. marginatus* suck out sap from the phloem tissues of plants along the veins of older leaves, on stems and fruits. They inject harmful substance into the leaves or plants resulting in chlorosis, plant stunting, leaf deformation, early leaf and fruit drop, and death of host plants. They secrete a heavy buildup of honeydew suitable medium for the growth of sooty mould reducing the normal photosynthesis and gaseous exchange. This leads to the death of the plants within a few months after infestation (Williams and Granara de Willink, 1992; Ben-Dov, 1994). In heavy infestations, the fruit are inedible due to the aggregation of thick white waxy appearance (Walker *et al.,* 2003; Heu *et al.,* 2007). On slightly infested plants, papaya mealybugs look like small pieces of cotton attached to the plant particularly on papaya trees and look like the oozing of milky sap (Plate 3 and 4).

Plate 3. Infesttion of *Paracoccus marginatus* on papaya fruits (left) and pepper (right)

Plate 4. Infestation of *Paracoccus marginatus* on Jatropha leaf

2.3 Others invasive species of mealybugs associated with *Paracoccus marginatus*

Beside the genus *Paracoccus*, the family Pseudococcidae encompasses further injurious species attacking various fruits and other cultivated plants among which the genera *Phenacoccus* and *Ferrisia*. To date the genus *Phenacoccus* accounts for 206 species and quite a few are polyphagous (Ben-Dov *et al.,* 2013). *Phenacoccus* species are widely distributed throughout the world's zoogeographical regions and most of the species originate from Palearctic, Nearctic and Neotropical areas (Danzig, 2003; Downie and Gullan, 2004; Granara de Willink and Szumik, 2007). Some species have been involved in serious pest outbreaks. An example is *P. manihoti* on cassava in Africa (Herren and Neuenschwander, 1991). During the last two decades *P. solenopsis* has spread from North America (Mexico and USA) to Central America, South America, Asia, etc. (Wang *et al.,* 2010). In Africa, it was described as a serious pest on *Hibiscus rosa-sinensis* in Nigeria (Akintola and Ande, 2008).

2.3.1 *Phenacoccus manihoti*, Matile-Ferrero (Hemiptera: Pseudococcidae)

2.3.1.1 Description of *Phenacoccus manihoti*

Adult females are ovoid, rose-pink and dusted with white powdery wax and with an apparent segmentation on their body. The latter bear very short lateral and caudal white wax filaments in the form of swellings that produce a toothed appearance to the body outline (Plate 5). Their eyes are relatively prominent. All instars bear well developed legs of equal size (Matile-Ferrero, 1978). Eggs are oblong, golden yellow and laid enclosed in woolly and sticky ovisacs located at the posterior end of the adult females (Matile-Ferrero, 1978; Nwanze, 1978). The first instars have 6-segmented antennae. The subsequent instars (second instars and third) have 9-segmented antennae.

The fourth instar or the newly emerged adult measures 1.1-2.6 in length mm and 0.5-1.4 mm in width (Matile-Ferrero, 1978; Nwanze, 1978).

Plate 5. Adult female of *Phenacoccus manihoti*

2.3.1.2 Origin and Global distribution of *Phenacoccus manihoti*

The cassava mealybug is native to South America where it is host-specific to cassava. It is present only in restricted areas of Paraguay, Brazil and Bolivia (Williams and Granara de Willink, 1992). It was described in 1977 by Matile-Ferrero (Löhr *et al.*, 1990). *P. Manihoti* was accidentally introduced into Africa where an outbreak of the pest was first observed near Kinshasa (Zaire) and Brazzaville (Congo) in the early 1970s and quickly it became the most important pest on cassava. In the absence of its natural enemies it spread rapidly across tropical Africa causing considerable yield loss (Herren, 1981; Herren and Neuenschwander, 1991). In Benin, *P. manihoti* was first observed in 1978 near Dangbo and Sakété and it rapidly spread throughout almost all the country

(Herren, 1981). Over the years, *P. Manihoti* has expanded throughout the entire cassava belt of Africa, with the major exception of Madagascar. It was also reported as a cassava pest in Argentina (Granara de Willink, 2003). In 2008, it reached Asia, being first detected in Thailand. Since, it invaded aggressively throughout Thailand's cassava-growing region (Winotai *et al.*, 2010) and invaded its neighboring countries and Indonesia (Muniappan *et al.*, 2009).

2.3.1.3 Biology and life cycle of *Phenacoccus manihoti*

Phenacoccus manihoti are generally located on the underside of the cassava canopy leave, mainly around major leaf veins and at low density inside growing tips. In Congo, the populations of *P. manihoti* begin to build up in February. They pass through nine generations with the largest numbers being observed during the dry season. Population densities drop at the onset of the rainy season, when many mealybugs are washed off the plant (Fabres, 1980; Fabres and Boussienguet, 1981). Within cassava fields, this mealybug occurs in a markedly aggregated distribution pattern, which differs between seasons (Herren and Neuenschwander, 1991).

Phenacoccus manihoti reproduces by parthenogenetic oviparity and thus produces only female offspring. It has four developmental instars and the entire life cycle from egg to adult takes about 21 days. The adult female lays up to 500 eggs in an ovisac (Nwanze, 1978). Under optimal conditions, adults can deposit between 200-600 eggs. (Iheagwam, 1981; Lema and Herren, 1985). The first instar is the most mobile stage (Nwanze, 1977) and is responsible for plant colonization within the same cultivated plot (Le Ru *et al.*, 1991).

The insect has a lower thermal threshold of 14.7°C, an optimal temperature of about 28°C and there is no development above 35°C (Iheagwam, 1981; Lema and Herren, 1985; Le Rü and Fabres, 1987; Schulthess *et al.,* 1987). The most favored sites for oviposition are terminal shoot tips, lower leaf surfaces and leaf petioles. Except for crawlers, all instars prefer the lower surfaces of fully expanded leaves (Nwanze, 1978) from where they move sluggishly to the stems and shoot tips. At low population densities, therefore, the insect is most abundant in the shoot tips (Schulthess *et al.*, 1987).

2.3.1.4 Host plants species infested by *Phenacoccus manihoti*

Phenacoccus manihoti is host-specific to cassava *(Manihot esculenta* Crantz). It has several non-preferred host that can support reproduction, but only cassava is known to experience significant damage by this insect. Although it has been collected on plants in various families, such as citrus and tomato, soybean, sweet potato, there is no evidence that it can survive for more than one generation on plants other than *Manihot* and perhaps certain other Euphorbiaceae (Williams and Granara de Willink, 1992). The other host crops and wild hosts are only marginally infested or apparently accidental (Herren and Neuenschwander, 1991). However, *Talinum triangulare*, Croton and Poinsettia species are particularly suitable for laboratory rearing and experiments. Also *P. manihoti* has been taken at quarantine inspection in the USA from Cuba (on *Sida*), Dominican Republic (on *Euphorbia*), Ecuador (on *Cucurbita*) and Mexico (on many hosts) (Ben-Dov *et al.*, 2006).

2.3.1.5 Damage symptoms of *Phenacoccus manihoti*

Phenacoccus manihoti causes serious damage to cassava leading to famine in Africa (Herren and Neuenschwander, 1991). The insect is more abundant and its damage severity is greater in the dry than in the wet season. During feeding, the mealybug feeds by sucking phloem fluids on the cassava stem, petiole, and leaf near the growing point of the cassava (Plate 6). Thereby, it injects a toxin that causes leaf curling, slowing of shoot growth, and eventual leaf withering (Egho *et al.*, 2013). As result, the internodes are reduced and the plants are stunted and bunchy (Plate 7).

At the peak population, cassava leaves wear a dry look and the plant progressively withers and turns to a “candle stick” appearance due to the adult insect mass population, ovisacs and nymphs. Cassava farms at this stage look like “burnt farms” (Egho *et al.*, 2013). Yield loss in infested plants is estimated to be up to 60 percent of root and 100 percent of the leaves (Herren, 1981).

Plate 6. Clusters of *Phenacoccus manihoti*

Plate 7. Cassava plant infested by *Phenacoccus manihoti*

2.3.2 *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae)

The solenopsis mealybug, *P. solenopsis* was described by Tinsley (1898) from weed roots in a nest of the ant *Solenopsis geminata* Fabricius in New Mexico, U.S.A. The name *solenopsis* refers to a genus of these ants with which this mealybug is associated. To protect their supply of honeydew on which they feed, these ants spread the young instars, protect them from natural enemies and keep their colonies clean.

2.3.2.1 Description of *Phenacoccus solenopsis*

The female adult of *P. solenopsis* is wingless with a 3-4 mm long oval shaped body. The body is somewhat rounded in lateral view; yellowish-green; legs red; covered by thin, white hydrophobic mealy wax (water repellent). On the intersegmental areas of thorax and abdomen appear dark dorso-submedial bare spots. These areas form one pair of dark longitudinal lines and have 18 pairs of lateral wax filaments, posterior pairs longest, up to 1/4th of length of the body (Plate 8).

The adult male is about 1 mm long, with a pale grey body and a single pair of transparent wings. Four filaments of white wax project from the end of its abdomen (Plate 9). The adult male has no feeding mouthparts and causes no damage.

Plate 8. Adult female of *Phenacoccus solenopsis*

Plate 9. Adult male of *Phenacoccus solenopsis*

2.3.2.2 Origin and global distribution of *Phenacoccus solenopsis*

Phenacoccus solenopsis was not reported as serious pest of any economic crop in its native origin, Central America (Tinsley, 1898). In 1990, it became an invasive by spreading on cultivated cotton in Texas, USA (Fuchs *et al.,* 1991) followed by the reports in the Ecuador (Ben-Dov, 1994). *P. solenopsis* has a wide range of variation in morphological characters, biological adaptations and ecological adjustability (Hodgson *et al.,* 2008). It was reported from 35 localities of various ecological zones of the globe (Ben-Dov *et al.,* 2009). In 2000, the pest was found in the Caribbean region such as Barbados, Cuba, Dominican Republic, Jamaica and Panama (Watson and Chandler, 2000). Subsequently, it spread to Chile (Larrain, 2002), Argentina (Granara de Willink, 2003), Brazil (Culik and Gullan, 2005). In Pakistan and India, *P. solenopsis* has been described as a serious and invasive pest of cotton (Hodgson *et al.,* 2008). It was found on *Hibiscus rosa-sinensis* in Nigeria (Akintola and Ande, 2008). Latest report on the invasiveness of *P. solenopsis* has been from the Eastern region of Sri Lanka (Prishanthini and Vinobaba, 2009) on ornamentals, vegetable crops, and weeds, and in China on *H. rosa-sinensis* (Wang *et al.,* 2009; Wu and Zhang, 2009). It was found in Benin for the first time in 2006 (Hodges *et al.,* 2008; EPPO, 2014). Most recently, *P. solenopsis* was reported to be a potential pest in 17 provinces of the Peoples' Republic of China (Wang *et al.,* 2009).

2.3.2.3 Biology and life cycle of *Phenacoccus solenopsis*

Phenacoccus solenopsis is sexually dimorphic, having short-lived, winged males and longer-lived, wingless, larviform females (Abbas *et al.,* 2010). Mature females lay their eggs in cottony ovisacs located at posterior part of abdomen and containing between 150-600 eggs. The eggs are smooth, translucent, light creamy yellow in color and oblong in shape with tapering ends (Nikam *et al.*, 2010). They hatch after three to nine days into the first instar (crawlers), which are very mobile. Freshly emerged first instar larvae are oblong in shape, dorsally convex, light yellow in color with three pairs of legs and a pair of seven segmented filiform antennae. Body color of newly hatched larvae changes to pale white within two days after hatching from eggs.

The female crawler undergoes four larval instars before turning into an adult with no pupal stage. The total life span of a female mealybug is 30-48 days, which includes 21 days as adult. Male crawlers undergo three larval instars before passing through a pupal stage. The male mealybug lives for 24-30 days including three to five days as an adult. They can have 12-15 generations in a year, depending on temperature. Mealybugs can survive cold conditions, both on the host plant and in the soil. In warm climates, they reproduce all year round (Charleston *et al.,* 2010).

2.3.2.4 Host plants species infested by *Phenacoccus solenopsis*

Phenacoccus solenopsis is highly polyphagous and has a vast host range. It attacks numerous crops, weeds, ornamentals and medicinal plants (Hodgson *et al*., 2008; Arif *et al*., 2009; Prishanthini and Vinobaba, 2009; Wang *et al*., 2009; Wu and Zhang, 2009). The most favored host of *P. solenopsis* is *Gossypium hirsutum* (Dutt, 2007). It has been recorded on members of 31 major plant genera in 13 families including, *Helianthus, Cucurbita, Euphorbia*, *Hibiscus, Solanum,* etc. (Ben-Dov, 1994; Akintola and Ande, 2008; Hodgson *et al.*, 2008).

2.3.2.5 Damage symptoms of *Phenacoccus solenopsis*

Nymphs and adults of the Solenopsis mealybug are found on the young growth of host plants, including twigs, leaves, flower buds and petioles. During the winter months they move into the soil and live in the root zone of the host. They extract large amounts of plant sap resulting in leaves distortion, deformation. They exude a large amount of honeydew which form a sticky deposit on the leaves and stem. Honeydew promotes the growth a dense mat of sooty mould fungi which covers the leaves surface and inhibit photosynthesis. As result, infested plants are distorted and the leaves are twisted or crinkled (Charleston *et al.*, 2010) (Plate 10).

Plate 10. Cotton stem (left) and boll (right) covered by *Phenacoccus solenopsis*.

2.3.3 *Ferrisia virgata* Cockerell (Hemiptera: Pseudococcidae)

Ferrisia virgata, commonly known as the striped mealybug, was discovered and described by Theodore Dru Alison Cockerell in 1893. The genus *Ferrisia* is apparently of New World origin (Williams, 1996).

2.3.3.1 Description of *Ferrisia virgata*

The adult female of *F. virgata* has an elongated oval body of about in 3-5 mm length, greyish-yellow and covered by white mealy wax. It has dark brown legs (Plate 11). This species is called striped mealybug due to the presence of two submedial longitudinal dark stripes on dorsum showing through the waxy secretion. The dorsum also bears numerous straight, glassy threads of wax. The adult female possesses two caudal wax filaments nearly at one-half of its body length and many long crystalline rods when adult. The dorsal ovisac is absent but consists in a few filamentous strands on the venter, forming a pad. They bear 1 pair of conspicuous lateral wax filaments, about 1/2 as long as body. The reproduction is usually ovoviviparous with numerous thin crystalline rods protruding from dorsum. *F. virgata* may be recognized by the anal lobe cerarii, each with two conical setae associated with a tubular ducts incorporated in rim and the ventral multiloculars disc pores present on posterior 3 abdominal segments, with more than 8 multilocular pores on segment VI. The antennae are 8-segmented (Fig. 4) (Williams and Watson, 1988; Williams and Granara de Willink, 1992; *Miller et al.*, 2002; Williams, 2004).

Plate 11. Adult female of *Ferrisia virgata*

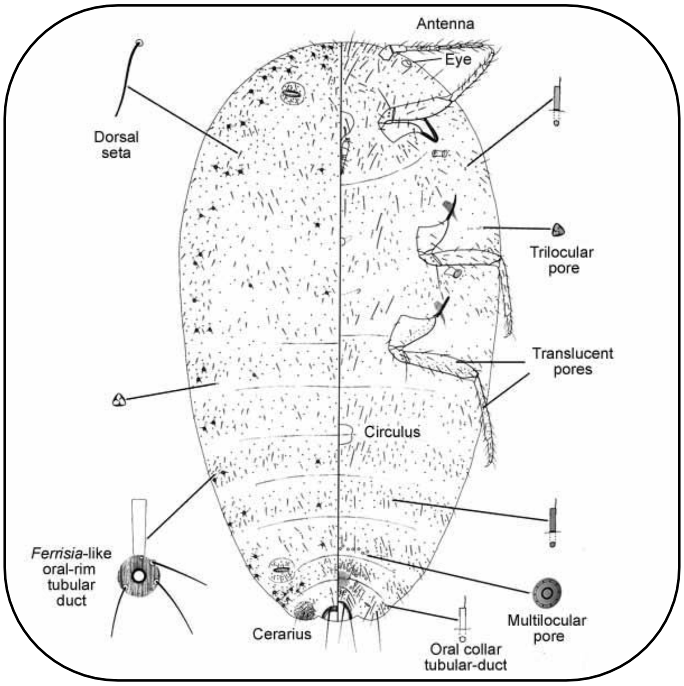


Figure 4. External morphological characters of *Ferrisia virgata* adult male.

Source. Williams and Granara de Willink, 1992.

2.3.3.2 Global distribution of *Ferrisia virgata*

Ferrisia virgata has spread to all zoo-geographical regions, mainly in the tropics, but often extends well into the temperate regions (Waterhouse, 1993; Ben-Dov, 1994; Foldi, 2000).

2.3.3.3 Biology and life cycle of *Ferrisia virgata*

The biology of *F. virgata* varies according to the natural environment in which it is found. *F. virgata* is parthenogenetic but it may reproduce sexually, especially when living in dense colonies. A complete life cycle required about 6-7 weeks at 30-35ºC and 65% relative humidity, and 13-14 weeks at 16.6ºC in Israel and each female produced 64-78 eggs (Ben-Dov, 1978).

In Lower Egypt, when infesting shrubs of *Acalypha macrophylla* (Euphorbiaceae), the pest completed 3 generations during summer. In autumn the females move to shrub cracks and other hidden sites (possibly including the soil), remaining there till next spring (Ammar *et al.*, 1979). In India, *F. virgata* can produce several overlapping generations a year (Nayar *et al.*, 1976). Mating took place only once and lasted for about 12-23 minutes. The female laid about 150 eggs in groups which lay beneath the body in a loose ovisac of waxy fibres (Schmutterer, 1969).

2.3.3.4 Host plant range of *Ferrisia virgata*

Ferrisia virgata is one of the most highly polyphagous mealybug known, attacking plant species belonging to some 150 genera in 68 families. Many of the host species belong to the Leguminosae and Euphorbiaceae. Among the hosts of economic importance are avocado, banana, betel vine, black pepper, cassava, cashew, cauliflower, citrus, cocoa, coffee, cotton, custard apple, egg-plant, grape-vine, guava, jute, lantana, Leucaena, litchi, mango, oil palm, pigeon pea, pineapple, soybean and tomato.

2.3.3.5 Damage symptoms of *Ferrisia virgata*

The stripped mealybug often occurs at the growing points, around the stem nodes, on the undersides of leaves and on the fruit. The damage is due to sucking out sap from terminal shoots, leaves and fruit resulting in yellowing, withering and drying of plants and shedding of leaves and fruit. The foliage and fruit also become covered with large quantities of sticky honeydew which serves as a medium for the growth of black sooty moulds, which together with waxy deposits result in a reduction of the photosynthetic area. Ornamental plants and their produce lose thereby their market value. Heavy infestations are conspicuous because of the white waxy ovisacs, white masses of male tests (waxy filamentous cocoon). *F. virgata* is a known vector of cocoa swollen shoot virus (CSSV) in West Africa and cocoa Trinidad virus (CTV, Diego Martin valley isolate) in Trinidad (Thorold, 1975).

2.4 Management of *Paracoccus marginatus*

An effective control of mealybugs requires a thorough knowledge of the targeted species and its bio-ecology. Full control is difficult to reach especially for polyphagous species and plant protection products have often a limited effectiveness against these pests because of the presence of waxy covering of their body. The following management options are in use against *P. marginatus*:

2.4.1 Cultural and Mechanical control of *Paracoccus marginatus*

Physical control and cultural control practices may be applied to make the environment less attractive or susceptible to the pest. It includes the use of barriers to keep away pests from crops (Abrol, 2013). To prevent the movement of crawlers, sticky bands or alkathene sheets or bands of insecticide may be applied on plant arms or on main stems. Field sanitation and sanitization of farm equipment before moving into uninfected crops is crucial. At the beginning of a local outbreak, severely infested branches should be cut off and burn immediately (Schmutterer, 1969). It is important to monitor and scout for early detection of the presence of the mealybug (Tanwar *et al.*, 2010).

2.4.2 Chemical control of *Paracoccus marginatus*

Mealybugs are generally difficult to control chemically. Adult mealybugs are more difficult to control than younger instars. A number of chemical has been described to control mealybug although none are currently registered specifically for control of papaya mealybug (Walker *et al.*, 2003). Active ingredients in registered pesticide formulations include acephate, carboryl, chlorpyrifos, diazinon, dimethoate, malathion, and white mineral oils. Typically, twice the normal dose is applied when treating for mealybugs because mealybugs are protected by thick waxy, cottony sacs, and often are concealed inside damaged leaves and buds. Thus, chemical controls are only partially effective and require multiple applications. Furthermore, problems with insecticide resistance and non-target effects on natural enemies make chemical control a less desirable control option to combat the papaya mealybug.

2.4.3 Biological control of *Paracoccus marginatus*

Biological control is the use of parasitoid, predator, pathogen, antagonist, or competitor populations to suppress a pest population, making it less abundant and thus less damaging (Van Driesche and Bellows, 1996). Biological control offers a self-sustaining solution for the suppression of invasive insect pests. It is widely accepted that there are three general approaches to biological control: importation, augmentation, and conservation of natural enemies. Importation is often referred to as "classical biological control" reflecting the historical predominance of this approach (Orr and Suh, 1998). The biocontrol of papaya mealybug has involved the use of natural enemies such as the mealybug destroyer, *Cryptolaemus montrouzieri* (Mulsant), *Scymnus coccivora* (Ayyar), other lady beetles, lacewings and hoverflies. Also, there is the larvae of the ape fly, *Spalgis epius* (Westwood), a hemipterophagous butterfly which feed on eggs, nymphs and adults of the papaya mealybug. *S. epius* is a member of the entirely entomophagous lycaenid subfamily Miletinae, most species of which feed on Hemiptera (Pierce *et al*., 2002). But the density dependent feeding behavior of those native predators warranted for the other alternative bio-control option for the effective management of this serious pest (Meyerdirk *et al.*, 2004).

2.4.4 Classical Biological Control of *Paracoccus marginatus*

Classical biological control is the introduction of natural enemies of pests from the place of origin of the pest, followed by successful establishment (Bellows *et al.*, 1999). It represents probably the best-known form of biological control worldwide. It is based on the concept of enemy-release, or enemy free space, experienced by a new invasive species when it is introduced into a new environment (Abrol, 2013). The most efficient natural enemies introduced for biological control of insects are parasitoids. These arthropods kill their hosts and are able to complete their development on a single host (Vinson, 1976). Most parasitoids that have been used in biological control are in the orders Hymenoptera and, to a lesser degree, Diptera (Van Driesche and Bellows, 1996). The most frequently used families in the Hymenoptera are Braconidae and Ichneumonidae in the Ichneumonoidea, and the Eulophidae, Pteromalidae, Encyrtidae, and Aphelinidae in the Chalcidoidea. In the Diptera, Tachinidae is the most frequently employed group (Greathead and Waage, 1986). Approximatively 20% of all biological control projects worldwide are considered to provide complete pest suppression (Abrol, 2013). The classical biological control strategy has met with some success in the control of mealybug species, such as *Phenacoccus manihoti* Matile-Ferrero (Neuenschwander, 2001), *Maconellicoccus hirsutus* (Green) (Kairo *et al.*, 2000), *Rastrococcus invadens* Williams (Agricola *et al.*, 1989; Neuenschwander *et al.*, 1994) and, more recently, *Paracoccus marginatus* Williams and Granara de Willink (Amarasekare *et al.*, 2009;

Muniappan *et al.*, 2006). Mealybug invasions have also been controlled by the accidental importation of natural enemies. At least six cases of biological control by fortuitous introductions of parasitoids and predators have been reported in mealybugs (Muniappan *et al.*, 2006).

The larvae of several species of parasitoid wasp in the family Encyrtidae attack the papaya mealybug in its native range. In 1999, the USDA Animal and Plant Health Inspection Service and Agricultural Research Service collected and reared four species of wasp from Mexico in a biological pest control experiment. They introduced them into Puerto Rico and the Dominican Republic and achieved a reduction of the papaya mealybug populations in both countries of over 95% (USDA-APHIS, 2000). All four wasps were observed parasitizing second and third instar of the papaya mealybug, and *Acerophagus papayae* Noyes (Encyrtidae) was the dominant species (Meyerdirk and Kauffman, 2001). All four have since been mass-reared and released in Florida (Walker *et al.*, 2003). Among the four parasitoids, *Acerophagus papayae* was found to be an efficient parasitoid for the suppression of papaya mealybug in its native range and has the ability to establish itself when introduced to new localities (Meyerdirk *et al.*, 2004; Muniappan *et al.*, 2006; Amarasekare *et al.*, 2008; Amarasekare *et al.*, 2009).

2.4.4.1 *Acerophagus papayae* Noyes and Schauff

Acerophagus papayae belongs to the order Hymenoptera, Superfamily Chalcidoidea and the family Encyrtidae. It is the smallest species out of the three detected parasitoids of papaya mealybug. The species *papayae* refers to the host plant, *Carica papaya* L. on which its host feeds.

2.4.4.2 Description of *Acerophagus papayae*

Acerophagus papayae is a tiny wasp with a pale orange body color observed for both the male and the female. Except for the un-segmented clava, and genitalia, males are very similar to their females (Noyes and Schauff, 2003). It has a transparent wings, grey/bluish eyes with three black triangular spots in the forehead. The female *A. papayae* is 0.58 to 0.77 mm in length including its ovipositor. The head and antennae, are generally pale orange with the base of the club slightly dusky. The compound eyes are greenish and the ocelli red. The thorax is pale orange with the neck of pronotum brown and having a translucent posterior margin and the side a little paler, almost yellow. The posterior margin of pronotum, mesoscutum and scutellum bear conspicuous brown setae; tegulae are very pale orange but apically pale greyish brown; side and ventral side of thorax and legs slightly are paler than the dorsal side of thorax. The abdomen is mostly pale orange but brown near cercal plates and dorsally along posterior margins of tergites III to V. Its ovipositor sheath is pale orange, apically brown (Plate 12) (Noyes and Schauff, 2003). The males are much smaller than the females, generally 0.44 to 0.66 mm in length (Noyes and Schauff 2003). They general appearance is similar to the females but the antennal club is un-segmented. The abdomen with apical tergites is more extensively darkened than in the female. The forewing has less conspicuous infuscate area below stigmal vein only and hardly extending towards posterior wing margin (Noyes and Schauff, 2003).

Plate 12. Adult female of *Acerophagus papayae*

Source: Noyes and Schauff, 2003.

2.4.4.3 Distribution of *Acerophagus papayae*

Acerophagus papayae is originated from Mexico. It was introduced for classical biological control of the papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink in many countries such as, Puerto Rico, Hawaii, Palau, Sri Lanka, and India and in Africa continent and Florida etc.

2.4.4.4 Biology of *Acerophagus papayae*

Acerophagus papayae is a solitary endoparasitoid of papaya mealybug. It is species-specific, preying only on the papaya mealybug. Its biology is partly influenced by the host crops on which *P. marginatus* feeds. It preferably parasitizes the second instar of the mealybug compared to third instar and adult female (Nisha *et al.*, 2014). The adult wasps lay their eggs inside the mealybug larvae (Plate 13). When the eggs hatch, the immature wasps eat the mealybug larvae until the larvae die. Each adult female lays about 50 eggs with a single egg deposit inside its host. The parasitized mealybug is mummifies usually in about nine (09) days following oviposition. (Plate 14 and 15).

Plate 13. *Acerophagus papayae* ovipositing on *Paracoccus marginatus*.

Plate 14. Papaya mealybug mummified by *Acerophagus papayae*

Source. Noyes and Schauff, 2003

Plate 15. Pupa of *Acerophagus papayae*

Source. Noyes and Schauff, 2003

CHAPTER THREE

3.0 Material and Methods

3.1 Study site

This study was conducted in the Biodiversity Center of the International Institute of Tropical Agriculture at Abomey-Calavi, Benin (IITA-Benin) where colonies of *Acerophagus papayae* are mass produced in laboratories to serve as a source for the West African sub-region.

3.2 Susceptibility of Paracoccus marginatus Williams and Granara de Willink (Hemiptera: Pseudococcidae) to four host plants species under laboratory conditions

3.2.1 *Paracoccus marginatus* mass rearing

A stock colony of *P. marginatus* was started on African egg-plants, S*olanum macrocarpon* L. (Solanaceae). The plants were allowed to sprout in pots and were then infested with ovisacs of *P. marginatus*. Potted plants were placed in rearing cages covered laterally with fine mesh, Plexiglas on top and plywood at the bottom. The dimensions of the cages were 50 by 50 by 50 cm. They were then held in growth chamber at 28° C, 75% Relative Humidity, and photoperiod of 12:12 (Day/Night) hour.

3.2.2 Host plant selection

Four of the most preferred host plants of *P. marginatus*, were selected, namely *Manihot esculenta* (cassava), *S. macrocarpon* (African egg-plant, Gboma), and *Vernonia amygdalina* (bitter leaf) including the primary host, *C. papaya* (papaya). The reproductive performance of *P. marginatus* on three of the plants was compared to that of its primary host. In order to maintain regular supply of the host plant, cutting of *M. esculenta*, *S. macrocarpon*, and *V. amygdalina* were allowed to sprout in plastic plots. While seedlings of papaya were sowed on small beds in the crop garden of IITA-Benin. Host plants were transplanted in plastic pots of 14.5 by 15 by 10 cm filled with mulched soil and perforated on three sides at the bottom to allow excess water to flow out (Plate 16 and 17). They were regularly watered each other day. Four to six weeks after sprouting, when expanded leaves were obtained, the potted plants were used for the host susceptibility study.

Plate 16. Potted plants of *Carica papaya* L. (papaya).

Plate 17. Potted plants of *Manihot esculenta* (cassava)

3.2.3 Isolation of the host plant leaf

All leaves were first cleaned with water to avoid any external infestation. Secondly, five (05) tender leaves were left on each potted host plants. To prevent the movement of immature mealybugs from one leaf used as an experimental unit to another, leaves were then isolated and a ring of non-poisonous entomological glue (TEMO STICK) was made on the petiole of each leach to maintain the mealybug on the experimental unit (leaf).

3.2.4 Comparative development of *Paracoccus marginatus* on four host plants

The effect of four different host plants on the developmental time of *Paracoccus marginatus* was assessed under laboratories conditions. Five isolated leaves of each of host plant were infested with the first instar larvae (crawler) of *P. marginatus*. Crawlers were obtained from ovisacs placed on African egg-plant leaves inside a large plastic box of 15 by 10 by 5 cm diameter with a lid with an opening of 6 cm in diameter. The opening was covered with fine nylon mesh for aeration. The plastic box was kept at 28° C in an incubator (PERCIVAL Model I-36 NL). After egg hatching, 100 crawlers of 24 hours old were transferred with a wet camel's hair brush onto each leaf of a newly prepared plant as described above for each host plant species. These were then placed in an incubator and kept at 28° C, 75% RH (Plate 18). The developmental time were assessed for one thousand (1,000) of *P. marginatus* crawlers for each host plant species. Their development to the adult instar was daily followed up daily by checking for the presence of exuviae as indication of moulting. Twenty (20) second instar mealybugs obtained after the eleventh day from each host plant were gently transferred to newly corresponding prepared potted plants. The developmental and survival time of each instar, and the number of mealybugs developing into adult male and female was recorded for each plant species. Sex determination of each individual mealybug was conducted on late second instars when males started to build a silken cocoon whereas females increased in size. From then on, male and female developmental time were recorded separately. Life table parameters such as developmental time, survival and fecundity, were compared among the four tested plants and their individual performances evaluated to determine the most suitable laboratory host plant.

Plate 18. Potted plant of Gboma (Left) and Cassava (Right) in the Incubator.

3.2.5 Fertility and Survival

To assess the oviposition capacity of *P. marginatus* adults, ovisacs were collected daily from the stock colony. The eggs were transferred then to potted plants and allowed to hatch. The newly hatched crawlers were observed till the development of pre-ovipositing females. On each plant the number of individuals was then reduced to 20 by removing surplus mealybugs. Each female was considered a replicate. Eggs laid per female were collected on a daily basis with a wet camel's hair brush and were counted using a hand held tally counter. This procedure lasted until the female stopped laying eggs. The female life span continued even though they stopped laying eggs. A female was considered to be dead when no reaction was anymore observed following contact with the camel's hair.

3.3 Host specificity of *Aceropghagus papayae* Noyes (Hymenoptera: Encyrtidae), introduced as parasitoid of *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae)

3.3.1 Non-target mealybug selection

The selection of potential non-target mealybug species used for this experiment was based on different criteria. *Paracoccus marginatus* as a notorious mealybug species is polyphagous and found to occur on several host plants. *P. marginatus* are consequently found to share the same host plant with other mealybugs. *A. papayae* is then exposed to a number of host species when searching for its target host, *P. marginatus*. Those non-target species are likely to be a good candidate in order to assess host specificity of *A. papayae*. Three different mealybug species were found to be commonly associated with *P. marginatus*. These are: *Phenacoccus manihoti* (cassava mealybug), *Phenacoccus solenopsis* (cotton mealybug), *Ferrisia virgata* (striped mealybug) sharing at least one host plant with the target insect pest, *P. marginatus*.

3.3.2 Host plant species selection

This experiment was aimed at simulating as close as possible natural conditions under which the selected non-target species are associated with the main mealybug host in the field. The choice of the host plant was based not only on the common relationships existing between *P. marginatus* and the non-target but also on the host plant, thus, the four selected host plants: *Carica papaya*, *Jatropha curcas*, *Solanum macrocarpon* and *Talinum triangulare* in the present experiment are found to be hosts of the *P. marginatus*. The following combinations were made for the host specificity test:

- *P. marginatus* on *C. papaya*
- *P. marginatus* and *P. manihoti* on the leaf of *T. triangulare*
- *P. marginatus* and *P. solenopsis* on the leaf of *T. triangulare*
- *P. marginatus* and *Ferrisia virgata* on the leaf of *J. curcas*

3.3.3 Mealybug and parasitoid rearing

Mass rearing of all mealybug species tested was initiated on potted plants that were maintained in a greenhouse for the availability of all life stages of each of the mealybug species all along the experiment period. They were maintained at 28 ºC and the plants were watered every other day. All the different host plants used were allowed to sprout prior to rearing. A colony of *P. manihoti* was established from ovisacs collected on cassava leaves in the field and transferred to potted plants of *T. triangulare*. *Phenacoccus solenopsis* was reared in the greenhouse on *Solanum* sp. and *T. triangulare* following its initial collection in the field on infested leaves of the same host plants. Ovisacs of *F. virgata* from field material on *J. curcas* were used to initiate a stock colony of the striped mealybug in the greenhouse. Weekly collected mummies of parasitized *P. marginatus* on *C. papaya* leaves originating from the mass rearing facility at their IITA station were gently transferred into a Petri Dish of 9 cm diameter with 3 cm diameter holes bored on the lid and covered by a muslin mesh to permit ventilation. They were maintained in a rearing chamber at 28 ºC, 75% HR and a 12:12 D/N photoperiod and checked daily for parasitoid emergence. Upon emergence, the parasitoids were fed with a 1:3 honey-water mixture soaked in blotting paper. Three day old mated females were used in the experiments and substituted with fresh ones after each test.

3.3.4 Host specificity testing

The suitability of the four selected mealybug species was evaluated under choice and no-choice test conditions in the laboratory. Tests were conducted in experimental enclosure using a 9 cm diameter Petri dish. The behavior of the parasitoid was recorded in one minute time segments over a full hour of observation and repeated 50 times. In case of oviposition, the host stage in which the female laid an egg was noted and egg-laying duration was recorded.

During this study, the behavior of the female parasitoid was assessed using foraging categories numbered from 0 to 10 as following: 0= resting; 1= moving; 2= jumping; 3= preening; 4= honey dew feeding; 5= arrestment and wax examination; 6= palpation and examination with antennae (host); 7= probing with the ovipositor; 8= oviposition; 9= smearing with the ovipositor; 10=host feeding.

3.3.4.1 No choice condition

No-choice testing provides the most constraining approach to determining host suitability for the parasitoid. In this test, a mated female parasitoid was confined to all life stages of only a single host species. It seems to provide the greatest chance of drawing out non-target effects because of the absence of the natural host. A leaf disk of each selected host plant prior to being infested with mealybugs was placed into a Petri Dish of 9 cm of diameter. The diameter of leaf covered almost the surface of the Petri Dish for which care was taken to make sure that all the life stages of the target or one of the non-target mealybug were available to the parasitoid. Missing instars were introduced with a fine camel’s hair brush. A single three day old mated female parasitoid was then introduced into the Petri Dish after an hour allowing the different instars to settle on the leaf. Fifty gravid parasitoid females were used for each tested host plant. A piece of papaya leaf was used to assess the foraging of the parasitoid on *P. marginatus*, this experiment serving as control. The host specificity test of *A. papayae* on *P. manihoti* was done on a leaf of *T. triangulare* while a leaf of *J. curcas* was used for *F. virgata*.

3.3.4.2 Choice condition

This test was conducted in order to monitor potential non-target effects in a semi-natural situation but with the possibility for the parasitoid to decide between host and non-host in a small enclosure. This experiment was carried out by paring all life stages of the natural host, *P. marginatus* with all life stages of one of the non-target host (*F. virgata*, *P. manihoti* or *P. solenopsis*) on the appropriated host plant. As in the previous set up, fifty gravid female parasitoids were used during fifty observation hours. To study the host specificity of *A. papayae*, infested leaves of *J. curcas* with all life stages of both *P. marginatus* and *F. virgata* species was placed into a Petri Dish (Plate 19). Again, missing instars were gently transferred with a fine camel's hair brush. A single three days old female was then introduced into the Petri Dish for each one hour observation period per test. Infested leaves of *S. macrocarpon* was used as host plants in testing the host specificity of *A. papayae on P. marginatus* and *P. solenopsis* (Plate 20) while infested leaves *of T. triangulare* were used on *P. marginatus* paired with *P. manihoti*.

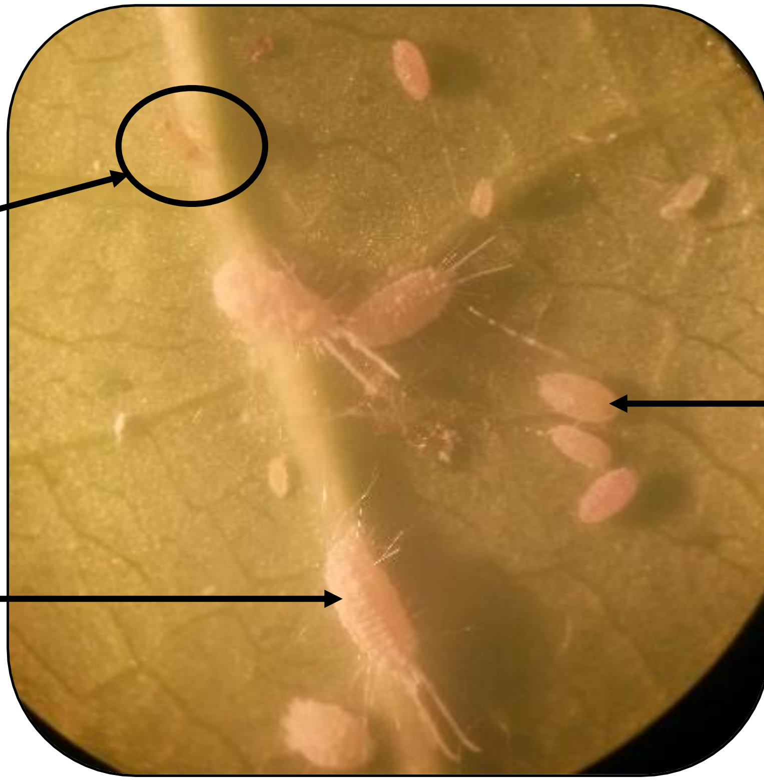


Plate 19. *Paracoccus marginatus* paired with *Ferrisia virgata* on *Jatropha curcas*.

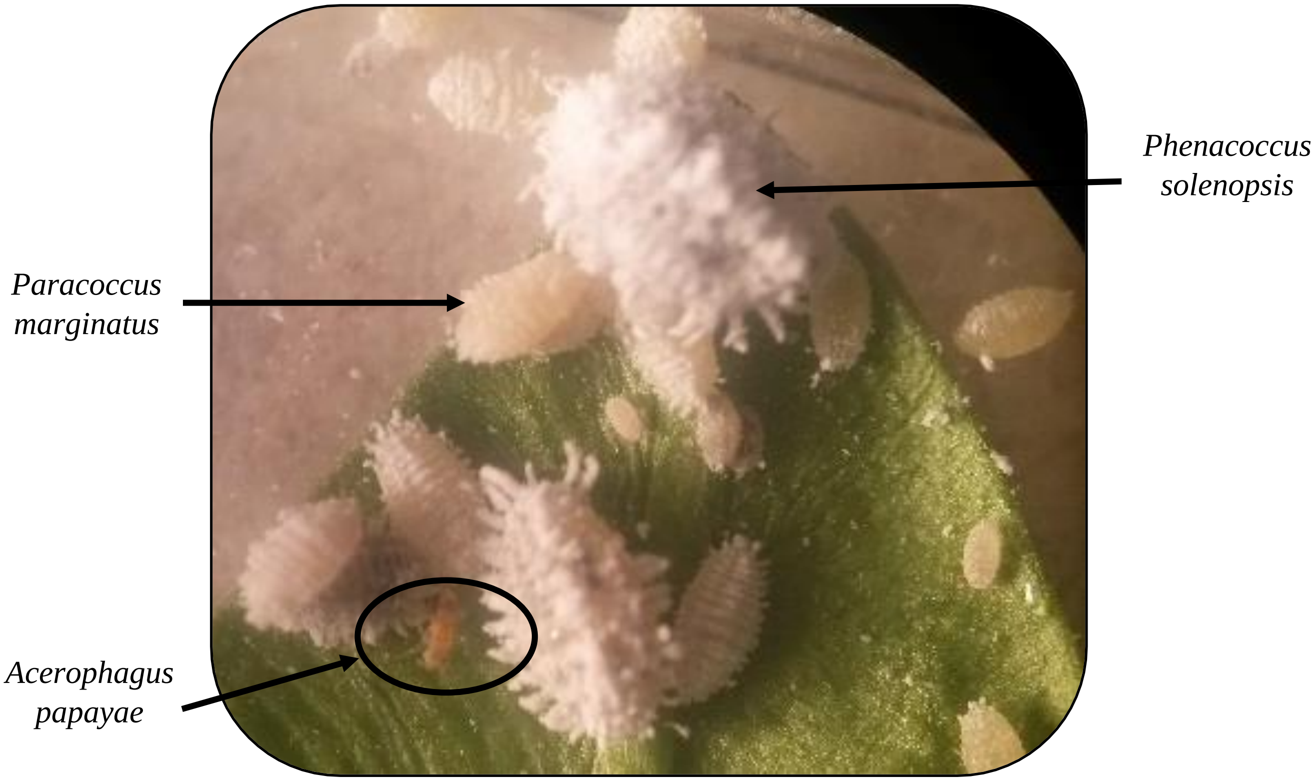


Plate 20. ***Paracoccus marginatus* paired with *Phenacoccus Solenopis* on *Talinum triangulare*.**

3.4 Statistical analysis

The data resulting from the effects of different host plants on host instars mortality of *P. marginatus*, host instars parasitized by *A. papayae* and the duration of oviposition were subjected to an Analysis of Variance (ANOVA). The means were compared using Tukey's HSD. Means pre-oviposition, oviposition and post ovipositon period as well as fecundity and the longevity of *P. marginatus* were compared using Kruskal-Wallis Rank Sum Test. Wilcoxon Rank Sum and Signed Rank Tests were used to compare means of two host plants. Excel was used for data entry and has permitted to present the descriptive analysis of the behavior. Test of Equal or Given Proportions (prop. test) was used to test the probabilities of success of a preferred host instar of non-target mealybug over another. All Chi-square were judged at P=0.05 and significant values marked with an asterisk. Means that were significantly from each other were marked by different letters. All the analyses were performed using R x 64 (Version 3.1.2) statistical software.

CHAPTER FOUR

4.0 Results

4.1 Susceptibility of Paracoccus marginatus Williams and Granara de Willink (Hemiptera: Pseudococcidae) to four host plants species under laboratory conditions

4.1.1 Development of *Paracoccus marginatus* reared on four different host plants species

The developmental times of *P. marginatus* (first instar to adult) reared on four host plants species were different. *P. marginatus* failed to complete full development on *V. amygdalina* where all observed individuals died at the second instar. Also, the development of adult males of *P. marginatus* was not completed on *M. esculenta* and *S. macrocarpon* where all observed individuals died at the third instar male. First instar developmental time was longer on *V. amygdalina* (9 days) and the shortest time was on *C. papaya* (5.98 days). The shortest cumulative developmental time for adult females was on *C. papaya* (15 days) and the longest on *M. esculenta* (19 days). Males had longer developmental time (19.42 days) than females (15 days) on *C. papaya* (Table 1).

Table 1. Mean number of days for each developmental instar of *Paracoccus marginatus* reared on four host plants species

Host plant species	Host Instars							
	First	Second		Third		Fourth	Cumulative	
		Female	Male	Female	Male	Male	Female	Male
Carica papaya	5.98	7.02	7.44	2.00	3.00	3.00	15.00	19.42
Manihot esculenta	7.76	5.24	9.24	6.00	-	-	19.00	-
Solanum macrocarpon	7.00	8.71	8.00	2.13		-	17.84	-
Vernonia amygdalina	9.00	6.00	6.00	-	-	-	-	-

4.1.2 Percentage mortality of each developmental instar of *Paracoccus marginatus* reared on four host plants species

Host instars mortality are presented in Table 2. Different host plants species affected significantly the survival of *P. marginatus* host instar development, however, there was no significant difference in the mortality of each developmental host instar among host plant species. The highest mortality of the first-instar (85.83%) was compensated by a low mortality of third instar females on *M. esculenta*. There was no mortality of third-instar males and fourth-instar male (Table 2).

Table 2. Percentage mortality of each developmental instar of *Paracoccus marginatus* reared different host plants species.

Host Plants species	First	Second	Third		Fourth
			Female	Male	
Carica papaya	62.46 a*	53.00 a	33.77 a	0	0
Manihot esculenta	85.83 b	50.52 a	21.28 a, b	-	-
Solanum macrocarpon	67.45 a	85.00 b	50.00 c	-	-
Vernonia amygdalina	66.29 a	58.00 a	0.00	-	-
df	3	3.00	3.00	-	-
F value	13.35	8.45	16.12	-	-
P value	0.0128	0.0002	0.0076	-	-

*Means within a column followed by the same letters are not significantly different at α = 0.05 (TukeyHSD).

4.1.3 Reproduction of *Paracoccus marginatus* adult female reared on different host plants species

4.1.3.1 Pre-oviposition, oviposition and post-oviposition periods of *Paracoccus marginatus* female reared on different host plants species

- **Pre-oviposition period**

The mean pre-oviposition period of *P. marginatus* was not significantly affected by the different plant species (P = 0.1268). The shortest pre-oviposition period was recorded on *C. papaya* (7.65 days) while the longest was on *M. esculenta* (15.08 days) (P = 0.0266). Most of females started egg-laying from 7 to 8 days on host plants tested (Fig. 5), however, female reared on *C. papaya* and *S. macrocarpon* showed a restricted pre-oviposition period of only 9 days (Fig. 6).

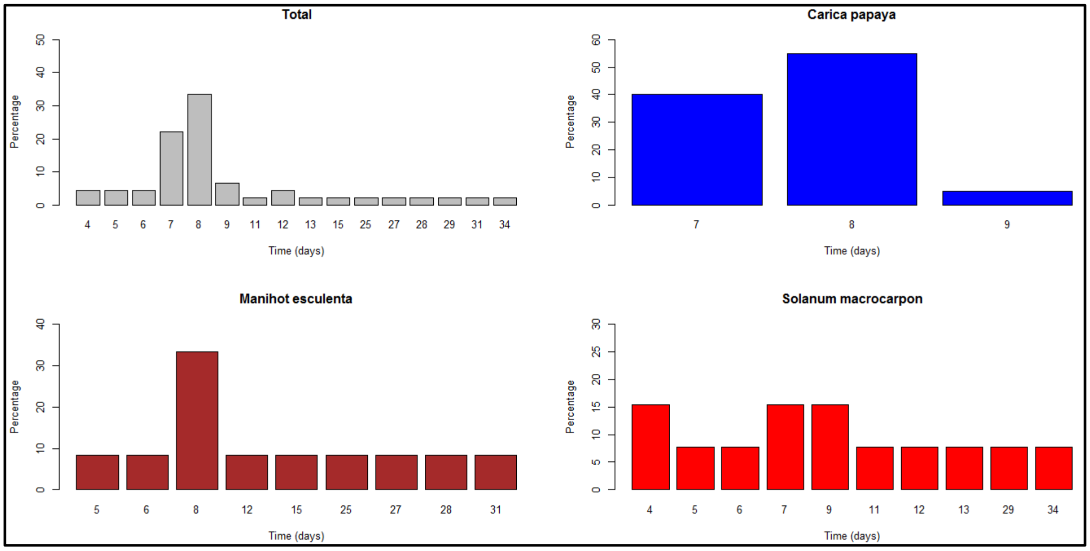


Figure 5. Percentage female pre-ovipostion period (days) on different host plant species.

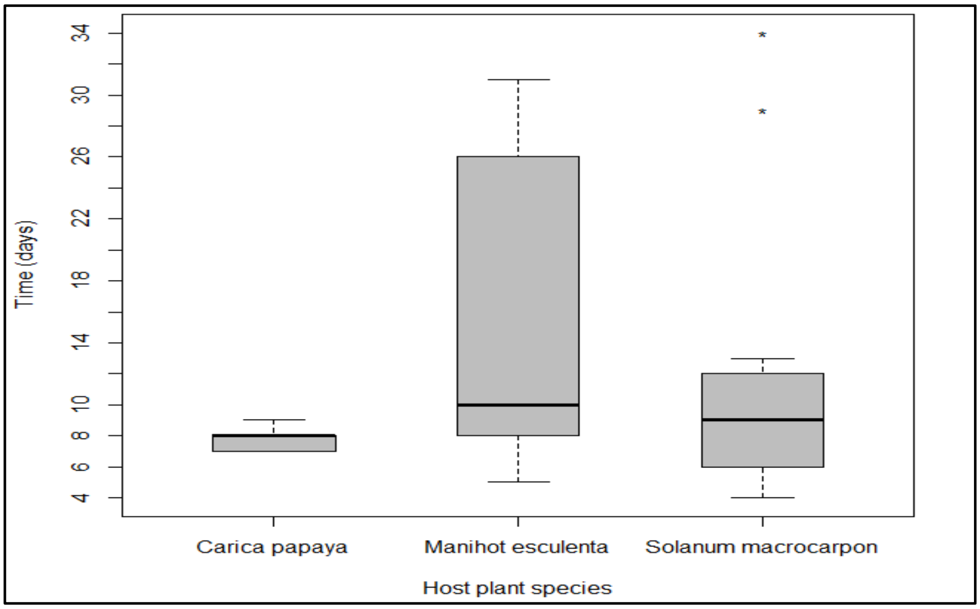


Figure 6. Distribution of female pre-oviposition period on different host plant species.

- **Oviposition (egg-laying) period**

The mean oviposition period was significantly affected by the host plants species (P = 0.0049). There was a significant difference between the oviposition period of *P. marginatus* reared on *C. papaya* (9.65 days) and those reared on *M. esculenta* (6.83 days) (P = 0.0363), and *S. macrocarpon* (4.54 days) (P = 0.0017). The variation in eggs laying period and its distribution are represented in Figures 7 and 8.

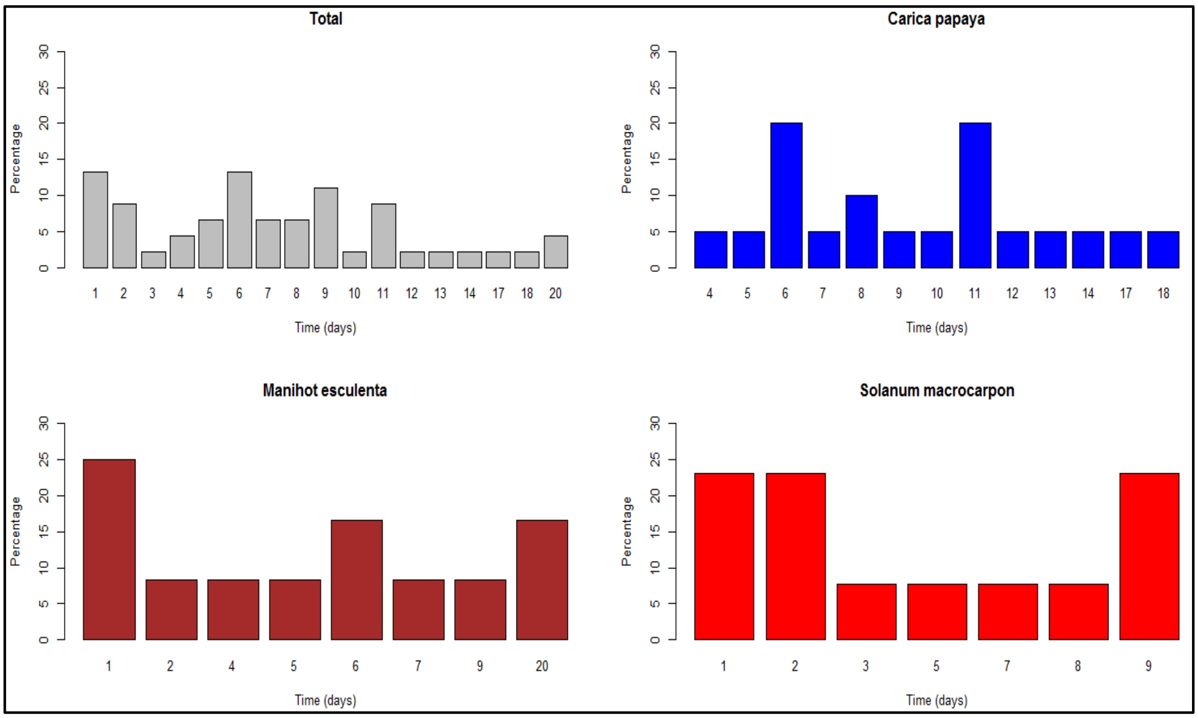


Figure 7. Percentage female ovipostion period (days) on different host plant species.

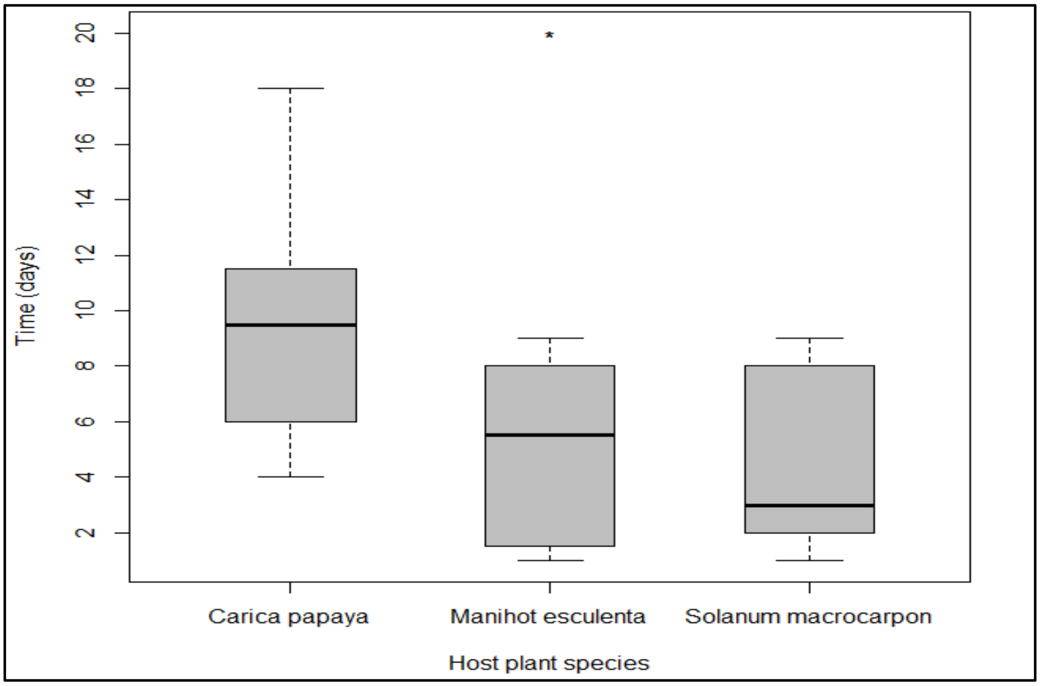


Figure 8. Distribution of female oviposition period on different host plant species.

- **Post-oviposition period**

The mean post-oviposition period was significantly affected by host plant species (P = 0.0366). There was a significant difference between the post-oviposition period of females reared on C. papaya (4.8 days) and M. esculenta (9.42 days) (P = 0.02659), however, the majority of females died nine days after oviposition while some lived up to 34 days after egg-laying (Fig. 9 and 10).

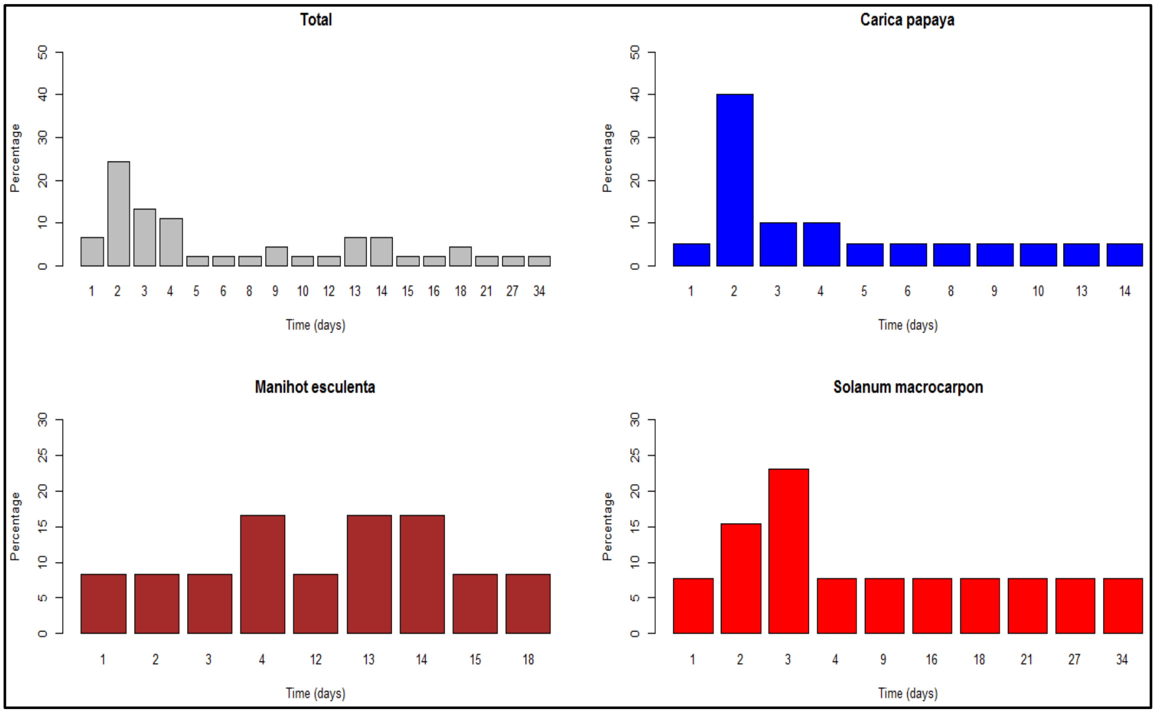


Figure 9. Percentage female post-ovipostion period (days) on different host plant species.

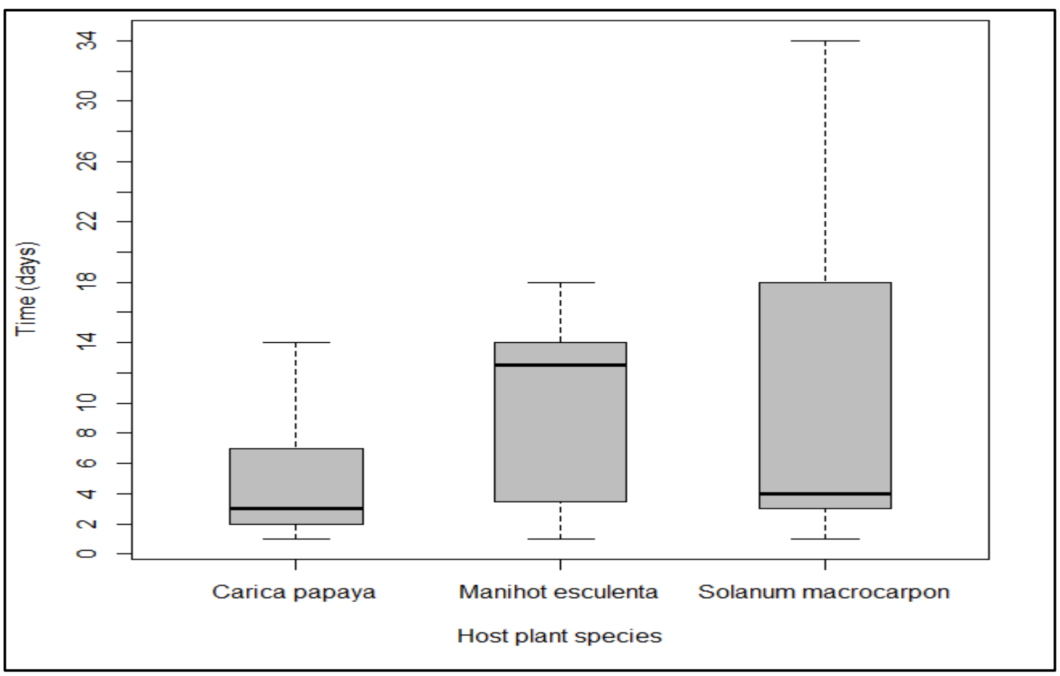


Figure 10. Distribution of female post-oviposition period on different host plant species.

4.1.3.2 Fecundity of Paracoccus marginatus adult females reared on different host plants species

Females fecundity was significantly influenced by the host plants tested (P = 0.033). The lowest number of eggs laid was on S. marcrocarpon (141.23 eggs) with the highest on C. papaya (277.40 eggs) (P = 0.005). On C. papaya, 75% of females laid between100 and 300 eggs while on M. esculenta, 50% of females laid between 50 to 100 eggs (Fig. 11, 12). The mean number of eggs laid per female daily is represented in figure 13. Female fecundity reached its peak on the fourth and seventh days on C. papaya and M. esculenta, respectively.

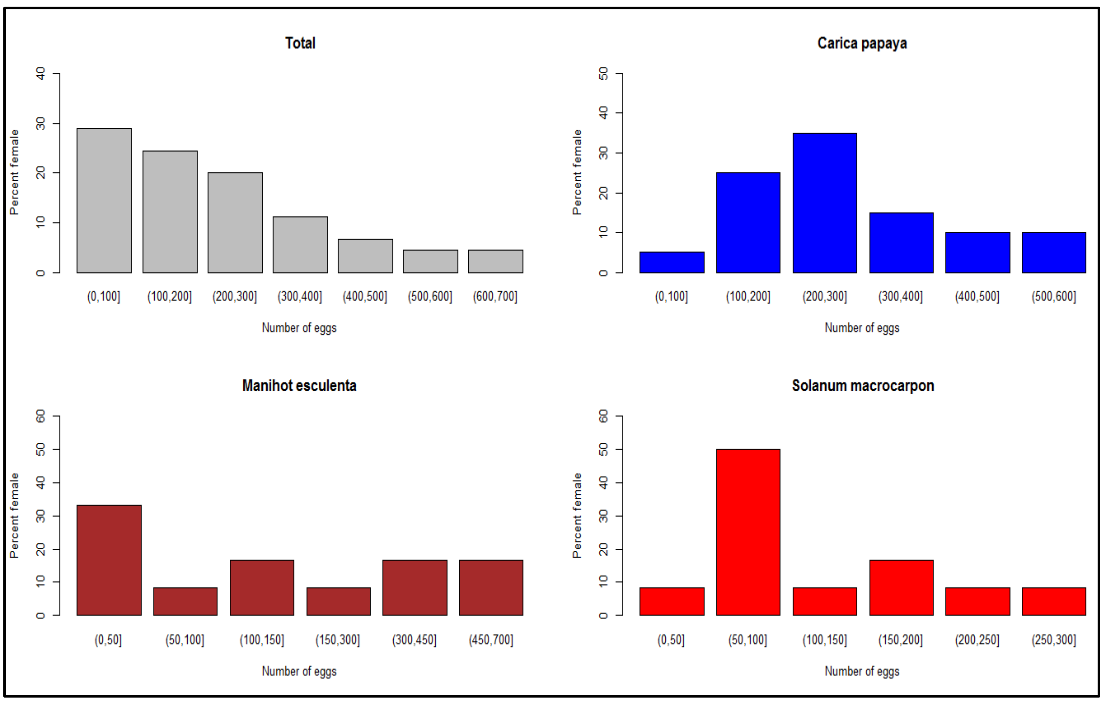


Figure 11. Number of eggs laid by female on different host plant species.

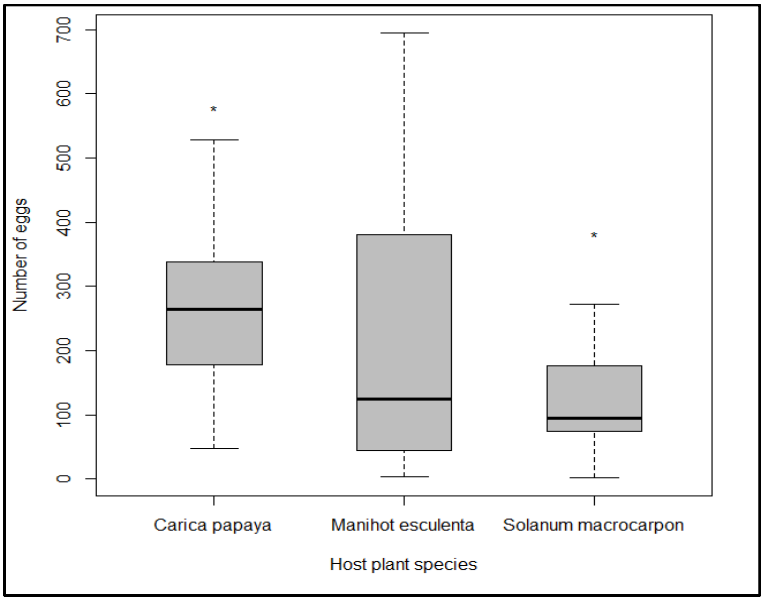


Figure 12. Distribution of female fecundity on different host plant species.

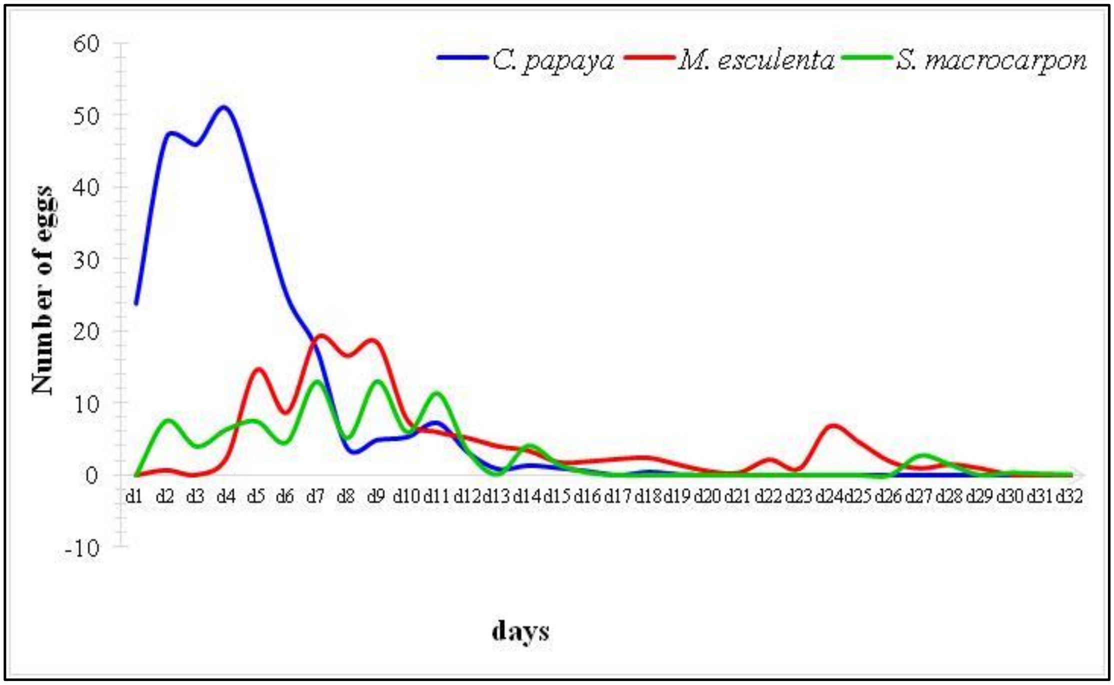


Figure 13. Daily mean number of eggs laid by adult female of *P. marginatus* on different host plants species.

4.1.3.3 Longevity of *Paracoccus marginatus* adult female reared on different host plants species

The longevity of adult female was significantly influenced by the different host plants tested (P = 0.0010) (Fig. 14). Females lived longer on *M. esculenta* (31.50 days) and *S. macrocarpon* (30.35 days) than on *C. papaya* (22.10 days) (Table 3). There was a significant difference between the longevity of the adult female reared on *C. papaya* and *M. esculenta* (P = 0.0004) and *S. macrocarpon* (P = 0.0054).

Table 3. Longevity (days) of *Paracoccus marginatus* adult female longevity reared on different host plants species.

Characteristic of the distribution	Host Plant Species		
	Carica papaya	***Manihot esculenta***	***Solanum marcrocarpon***
Minimum	15.00	12.00	14.00
First quartile	17.75	25.75	21.00
Mean	22.10 ± 1.84 a*	31.50 ± 4.08 b	30.35 ± 4.64 c, b
Third quartile	26.25	38.50	37.25
Maximum	36.00	46.00	48.00
Standard deviation	5.84	8.72	9.92

*Means within rows followed by the same letters are not significantly different at α = 0.05 (Wilcoxon).

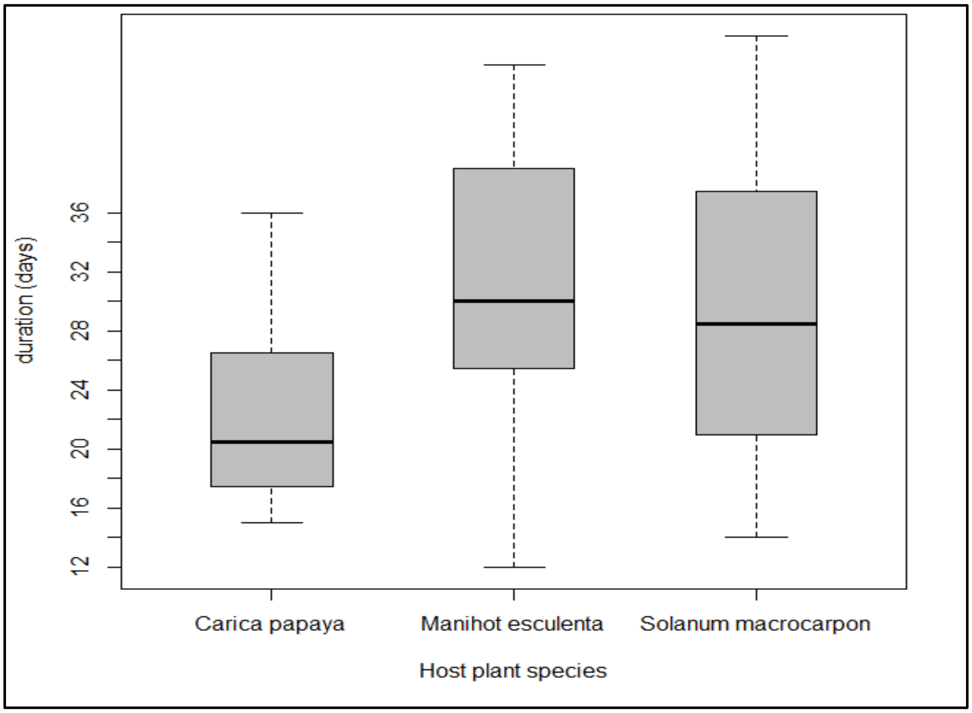


Figure 14. Distribution of female longevity on different host plants species.

4.2 Host specificity of *Aceropghagus papayae* Noyes (Hymenoptera: Encyrtidae), introduced as parasitoid of *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae)

4.2.1 Foraging behavior of *Acerophagus papayae* female in both choice and no choice condition

The profile of female *A. papayae* was similar in both experimental conditions with a light difference in no choice condition. The female parasitoid was more active in the presence of its target host than non-target host (Fig. 15). The foraging behavior of *A. papayae* based on observations made during both no-choice and choice conditions are schematized in Figure 16 (capital letters in brackets referring to this figure). The female of *A. papayae*, after its introduction into the enclosure in choice or no choice conditions started searching for the host (mealybug), walking on the leaf without any apparent direction. Frequently it passed close to hosts at a distance of a millimeter, or even walking directly over them without detecting them (A). The female often jump and after sudden movement of the host usually the non-target mealybug (B). Host searching continued until the female encountered a host. It made an arrestment and touched the host with its antennae for wax examination (C). Host encountered was carefully examined by drumming with the antennae (D). An already attacked host may be reexamined by the female. Searching was sometimes interrupted by bouts of resting (E) which was frequent with non-target host and scarce with target mealybug. Resting position was defined as sitting almost motionless with the antennae held in “V” shape or moving them only slightly (Bokonon-Ganta, 1996). The female parasitoid may feed on the host honeydew (F) and cleaned its body with the antennae (G).

After host examination, the female accept or reject the host. In case of host acceptance, the female probed the host with its ovipositor (H). This was followed by ovipostor insertion and oviposition occurring either on the anterior, middle or posterior part of the host sometimes in several attempts (I). Egg-laying was noted with ovipositor insertion followed by pumping. Ovipostion was followed by smearing of the host with the ovipositor (J). Successful attacks by the female parasitoid were detected by a vibration of its ovipositor. The same host was often attacked by the single female parasitoid. Rarely, host feeding was observed after the female parasitoid ovipositing into the same host (K). Some unsuccessful attacks were observed. This was due to the rejection (L) of the host by the female parasitoid after several attempts at inserting the ovipositor. It was either due to the defense behavior of the mealybug or imprecise insertion of the ovipositor.

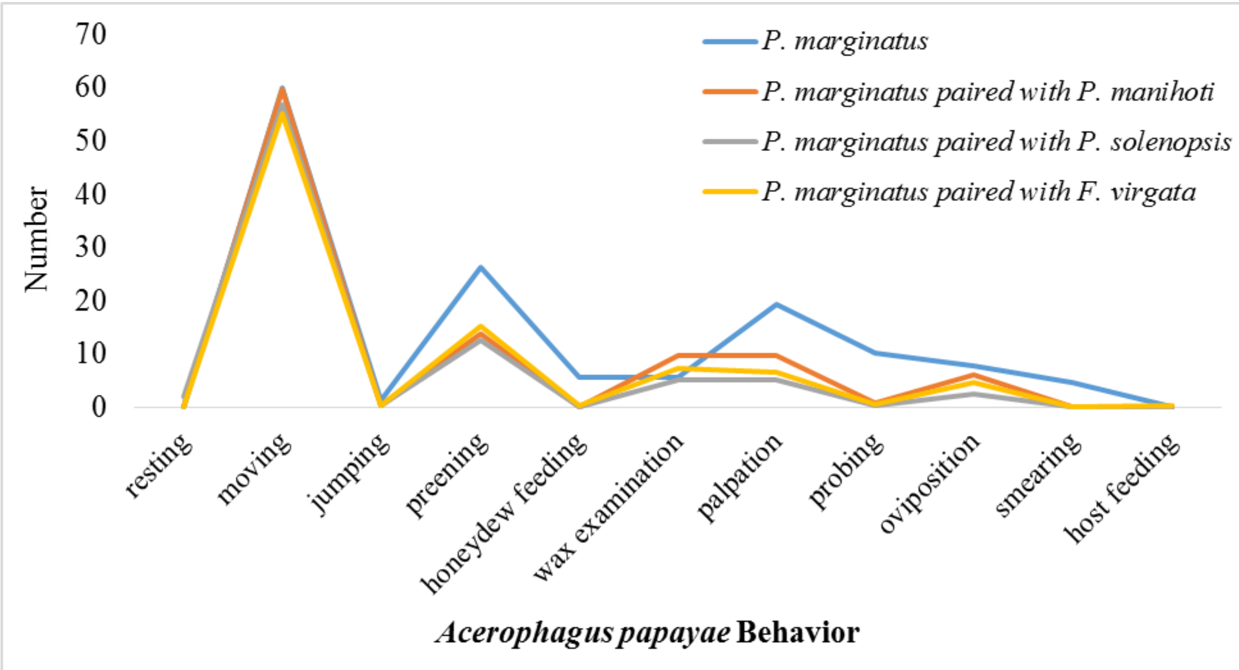


Figure 15. Comparative searching behavior of *Acerophagus papayae* in no choice condition and choice condition.

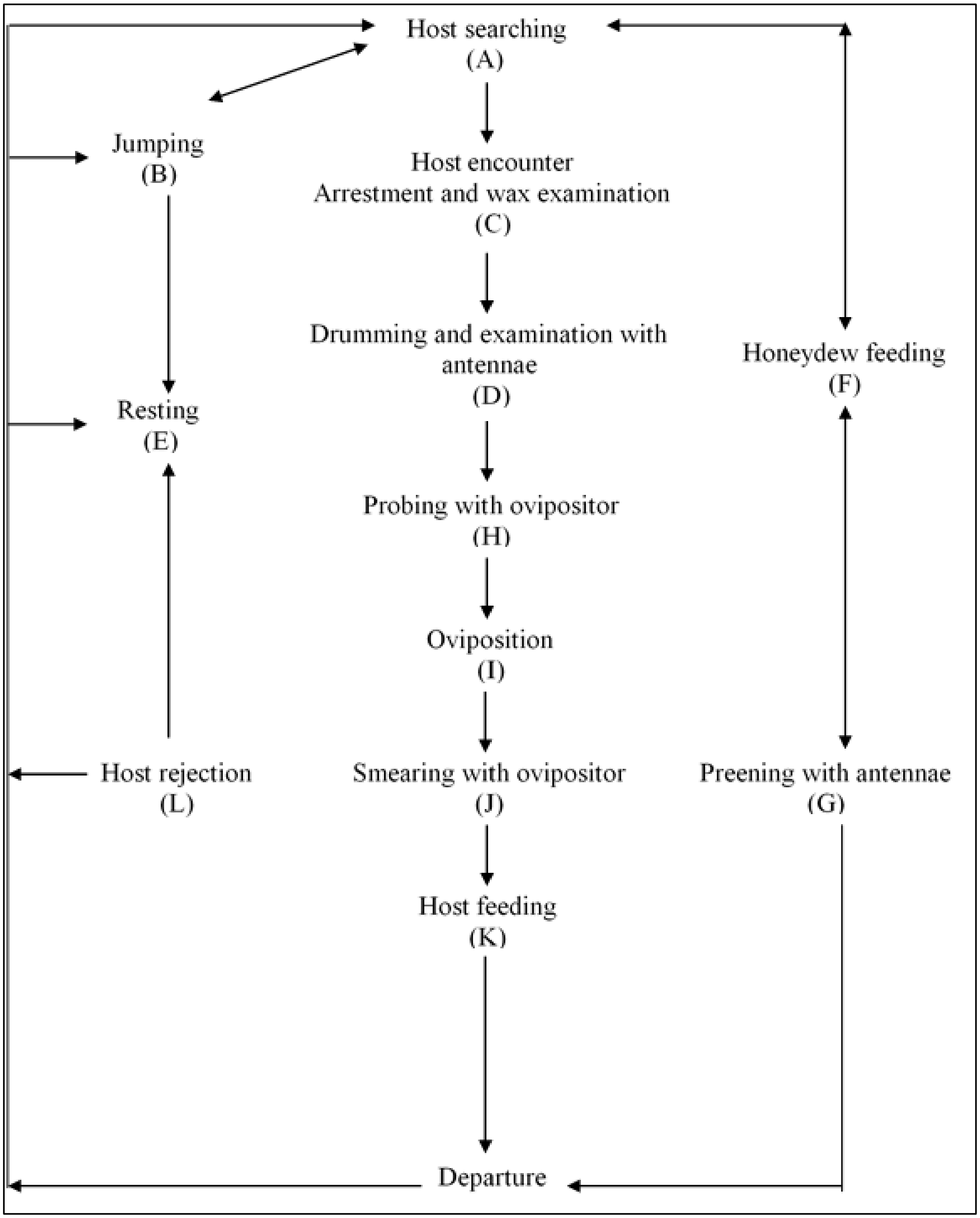


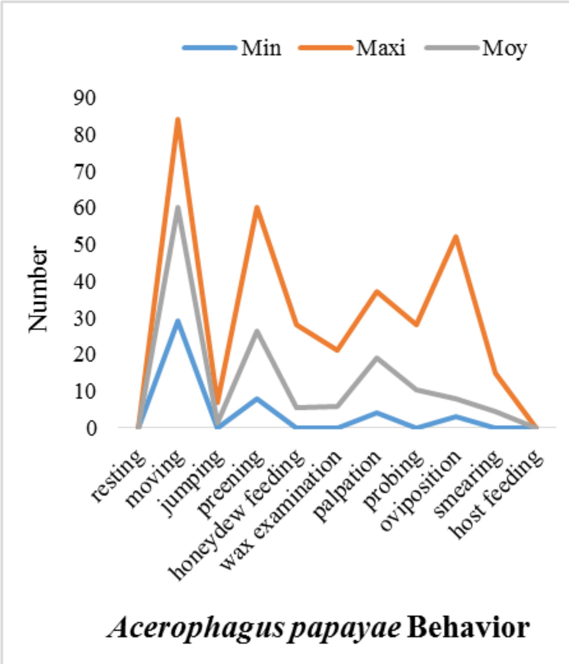
Figure 16. Components of the behavior of the female parasitoid, *Acerophagus papayae* when foraging for host.

4.2.2 Comparative searching behavior of *Acerophagus papayae* in no choice condition

In the presence of the target host, *P. marginatus*, the female parasitoid had an intensive activity. Host searching was made through repeated moving. Once the host was found, the female proceeded to its wax examination, palpation with antennae, turned up by probing the host with its ovipositor. This was followed immediately by insertion of the ovipositor with or without pumping (Fig. 17). The female may smear its host with its ovipositor to either clean up the ovipositor or to oviposit once again. Sometimes, before looking for a new host, the female fed on the honeydew. No resting position was observed all along the searching process of the female.

By contrast, the moving behavior observed in the presence of *P. manihoti*, a non-target host was due to the non-recognition of the host by the female parasitoid (Fig. 17). This was often followed by a resting position accompanied with pruning with antennae. After the host encounter, the female made a quick or sometimes long wax examination. Occasionally, the female went further to palpate its host or use its ovipositor, which was quickly interrupted by a sudden movement of the host. Female jumped away and cleaned up its body.

The host searching behavior of *A. papayae* female in no choice condition is summarized in Figure 18.



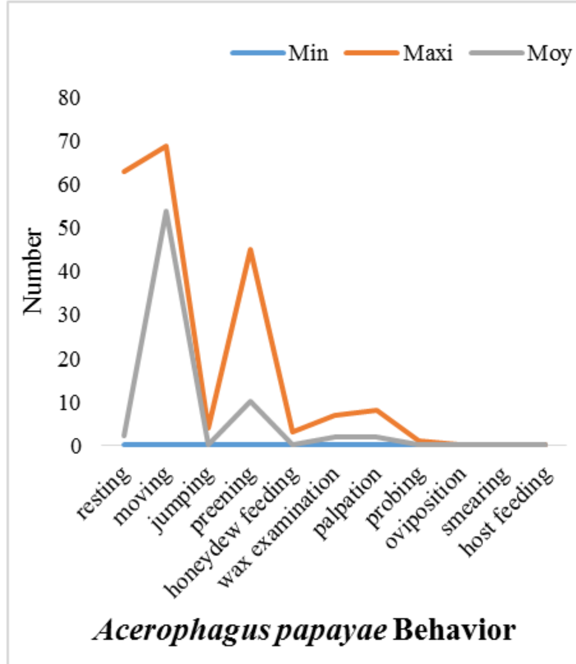


Figure 17. Mean number of different actions taking by *A. papayae* observed during an hour in presence of *Paracoccus marginatus* (Left) and *Phenacoccus manihoti* (Right).

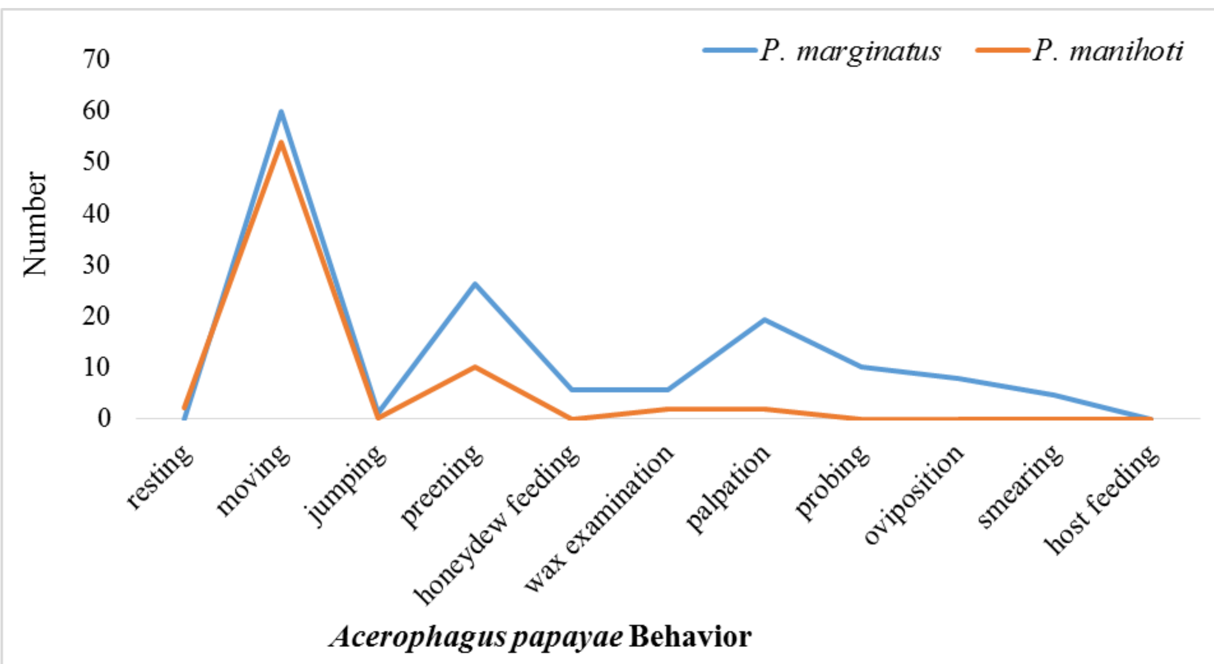


Figure 18. Searching behavior of *A. papayae* in no choice condition when in presence of *Paracoccus marginatus* and *Phenacoccus manihoti.*

4.2.3 Searching behavior of *Acerophagus papayae* in choice condition

Searching behavior of female parasitoids was practically the same in choice condition. Resting position was noticed only when the female was in the presence of *P. marginatus* paired with *P. solenopsis*. During host searching, the female made an arrestment to examine its host. Sudden movement or defense behavior of the non-target host involved the jumping of the female (Fig. 19 and 20). Occasionally, the female succeeded in inserting its ovipositor into the three non-target mealybugs tested after palpation with antennae and probing with ovipositor.

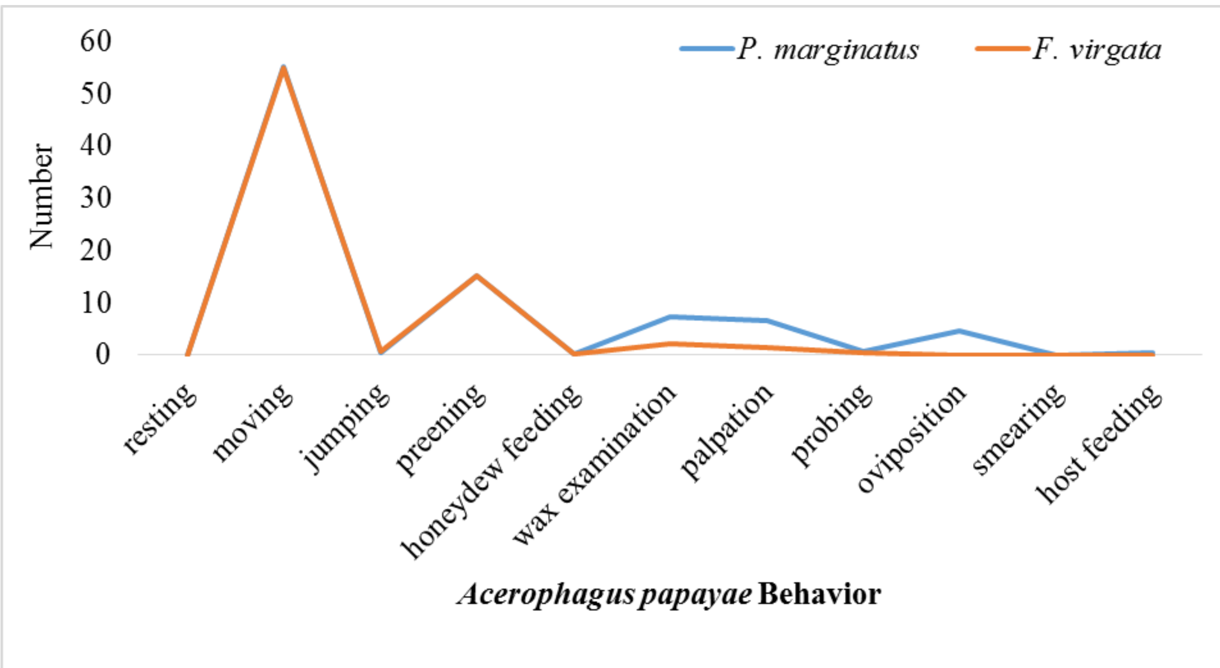
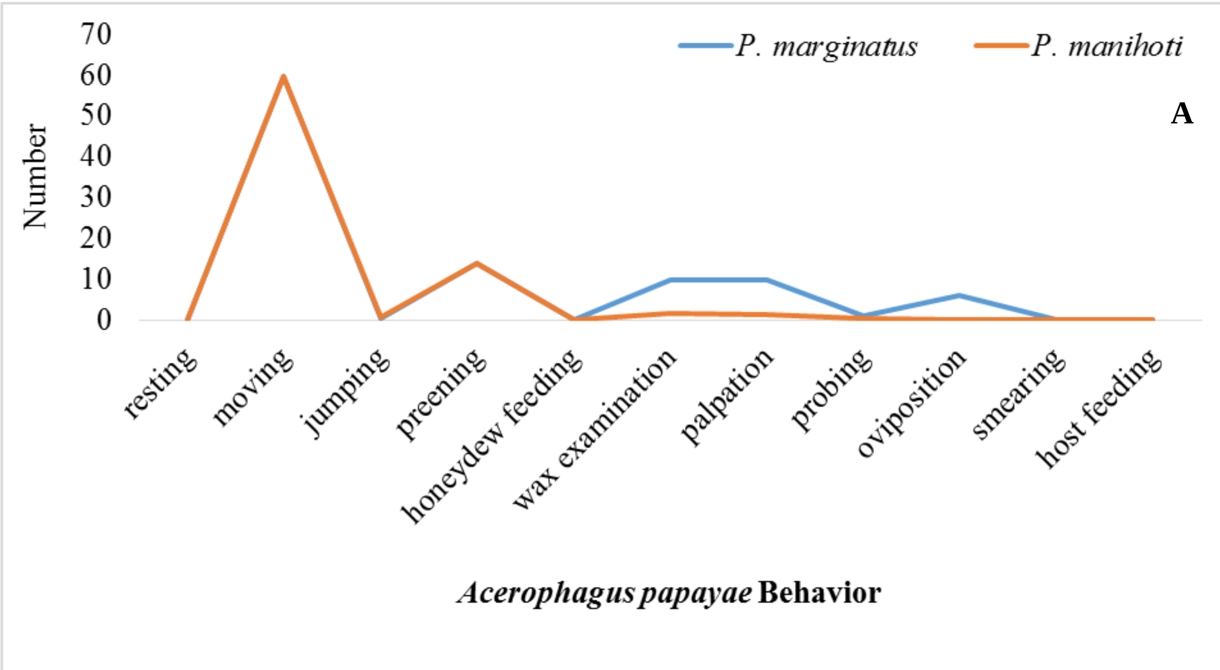


Figure 19. Searching behavior of *A. papayae* in choice condition when in presence of *Paracoccus marginatus* paired with *Ferrisia virgata.*



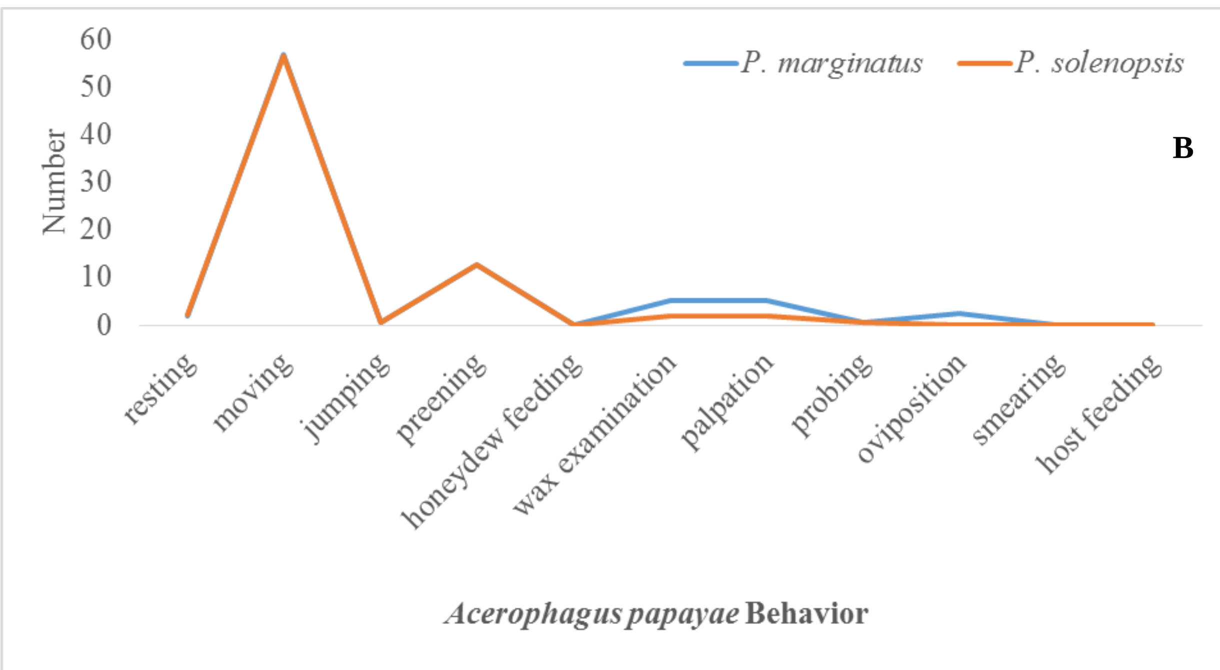


Figure 20. Searching behavior of *A. papayae.* A = Under choice condition in the presence of *Paracoccus marginatus* paired with *Phenacoccus manihoti,* B = Under choice condition in the presence of *Paracoccus marginatus* and *Phenacoccus solenopsis.*

4.2.3 Host instars parasitized by *Acerophagus papayae*

Female parasitized all development instars of *Paracoccus marginatus* excepted ovisacs in both choice and no choice conditions. There was no significant difference in host instars parasitized by *A. papayae* in no choice compared to choice conditions with *P. solenopsis* (P = 0.0581), *P. manihoti* (P = 0255) and *F. virgata* (P = 0.616). Because the frequency of encounters and attacks of the parasitoid with an adult female with ovisac was very low, this instar was excluded for the final analysis.

4.2.3.1 Host instar preference of *Acerophagus papayae* in no choice condition

Female parasitoid encountered (host examination without parasitisism) 69 instars of *P. manihoti* while 391 instars of *P. marginatus* were parasitized. Female + ovisac were less parasitized (N=28) while second and young female instars was more parasitized (Tables 4 and 5).

Table 4. *Paracoccus marginatus* instars parasitized by a female *A. papayae* in no choice condition.

Host instars	Number attacked	Mean number/fem	Percentage
Ovisac	0	0.00	0.00
First	64	1.28	16.37
Second	112	2.24	28.64
Third	76	1.52	19.44
Young female	111	2.22	28.39
Female + Ovisac	28	0.56	7.16
Total	**391**	**7.82**	**100**

Table 5. Percentage of *P. marginatus* instars A parasitized over B by a single female *A. papayae*

Host instars			% of B	Chi^2	P value
A	**N instar**	**B**			
First	64	Second	28.64	28.23*	0.0001
		Third	19.44	2.16	0.1416
		Young Female	28.39	27.20*	0.002
		Female + ovisac	7.16	48.48*	0.00002
Second	112	Third	19.44	20.58*	5.71e-06
		Young Female	28.39	0.0031	0.9553
		Female + ovisac	7.16	268.22*	<2.2e-16
Third	76	Young Female	28.39	14.97*	0.0001
		Female + ovisac	7.16	86.80*	<2.2e-16
Young female	111	Female + ovisac	7.16	261.83*	<2.2e-16

* Significant difference at 5%.

4.2.3.2 Host instar preference of *Acerophagus papayae* in choice condition

In the choice condition, *P. marginatus* was significantly preferred to all other mealybugs species in terms of number of hosts parasitized. The low number of host instars of *P. marginatus* parasitized was observed in the presence of *P. solenopsis*. In the presence of *P. solenopsis*, second and third instars of *P. marginatus* were more preferred. First instars were not parasitized (Table 6), whereas the first and second instars were the most parasitized in presence of *F. virgata* (Table

7). Host instars of *P. marginatus* were more parasitized by female parasitoids when paired with *P. manihoti*, N = 302 (98.69%) with only N = 4 (1.30%) of *P. manihoti* instars. First instars were less parasitized (0.33%) than the third instars (43.14%) (Table 8).

In summary, the results show that *A. papayae* females may oviposit in all instars of *P. marginatus* but the first instars hosts are less often parasitized. Thus, *A. papayae* prefers hosts older (third and young adult) than first and second instar. The presence of non-target mealybug did not influence the behavior of the female parasitoid in term of host finding.

Table 6. Percentage of *P. marginatus* instar A parasitized over B by a single female *A. papayae* when exposed to *P. marginatus* and *P. solenopsis*

Host instars			% of B	Chi^2	P value
A	**N instar**	**B**			
Second	38	Third	41.67	8.73*	0.0031
		Young Female	21.97	3.23	0.0724
		Female + ovisac	3.79	219.91*	< 2.2e-16
Third	55	Young Female	21.97	29.06*	7.03e-08
		Female + ovisac	3.79	510.13*	< 2.2e-16
Young Female	29	Female + ovisac	3.79	114.98*	< 2.2e-16

* Significant difference at 5%

Table 7. Percentage of *P. marginatus* instars A parasitized over B by a single female *A. papayae* when exposed to *P. marginatus* and *F. virgata.*

Host instars			% of B	Chi^2	P value
A	**N instar**	**B**			
First	58	Second	29.71	3.14	0.0763
		Third	18.41	5.09*	0.02411
		Young Female	20.08	2.36	0.1247
		Female + ovisac	6.69	115.43*	< 2.2e-16
Second	71	Third	18.41	19.60*	9.55e-06
		Young Female	20.08	13.23*	0.0002762
		Female + ovisac	6.69	199.08*	< 2.2e-16
Third	44	Young Female	20.08	0.32*	5.72e-01
		Female + ovisac	6.69	50.69*	1.08e-12
Young Female	48	Female + ovisac	6.69	66.51*	3.49e-16

* Significant difference at 5%

Table 8. Percentage of *P. marginatus* instar A parasitized over B by a single female *A. papayae* when exposed to *P. marginatus* and *F. virgata.*

Host instars			% of B	Chi^2	P value
A	**N instar**	**B**			
First	1	Second	17.32	60.69*	6.67e-15
		Third	43.14	229.20*	< 2.2e-16
		Young Female	31.37	136.37*	< 2.2e-16
		Female + ovisac	6.54	18.33*	1.86e-05
Second	53	Third	43.14	82.93*	< 2.2e-16
		Young Female	31.37	27.58*	1.51e-07
		Female + ovisac	6.54	56.56*	5.46e-14
Third	132	Young Female	31.37	19.25*	1.15e-05
		Female + ovisac	6.54	665.70*	< 2.2e-16
Young Female	96	Female + ovisac	6.54	305.23*	< 2.2e-16

* Significant difference at 5%

4.2.4 Host feeding

Host feeding occurred once on first instars of *P. marginatus* when paired with *F. virgata*. After oviposition, female parasitoid turned around, held the already parasitized host with the forelegs and fed on it throughout the opening made with the ovipositor. The content of the mealybug was exhausted resulting in the death of the host.

4.2.5 Duration of oviposition

Oviposition was noted after ovipositor insertion was followed by pumping. Egg-laying duration was timed in both choice and no choice condition. The least oviposition duration was recorded on the third-instar (9.5 s) and the highest on the second-instar (222.5 s). (Table 9).

Table 9. Mean oviposition duration (Second) of *A. papayae* in choice and no-choice condition

Host instars	No-choice condition			Choice condition			No-choice and choice condition		
	Min	**Mean ± SD**	**Max**	**Min**	**Mean ± SD**	**Max**	**Min**	**Mean ± SD**	**Max**
First	21.3	70.7 ± 59.4	76.6	17.6	97.7 ± 104.7	130.0	17.6	84.5 ± 85.8	86.0
Second	13.5	156.9 ± 109.3	222.5	12 52	128.1 ± 92.4	175.3	12.5	138.6 ± 99.3	189.3
Third	26.4	133.3 ± 81.0	181.0	9 5	122.2 ± 99.4	154.0	9.5	124.9 ± 95.1	163.0
Young female	23 4	116.9 ± 76.1	142.5	15 30	111.7 ± 63.4	150.4	15.4	113.7 ± 68.3	150.0
Female + ovisac	27 4	115.8 ± 95.6	108.8	13 40	120.4 ± 92.0	157.3	13.4	118.5 ± 92.4	152.6

CHAPTER FIVE

5.0 Discussion

Paracoccus marginatus is a polyphagous insect and has a large range of host plants species (Miller *et al.*, 1999; Meyerdirk *et al.*, 2004; Muniappan *et al.*, 2008, Tanwar *et al.*, 2010; Goergen *et al.*, 2011). In such polyphagous insects, the life history may vary with the host plant and the quality of host plants may influence some insect's life history parameters such as developmental time, fecundity, longevity and survival (Awmack and Leather, 2002). This justifies the variation in some life history parameters of *P. marginatus* in this study. Host plants especially food limitation also play an important role in regulating insect populations (Umbanhowar and Hastings, 2002). There was a significant difference in the development time of *P. marginatus* reared on four host plants species tested. The cumulative development time determined was from the first instar to adult. However, *P. marginatus* was however not able to develop, survive and reproduce on all four plants tested. Only *C. papaya* permitted the full development of both male and female.

At 28 °C, it takes 15.00, 19.00 and 17.84 days for the female adult to complete its development on *C. papaya*, *M. esculenta* and *S. macrocarpon* respectively. The cumulative developmental time of female *P. marginatus* were 25.9 and 23.2 days including an egg hatching time of 8.7 and 7.3 days at 25 °C and 30 °C respectively (Amarasekare, 2007). Male had longer developmental time than female. This is somewhat in agreement with previous studies in which at 25 °C, it takes 27-30 days for the male to complete its development and 24-26 days for the female (Walker *et al.*, 2003; Amarasekare, 2007; Tanwar *et al.*, 2010). Different host plant species have affected the development time of *P. marginatus* and others mealybugs. Female develops more rapidly on Acalypha and Parthernium than on Hisbiscus and Plumeria species (Amarasekare, 2007). Similar trends were found by Nagrare *et al.*, (2011) with *Phenacoccus solenopsis* where developmental period from immature crawler to adult stage was 13.2 days at mean temperature and relative humidity of 23.3-30.2 °C and 40.5-92.5% RH. Similar results were found with *Planococcus kraunhiae* (Kuwana) where female developmental time was shorter when reared on germinated *Vicia faba* L. seeds than on leaves of a *Citrus* sp. L. and on *Cucurbita maxima* Duchesne (Narai and Murai, 2002).

On the other hand, the development of *P. marginatus* was not completed on *Vernonia amygdalina*. The development was interrupted at the second instar. It may be suggested that *P. marginatus* does not prefer this host plant. In the field, *V. amygdalina* may have been used to tide over a difficult period pending a favorable time when the preferred host plants become available. This result agrees with Hsiao and Fraenkel (1968a) who had postulated that the initial acceptance of a plant as food by an insect does not necessarily mean that the plant will support growth and development. Also, the sticky layer observed on the leaves of *V. amygdalina* may have contributed to this interrupted development of *P. marginatus*. It may also be due to the low sap content and, the bitter taste of the host plants which may be involved in the variation in plant suitability, although these factors were not investigated for *P. marginatus* in this study. The male development of *P. marginatus* lasted to third instar when reared on *S. macrocarpon* and *M. esculenta*. Studies done by Wigglesworth (1965) and Chine and Highland (1985), have explained that all the requirements for growth and development in adult insects depend upon the adequacy and quality of its nutrition during the larval stages. Again the biochemical properties and the quality of the nutrients available in the host may not have promoted the formation of the cocoon (pre-pupa) from which the adult male emerged.

The survival of *P. marginatus* first-instar was low on different host plants species tested as compared to the second-instar. In regard to each host plant tested, the loss of the first instar was offset in the second instar. The highest loss of the third instar female was observed on *M. esculenta*. A loss of 17 to 18% of the first instars was also observed on hibiscus, acalypha, and parthenium (Amarasekare, 2007). A low survival rate of first-instar mealybugs was also observed when *Planococcus kraunhiae* were reared on *V. faba* seeds (Narai and Murai, 2002). The loss of first instar *P. marginatus* may be due to the movement of crawlers (first instars) away from the leaf tissues and their falling off the plants. This movement was observed on all plant species, although it was more evident on *C. papaya.*

The reproduction of *P. marginatus* was also affected by different host plants species. This variation in *P. marginatus* response has previously been shown to be attributed to the host plants and the condition under which the plants were grown, therefore, the number of eggs laid by *P. marginatus* varied from 186.3 on plumeria to 244.4 on hibiscus (Amarasekare, 2007). Chong *et al.*, (2003) reported that *P. marginatus* can produce as many as 300 eggs in approximately 11 days at 25 °C. Females usually lay 100 to 600 eggs with egg-laying usually occurring over the period of one to two weeks (Walker *et al.*, 2003). The differences in the production of egg by *P. marginatus* on the different host plants used in this study also confirmed the finding of Wigglesworth (1965), Engelmann (1970), Chine and Highland (1985), that the food an insect has eaten as a larva helps to determine the oviposition responses of the adult female. However, difference between our study and the results of above-mentioned researchers to might however be as result of host plant differences.

The longevity of adult females was significantly influenced by the different host plants tested. The longevity of adult female range from 12-46 days, 15-36 days and 14-48 days on *M. esculenta*, *C. papaya* and *S. macrocarpon* respectively. The differences in the longevity of *P. manihoti* reared on the different host plants probably reflects the differences in the quality of food on which they were reared. Similar results were found by Chine and Highland (1985) in the life span of stored product insects on various food components. Lukefahr and Martin (1964) had also observed variations on the life span of adult bollworm reared on three different diets.

Understanding the variations in host plants and food quality among different host plants could have useful implications for the management of insect pests including *P. marginatus* (Greeberg *et al.*, 2001; Saeed *et al.*, 2009).

Host searching behavior of *Acerophagus papayae* was similar in both choice and no-choice conditions. Searching started by walking around until encountering a host. Wax examination was with antennae and this was always done by the parasitoid. This was observed with *Gyranusoidea tebygi* Noyes when in contact with its host, the mango mealybug *Rastrococcus invadens* Williams (Boavida *et al.,* 1995). Frequently, female passed close to host at a distance of one millimeter, or even walking directly over them without detecting them. A similar attitude was noticed by Bokonon-Ganta *et al.,* (1995) with *Anagyrus mangicola* searching for its host, *R. invadens*. Sometimes the encyrtid parasitoid *Coccidoxenoides peregrinus* (Timberlake) walked over its hosts *Planococcus ficus* (Signoret) without any notable sign of recognition (Joyce *et al.*, 2001), however, the presence of a non-target mealybug did not affect the host recognition. It is suggested that wax examination is a prior host recognition behavior of *A. papayae*. Mealybug's wax on the body surface of *P. marginatus* may contain chemical cues important in host recognition as suggested by Chong (2005) when dealing with *Anagyrus loecki* and its host, *Phenacoccus madeirensis*.

All three non-target mealybug species were parasitized but no eggs were found after host instar dissection. Similar results were recorded by Sagarra *et al.,* (2001) where *Anagyrus kamali* parasitized two non-target mealybugs (*Planococcus citri* and *Planoccocus halli*) out of eights tested. They found that the parasitoid did not complete its development in the latter two hosts. Of eight mealybug species, *M. hirsutus* was the only suitable host for the complete development of *A. kamali* progeny. Individually exposed, *P. manihoti* did not induce oviposition by *A. papayae*. Again, *P. solenospis* and *F. virgata* in no-choice condition were not parasitized. The parasitoid discriminated among different host species and selected the most suitable host for the development and survival of its progeny. This confirmed the host specificity of *A. papayae* to *P. marginatus* even though other co-existing non-targets mealybugs may be found and tested. The absence of alternative hosts for *A. papayae* development might reduce the efficiency of the parasitoid as a biological control agent since other alternative host species will not be available for *A. papayae* when *P. marginatus* is at low densities. This however, is an advantage from the point of view of preservation of native mealybug biodiversity, as the introduction of this new natural enemy should not disturb indigenous species because *A. papayae* is relatively specific to *P. marginatus*, and therefore not competing with indigenous species of natural enemies. In addition, female parasitoids of *A. papayae* were able to select their host instars for oviposition when they had a choice. Host selection behavior is most important because a female parasitoid can manipulate the offspring sex ratio at oviposition by regulating fertilization (King, 1987). The biology of the parasitoids may be greatly influenced by the quality of the host (Doutt, 1959). Host instar selection was partly the same in both choice and no-choice condition. All host instars except the ovisacs were susceptible to parasitism by *A. papayae*. The first instar and female + ovisacs being the least parasitized. Though a wide range of hosts range were used for parasitism, older instars were more preferred. This may be justified by the fact that the host size is one of the important factors that solitary endoparasitoids consider when they select host stages for oviposition (Vinson and Iwantsch, 1980). The same host selection behavior was observed *with Anagyrus mangicola* where second and fourth instars were equally often used for oviposition (Bokonon-Ganta *et al.,* 1995). It has been found that females are produced in mainly hosts older than second instars and male offsprings in smaller hosts (Bokonon-Ganta *et al.,* 1995). Other solitary encyrtids such as *Anagyrus pseudococci* (Girault) (Avidov *et al.,* 1967), *Apoanagyrus* (Epidinocarsis) *Lopezi* De santis (Kraaijeveld and van Alphen, 1986; Löhr *et al.,* 1988) have exhibited similar behavior in producing more males offspring than females into smaller hosts. 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 sex ratio from the smaller hosts (King, 1987). *Anagyrus loecki* Noyes and Schauff has shown male-biased sex ratio in second-instar hosts and female-biased sex ratio in third-instar and adult female mealybugs (Amarasekare, 2007). Also, the solitary endoparasitoid, *Aenasius vexans* Kerrich (Hymenoptera: Encyrtidae), which was able to oviposit in second-instar nymphs of *Phenacoccus herreni* Cox and Williams (Hemiptera: Pseudococcidae), also recorded a considerably higher proportion of males in the second instar than in the larger instars of *P. herreni* (Bertschy *et al.,* 2000). *Acerophagus papayae* female was able to parasitize as many as host within the frame of time of the observation. The time spent on a single female varied from host instar to another. The oviposition may last as long as 10 minutes when the host is most suitable. The least duration was 9.5 seconds. The short time often recorded was reflected in more host instars parasitized. Bokonon-Ganta *et al.,* (1995) found that even after the shortest oviposition time recorded, which lasted only 5.4 seconds, an egg was deposited by *Anagyrus mangicola*. Another factor that influences the host-parasitoid population dynamics is host feeding (Godfray, 1994). Egg-laying behavior in parasitoid demands protein consumption. Female may feed upon its host during host searching andhost feeding behavior was recorded on first instar hosts. This was supplemented by honeydew feeding by the female parasitoid in this study. Previous studies on parasitoid behavioral studies have shown that first instars were more often host-fed upon. First instars of *Rastrococcus invadens* Williams (Hemiptera: Pseudococcidae) were preferred for host feeding by the parasitoid *Anagyrus mangicola* Noyes (Hymenoptera: Encyrtidae) (Bokonon-Ganta *et al.*, 1995). This has also been recorded on *A. pseudococci* (Girault) (Avidov *et al.*, 1967) and *Apoanagyrus* (Epidinocarsis) *lopezi* De santis (Kraaijevekd and van Alphen, 1986; Löhr *et al.*, 1988). Female wasps of *Leptomastix epona* may host feed on small mealybugs (second and third instar nymphs) that they do not use for oviposition (Farahani *et al.*, 2011). This is in contrast with our findings in which the female parasitoid fed upon its host immediately after oviposition. If such hosts were not available, females may feed on plant nectar or plants exudates, honeydew feeding. This indicates that feeding is important to the parasitoid.

Acerophagus papayae is effective in searching for and parasitizing *P. marginatus* even when associated with another mealybug. Foraging behavioral observations on *A. papayae* suggested that the parasitoid was able to parasitize all developmental stages of *P. madeirensis* but preferred the third-instar immature and young female mealybugs. This result suggested *A. papayae* was especially effective in searching for its most preferred host stages. Overall, *A. papayae* possesses suitable characteristics with regard to host specificity and host instar selection. It showed better adaptability by being able to oviposit in second to young female.

CHAPTER SIX

6.0 Conclusion and Recommendations

6.1 Conclusion

Biological control program for one pest involves an understanding of the variations in host plants and food quality among different host plants. It must also be compatible with the production practices and the management program against other pests. This may be for the management of insect pests including *Paracocccus marginatus.* Host suitability of *P. marginatus* under laboratory conditions have shown significant variations in the pest response to the different host tested. Of the four host plants species tested (*V. amygdalina, M. esculenta, S. macrocarpon* and *C. papaya*), *C. papaya* was the most suitable for the development of both male and female. Host plants have affected in a different manner the development, reproduction and survival of *P. marginatus*. As an alternative host, *M. esculenta* may be useful for mass production of *P. marginatus*. Results show that *C. papaya* remains the preferred host of *P. marginatus*.

Furthermore, the host specificity study suggest that *Acerophagus papayae* is specific to *P. marginatus* among the three mealybug species tested (*Ferrisia virgata*, *Phenacoccus manihoti* and *P. solenopsis*). The effect of *A. papayae* on those non-target mealybug species is found to be minimal. Searching behavior of *A. papayae* in both choice and no-choice condition is similar and it is more effective in *P. marginatus* encountered. This shows that *A. papayae* is able to parasitize all developmental stages of *P. marginatus* but preferred the third-instar and young female mealybugs. In general, *A. papayae* possesses suitable characteristics with regard to host specificity and host instar selection. It showed better adaptability by being able to oviposit in second to young female. In conclusion, our findings will help to perceive the performance of *P. marginatus* and could help in its management and control.

6.2 Recommendations

Future studies should focus on the following:

- The wide range of common host plants species for the development, reproduction and survival of *P. marginatus* under laboratory condition
- Assessment of the component of host plants species of *V. amygdalina* as control measure of *P. marginatus*
- The evaluation of *M. esculenta* as host plant for the mass production of *A. papayae*
- The interactions between *A. papayae* and other biological control agents of mealybugs.
- The non-target effect of others mealybugs associated with *Paracoccus marginatus*
- The potential of the different non-target mealybugs that prevent *A. papayae* from attacking them.

Therefore, future studies should focus on testing a wider range of host plant species available in our agro-ecological area for the development of *P. marginatus.* In addition, assessing the chemical components of the hosts plants would help to better understand the mechanism of host suitability.

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APPENDICES

Appendix 1. Tables of stastical analysis

Table 1. Mean square of the analysis of variance of the percent mortality for the first instar of *P. marginatus* reared different host plants species

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Host Plant	3	0.5678	0.18926	13.35	5.18e-06
Residuals	36	0.5105	0.01418		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 2. Mean square of the analysis of variance of the percent mortality for the second instar of *P. marginatus* reared different host plants species

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Host Plant	3	0.7662	0.25539	8.451	0.000221 ***
Residuals	36	1.0879	0.03022		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 3. Mean square of the analysis of variance of the percent mortality for the third instar female of *P. marginatus* reared different host plants species

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
HostPlant	3	13.72	4.573	16.12	8.32e-07 ***
Residuals	36	10.21	0.284		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 4. Mean (±SE) of pre-oviposition, oviposition and post-oviposition period of *P. marginatus* reared on three host plants species

Parameters	Host plants species			
	Carica papaya	*Manihot esculenta*	*Solanum marcrocarpon*	*Vernonia amygdalina*
Pre-oviposition period	7.65 ± 0.27	15.08 ± 6.22	11.54 ± 5.66	00.00
Oviposition period	9.65 ± 1.82	6.83 ± 4.24	4.54 ± 2.04	00.00
Post-oviposition period	4.8 ± ± 1.84	9.42 ± 3.85	11 ± 6.70	00.00
df	19	11	12	00.00

Table 5. Characteristic of the distribution of the pre-oviposition period of *P. marginatus* reared on three host plants species

Characteristic of the distribution	Host plant species		
	Carica papaya	*Manihot esculenta*	*Solanum marcrocarpon*
Minimum	7.00	5.00	4.00
First quartile	7.00	8.00	6.00
Median	8.00	10.00	9.00
Mean	7.65	15.08	11.54
Third quartile	8.00	25.50	12.00
Maximum	9.00	31.00	34.00
Standard deviation	0.59	9.79	9.37

Table 6. Characteristic of the distribution of the oviposition period of *P. marginatus* reared on three host plants species

Characteristic of the distribution	Host plant species		
	Carica papaya	*Manihot esculenta*	*Solanum marcrocarpon*
Minimum	4.00	1.00	1.00
First quartile	6.00	1.75	2.00
Median	9.50	5.50	3.00
Mean	9.65	6.83	4.54
Third quartile	11.25	7.50	8.00
Maximum	18.00	20.00	9.00
Standard deviation	3.88	6.67	3.38

Table 7. Characteristic of the distribution of the post-oviposition period of *P. marginatus* reared on three host plants species

Characteristic of the distribution	Host plant species		
	Carica papaya	*Manihot esculenta*	*Solanum marcrocarpon*
Minimum	1.00	1.00	1.00
First quartile	2.00	3.75	3.00
Median	3.00	12.50	4.00
Mean	4.80	9.42	11.00
Third quartile	6.50	14.00	18.00
Maximum	14.00	18.00	34.00
Standard deviation	3.93	6.07	11.05

Table 8. Characteristic of the distribution of longevity of *Paracoccus marginatus* adult female of longevity reared on four different host plants species

Characteristic of the distribution	Host plants species			
	Carica papaya	*Manihot esculenta*	*Solanum marcrocarpon*	*Vernonia amygdalina*
Minimum	15.00	12.00	14.00	00.00
First quartile	17.75	25.75	21.00	00.00
Median	20.50	30.00	28.50	00.00
Mean	22.10 [a]	31.50 [b]	30.35 [b]	00.00
Third quartile	26.25	38.50	37.25	00.00
Maximum	36.00	46.00	48.00	00.00
Standard deviation	5.84	8.72	9.92	00.00

Table 9. Mean (±SE) of the fecundity of *Paracoccus marginatus* adult female reared on four different host plants species

	Host plants species			
	Carica papaya	*Manihot esculenta*	*Solanum marcrocarpon*	*Vernonia amygdalina*
Fecundity	277.4 ± 65.67551	227.75 ± 156.7509	141.2308 ± 63.0521	00.00

Table 10. Characteristic of the distribution of the fecundity of *Paracoccus marginatus* adult female reared on four different host plants species

Characteristic of the distribution	Host plant species		
	Carica papaya	*Manihot esculenta*	*Solanum marcrocarpon*
Minimum	47.00	0.00	0.00
First quartile	189.00	0.00	0.00
Median	265.00	13.50	82.00
Mean	277.40	58.08	180.90
Third quartile	326.50	54.75	160.00
Maximum	579.0	336.00	695.00
Standard deviation	140.328	100.84	250.32

Table 11. Different host instars of *P. marginatus* and *P. solenopsis* parasitized by a female *A. papayae* in choice condition

Host instar	Number parasitized		Mean number/fem		Percentage	
	P. marginatus	*P. solenopsis*	*P. marginatus*	*P. solenopsis*	*P. marginatus*	*P. solenopsis*
Second	38	1	0.76	0.02	28.79	0.76
Third	55	3	1.1	0.06	41.67	2.27
Young adult	29	1	0.58	0.02	21.97	0.76
Female + Ovisac	5	0	0.1	0	3.79	0
Total	127	5	2.54	0.1	96.21	3.79

Table 12. Different host instars of *P. marginatus* and *F. virgata* parasitized by a female *A. papayae* in choice condition

Host instar	Number parasitized		Mean number/fem		Percentage	
	P. marginatus	*F. virgata*	*P. marginatus*	*F. virgata*	*P. marginatus*	*F. virgata*
Ovisac	0	0	0	0	0	0
First	58	0	1.16	0	24.27	0
Second	71	2	1.42	0.04	29.71	0.84
Third	44	0	0.88	0	18.41	0
Young adult	48	0	0.96	0	20.08	0
Female + Ovisac	16	0	0.32	0	6.69	0
Total	237	2	4.74	0.04	99.16	0.84

Table 13. Different host instars of *P. marginatus* and *P. manihoti* parasitized by a female *A. papayae* in choice condition

Host instar	Number parasitized		Mean number/fem		Percentage	
	P. marginatus	*P. manihoti*	*P. marginatus*	*P. manihoti*	*P. marginatus*	*P. manihoti*
Ovisac	0	0	0	0	0	0
First	1	0	0.02	0	0.33	0
Second	53	0	1.06	0	17.32	0
Third	132	4	2.64	0.08	43.14	1.31
Young adult	96	0	1.92	0	31.37	0
Female + Ovisac	20	0	0.4	0	6.54	0
Total	302	4	6.04	0.08	98.69	1.31

Appendix 2. Figures

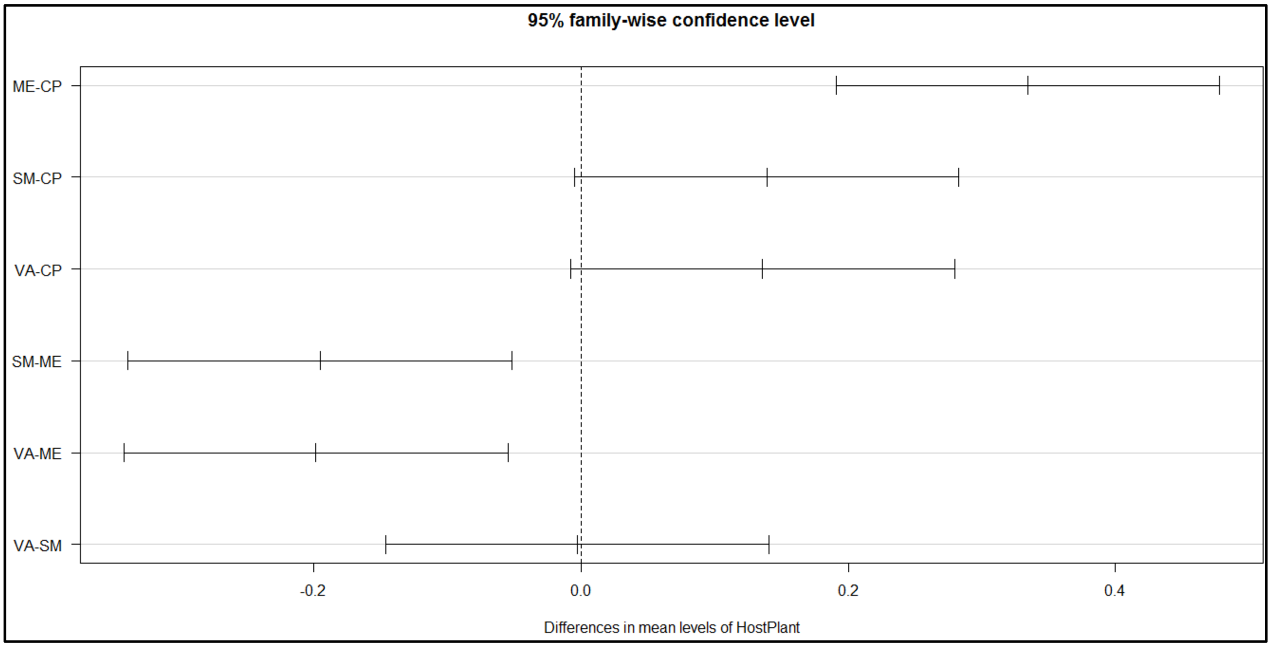

Figure 1. Difference in Mean level of the percent mortality for the first instar of *P. marginatus* reared different host plants species.

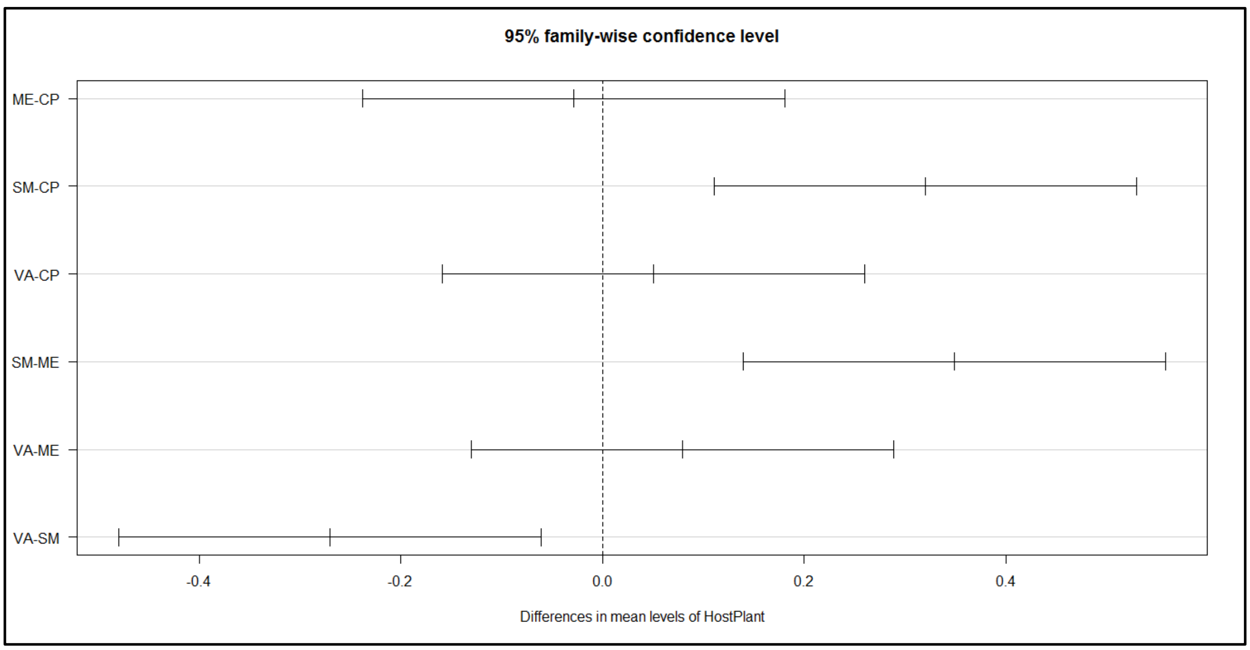

Figure 2. Difference in Mean level of the percent mortality for the second instar of *P. marginatus* reared different host plants species.

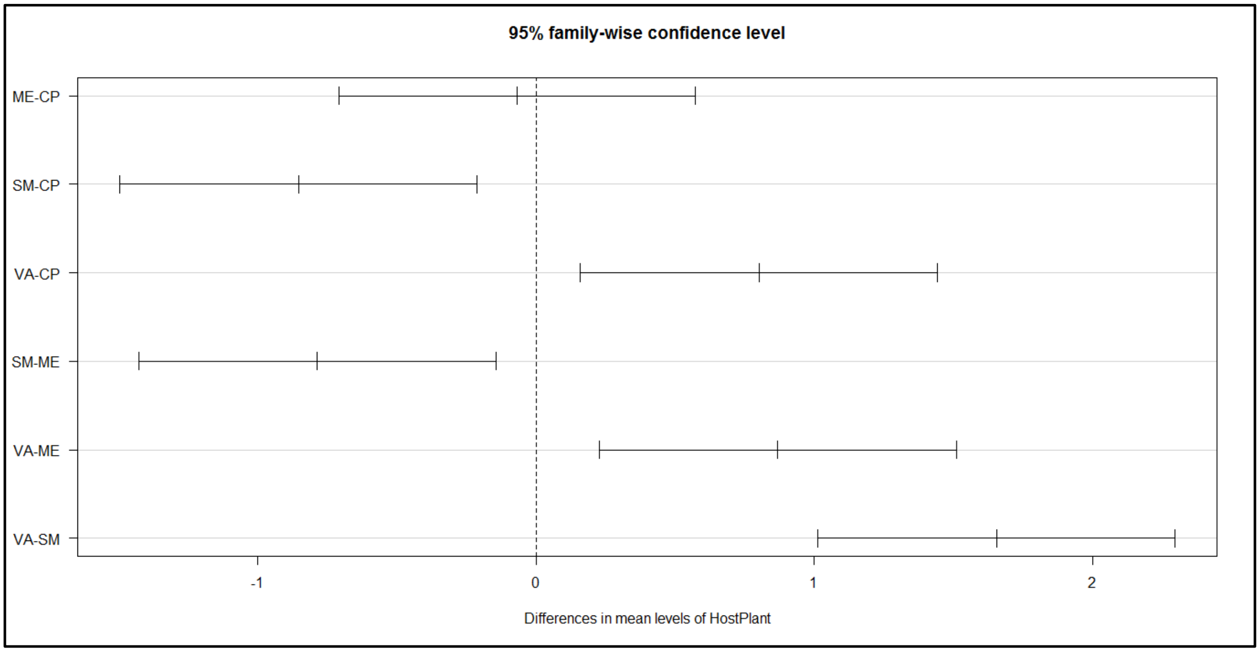

Figure 3. Difference in Mean level of the percent mortality for the Third instar of *P. marginatus* reared different host plants species.

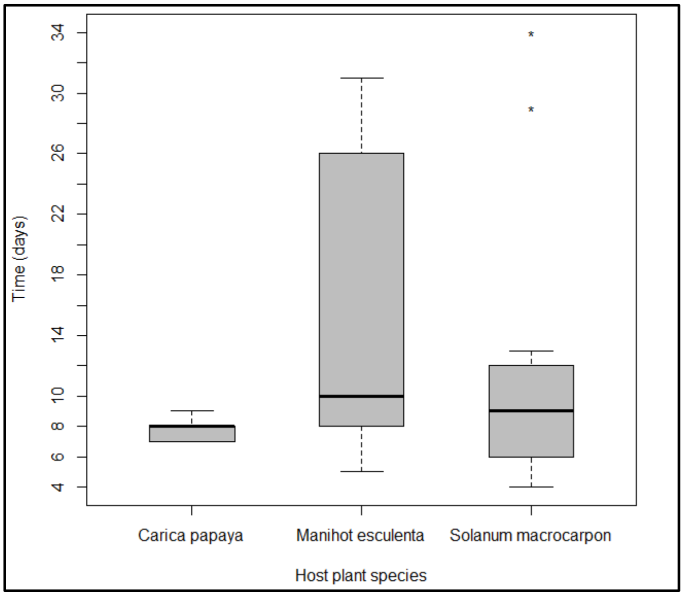

Figure 4. Distribution of female pre-oviposition period on different host plant species.

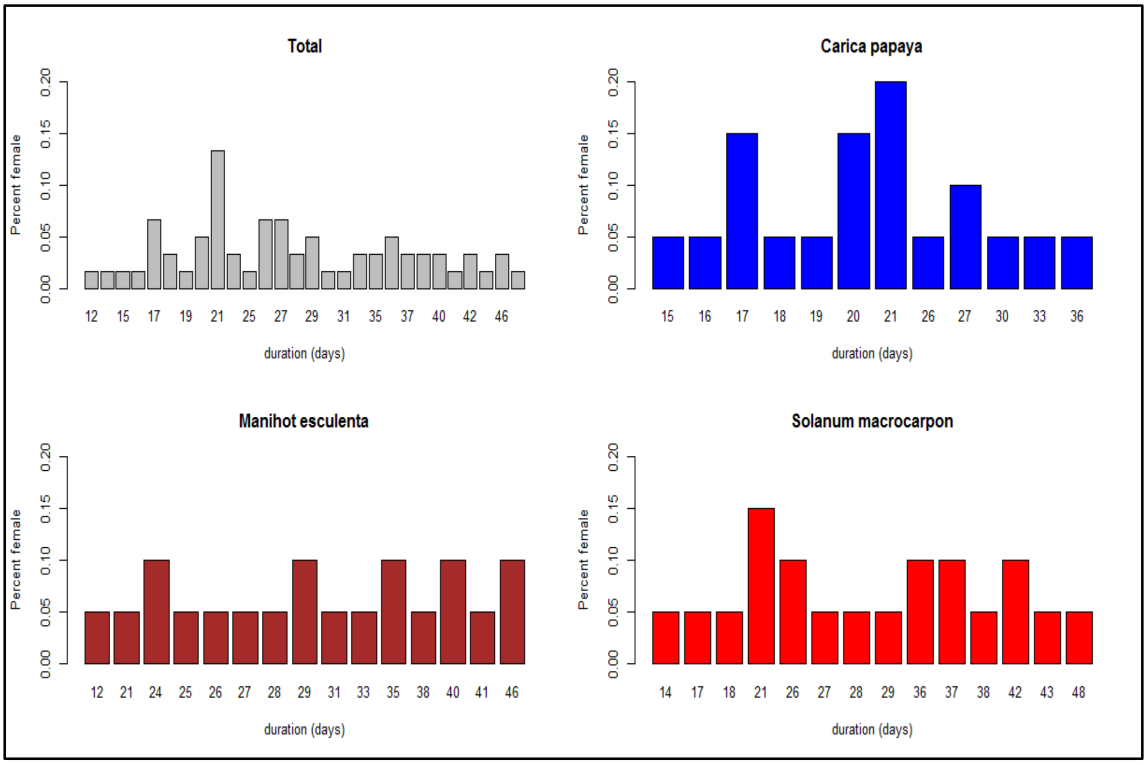

Figure 5. Longevity of female adult on different host plant species.

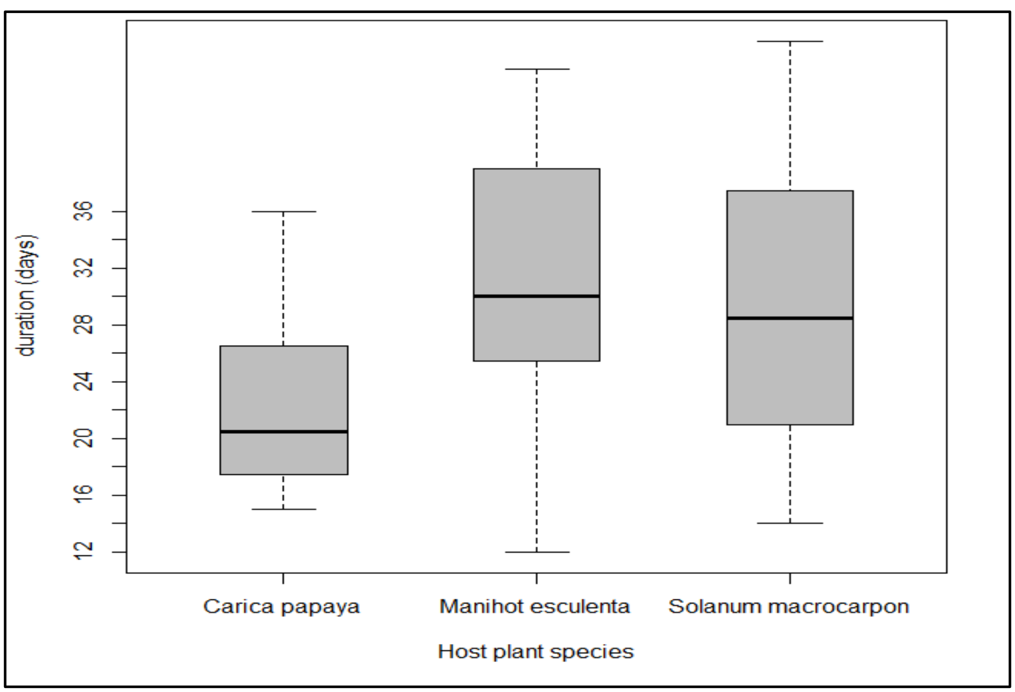

Figure 6. Distribution of the female longevity on different host plant species.

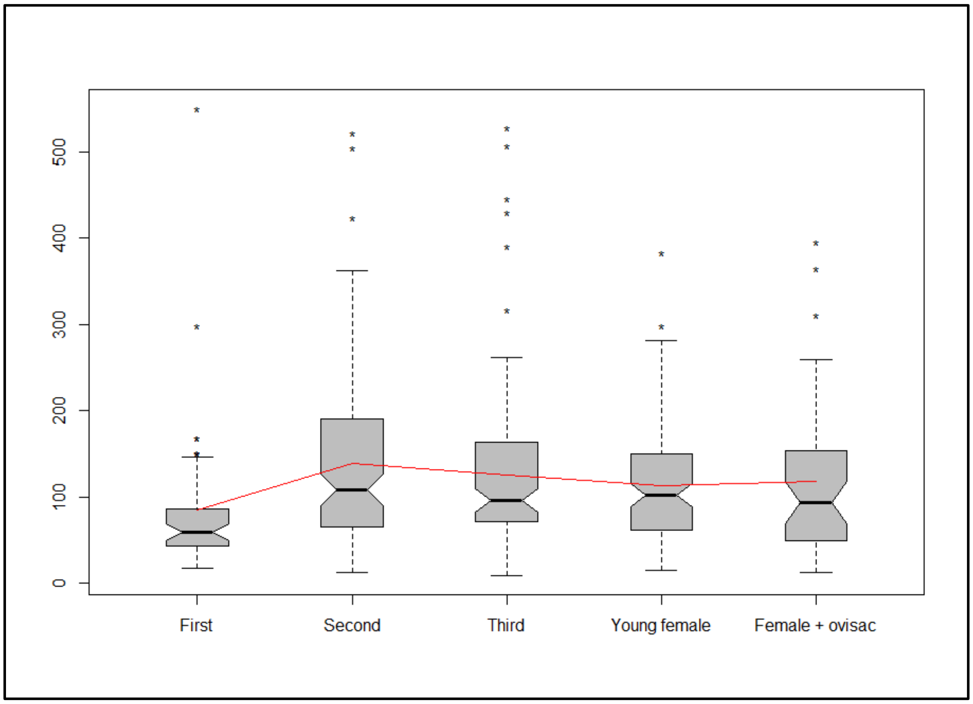

Figure 7. Distribution of the oviposition duration on different host plants species in choice and no-choice condition.

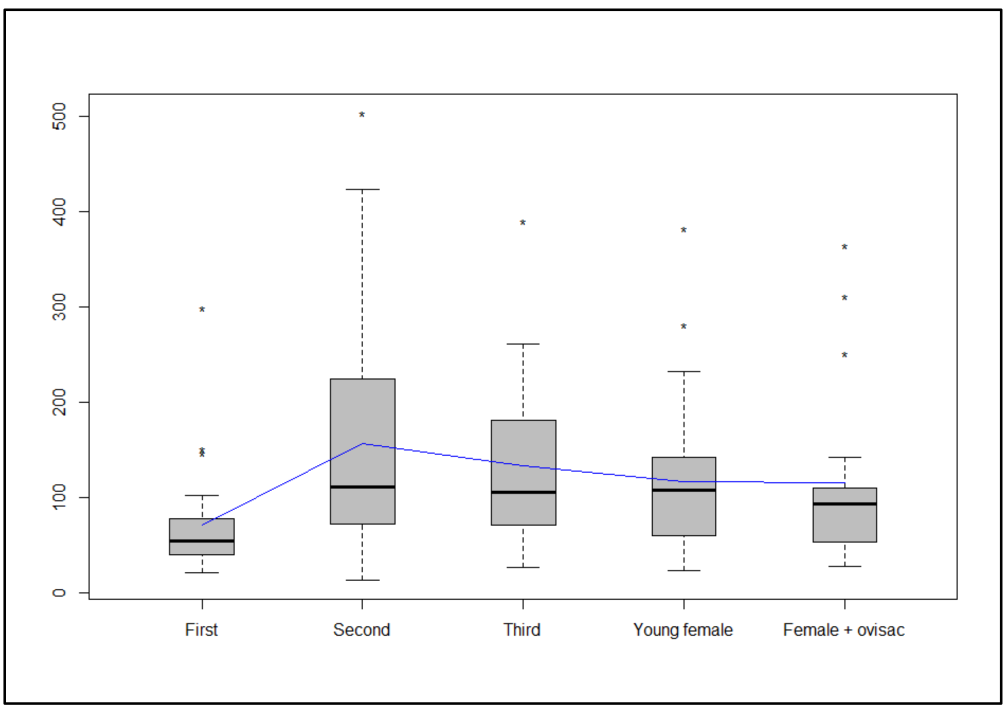

Figure 8. Distribution of the oviposition duration on different host plants species in no-choice condition.

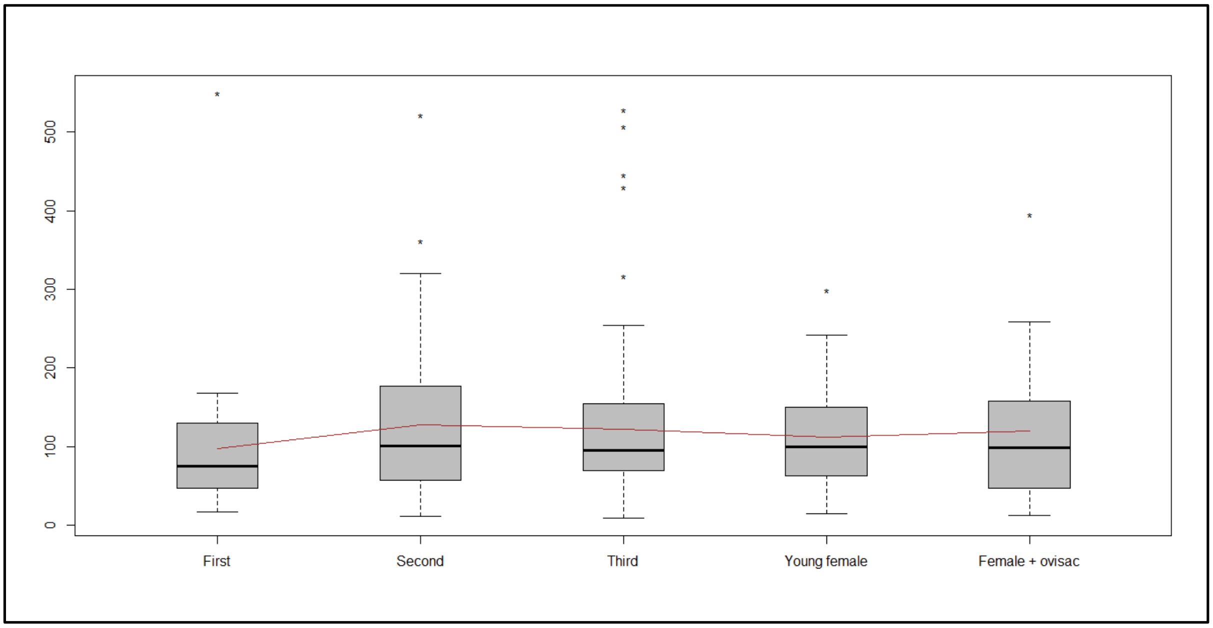

Figure 9. Distribution of the oviposition duration on different host plants species in choice condition.