INFESTATION INDUCED REACTIONS OF PAPAYA MEALYBUG, *Paracoccus marginatus* WILLIAMS AND GRANARA DE WILLINK, (HEMIPTERA: PSEUDOCOCCIDAE) ON PAPAYA AND AMARANTHUS

By

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THESIS

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DECLARATION

I, hereby declare that the thesis entitled **"Infestation induced reactions of papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink, (Hemiptera: Pseudococcidae) on papaya and amaranthus"** is a bonafide record of research work done by me during the course of research and that it has not been previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

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We, the undersigned members of the advisory committee of **Ms. Jimcymaria T. (2010-11-117)** a candidate for the degree of **Master of Science in Agriculture** with major field in **Agricultural Entomology** agree that this thesis entitled **"Infestation induced reactions of papaya mealybug *Paracoccus marginatus* Williams and Granara de Willink, (Hemiptera: Pseudococcidae) on papaya and amaranthus"** may be submitted by **Ms. Jimcymaria T.,** in partial fulfillment of the requirement for the degree.

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Introduction

1. INTRODUCTION

Papaya (*Carica papaya* L.) is valued for its nutritive and medicinal properties. Its fruit is a rich source of vitamin A and C. Though papaya cultivation had its origin in South Mexico and Costa Rica, India leads the world in papaya production with an annual output of about three million tonnes compared to an estimated annual world production of six million tonnes of fruit. Brazil, Mexico, Nigeria, India and Indonesia have consistently been the top producers contributing more than 71 per cent of the total world production. In India, papaya is grown in an area of 95749 hectares wherein, Kerala contributes 17723 hectares with 2.06 per cent of total production (National Horticulture Board, 2010). In Kerala, the limiting factors for commercial cultivation are high rainfall and severe drought in summer. However, this is best suited as a homestead fruit crop. The papaya prefers a rich, well-drained soil. It will not tolerate water logging around the trunk.

Papaya got popularity among the farmers for its export potential of the dried latex (papain) and relative freedom from pests and diseases. Some of the insect pests recorded in papaya are, papaya fruit fly (*Toxotrypana curvicauda* Gerstaecker), webworm (*Davara caricae* Dyar), whitefly (*Trialeuroides variabilis* Quaintance), papaya scale (*Philephedra tuberculosa* Nakahara and Gill), leafhopper (*Empoasca stevensi* Young), aphids (*Myzus persicae* Sulzer and *Lipaphis erysim*i Katenbach) and mites (*Tetranychus kanzawai* Kishida and *Panonychus citri* McGregor). In the recent past a new invasive insect, papaya mealybug *Paracoccus marginatus* Williams and Granara de Willink, has been first reported from Coimbatore, Tamil Nadu, on papaya, jatropha and other plants in the neighbourhood (Regupathy and Ayyasamy, 2011). The insect assumed the status of a major pest in 2009 when it caused severe damage to economically important crops and huge losses to farmers in Coimbatore, Erode, Tirupur and Salem districts of Tamil Nadu. Earlier reports revealed that apart from infesting papaya, *Paracoccus marginatus* also attacks several other genera of host plants, including economically important tropical fruits and ornamentals (Miller *et al*., 1999). Mealybugs are sexually dimorphic, where the sexes have distinct morphological differences. Females are neotenic (exhibiting nymphal characteristics). Mealybug females often retain legs and can move unlike the related female scale insects. Males are winged

and do change completely during their lives. The papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae) is a small polyphagous sucking insect with pest status that attacks several genera of host plants, including economically important tropical fruits, vegetables and ornamentals.

Papaya mealybug infestations are typically observed as clusters of cotton-like masses on the above-ground portion of plants. Immature and adult stages of *P. marginatus* suck the sap by inserting its stylets into the epidermis of the leaf resulting in curling, crinkling, rosetting, twisting and general leaf distortion. The honey dew excreted by the bug and the associated black sooty mould formation impairs photosynthetic efficiency of the affected plants. Flowers fail to open and petals become twisted or malformed. Fruits may be unusually small and such fruits eventually shrivel and drop. Premature flower drop and poor fruit set occur. Fruit quality gets affected due to large volume of honey dew production resulting in black sooty mould over the infected fruits which reduce the marketability and market value.

Persistent increase in the population of this mealybug and its invasive nature on large number of weed and wild hosts serving as statutory inoculum are likely to be a major threat to the economical production of papaya, mulberry, etc., effective precautionary measures are taken. Commercially available generalist predator, *Cryptolaemus montrouzieri* Mulsant and naturally occurring other lady beetles, lacewings and hover flies have a potential impact on mealybug populations. Biological control through release of laboratory cultured *C. montrouzieri* had been successfully followed for the management of mealybugs on grapes, citrus, mango, guava, coffee, rubber, cocoa and mulberry. Three species of exotic parasitoids namely *Acerophagous papaya* Noyes & Schauff, *Anagyrus loecki* Noyes & Menezes and *Pseudoleptomastrix mexicana* Noyes & Schauff were found to be very effective in controlling the infestation of papaya mealybug. Injuries to plants by insects result in different physiological and biochemical responses than mechanical damage alone. Generally in plants, the endogenous growth hormone, auxin is synthesized in young expanding leaves at the shoot apex and is actively transported down to the plant. Saikia *et al.* (2011) observed that the attack of *Helopeltis theivora* Waterhouse on the axillary vegetative buds and young leaves of tea resulted in

decreased level of auxin than non infested plants. However, very little is understood about the reactions in host plants due to the *P. marginatus* infestation.

Studies on the infestation induced reactions in host plants would help to know the hypersensitivity of plant species to the infestation, the pest load that a host plant could bear and to evolve appropriate management practices to reduce such reactions. Recognizing this need, the present study was initiated to study the infestation by *P. marginatus* and consequent reactions on papaya and amaranthus with the following objectives

1. To assess the pest load on the seedlings of three month old papaya and one month old amaranthus seedlings
2. To assess plant reaction by visual scoring on leaf deformities
3. To estimate the plant hormones (IAA and GA) and protein content in the infested and uninfested leaves of papaya and amaranthus.

Review of Literature

2. REVIEW OF LITERATURE

Papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae), a new invasive species of insect in major agricultural crops in India is causing serious economic threat to the agricultural industry. It causes extensive yield loss in papaya and has the ability to infest several other field and fruit crops. Various earlier works on the very important insect is reviewed hereunder.

2.1. Status of papaya mealybug

2.1.1. Worldwide scenario

Mealybugs (Hemiptera: Pseudococcidae) occur worldwide (Mckenzie, 1967; Williams, 1985; Williams and Granara de Willink, 1992; Miller *et al*., 2005) as an important pest on crops. Ten per cent of world's known species of coccoids has been reported from India. In the current decade, upward trend in the buildup of various mealybug species in crop plants and in the wild hosts was observed mainly due to certain abiotic changes in climate and environment (Tanwar *et al*., 2007).

Papaya mealybug, a native of Mexico and/or Central America (Miller *et al*., 1999) was first described in 1992 (Williams and Granara de Willink, 1992) and was re-described by Miller and Miller (2002). Later, it was reported in St. Martin in the Caribbean in 1995 and since then it had spread to 13 countries in the Caribbean, Florida in the US, and three countries each in Central and South America by 2000 (Miller *et al*., 1999; Matile- Ferrero *et al*., 2000; Kauffman *et al*., 2001). Heavy infestations of *P. marginatus* on papaya were recorded in Guam in 2002 (Meyerdirk *et al*., 2004; Walker *et al*., 2006), in the Republic of Palau in 2003 (Muniappan *et al*., 2006; Walker *et al*., 2006) and in Hawaii in 2004, on papaya, plumeria, hibiscus and jatropha (Heu *et al*., 2007).

2.1.2. India

Incidence of papaya mealybug was first observed in the orchards of Tamil Nadu Agricultural University on July 10, 2008, and it was the first report of the papaya mealybug in India and South Asia (Muniappan, 2009). Papaya mealybug was first recorded in Kerala in 2009. The pest attacks several plants, including economically important tropical fruits and ornamentals (Lyla and Philip, 2010).

Subsequently, surveys conducted during May 2009 in Coimbatore district of Tamil Nadu registered heavy infestations of *P. marginatus* in the orchards of papaya (*Carica papaya* L.; Caricaceae), mulberry (*Morus alba* L.; Moraceae), jatropha (*Jatropha curcus* L.; Euphorbiaceae) and tapioca (*Manihot esculenta* C.; Euphorbiaceae) besides moderate to low infestations on shoe flower (*Hibiscus rosa-sinensis* L.; Malvaceae), guava (*Psidium guajava* L.; Myrtaceae), brinjal (*Solanum melongena* L.; Solanaceae) and tomato (*Lycopersicon esculentum* L.; Solanaceae) (Anonymous, 2009).

2.2. Host range

Papaya mealybug was reported to cause serious damage to tropical fruit, especially papaya, and had been detected most frequently on *Hibiscus* (Miller *et al*., 1999). The mealybug was recorded from 22 plant families including economic and weed plants in genera such as *Acacia, Acalypha, Ananas, Annona, Bidens, Capsicum, Hibiscus, Ipomoea, Mangifera, Manihot, Persea, Plumeria, Punica, Solanum and Vigna* (Muniappan *et al*., 2009). The following table (Table. 1) shows the host range of papaya mealybug in Kerala.

Table.1. Major host plants of *P. marginatus* in Kerala

S. No.	Common name	Scientific name	Parts affected
I	**Vegetables**		
1	Amaranthus	*Amaranthus cruentus* L.	Leaf, stem and inflorescence
2	Brinjal	*Solanum melongena* L.	Stem, leaf and fruits
3	Cowpea	*Vigna unguiculata* (L.) Walp	Tender shoots and flowers
4	Dolichos bean	*Dolichos* sp.	Pods and leaves
5	Red gram	*Cajanus cajan* (L.) Millsp.	Leaves, pods and entire branches
6	Ash gourd	*Benincasa hispida* (Thunb) Cong.	Tender leaves, flowers, vine and fruits
7	Tomato	*Lycopersicum esculentum* L.	Leaves
8	Chillies	*Capsicum* sp.	Leaves and tender parts
9	Malabar spinach/ Water leaf	*Basella* sp.	Leaves
10	Curry leaf	*Murraya koenigii* (L.)	Leaves
II	**Tuber**		
1	Cassava	*Manihot esculenta* Crantz.	Leaves and tender parts
III	**Fruits**		
1	Guava	*Psidium guajava* L.	Entire plant
2	Banana	*Musa* sp.	Tender leaf tip and fruit bunches

3	Jack fruit	*Artocarpus heterophyllus* Lam.	Fruits and trunk
4	Indian gooseberry	*Phyllanthus emblica* L.	Entire plant
IV	**Flowering plants**		
1	Hibiscus/sorrel	*Hibiscus rosa-sinensis*	Entire plant
2	West Indian Jasmine	*Ixora* sp.	Leaves
3	Crape jasmine	*Tabernaemontana* sp.	Entire plant
4	Yellow Trumpet bush/ yellow bell	*Tecoma stans*	Leaves
5	Nerium	*Nerium indicum* Mill.	Leaves
6	Pagoda tree	*Plumeria* sp.	Entire plant
V	**Plantation crops**		
1	Rubber	*Hevea brasiliensis*	Trunk, leaves, Inflorence, fruit, etc.
2	Teak	*Tectona grandis* Linn.	Fresh and old leaves
3	Jatropa	*Jatropa curcas* L.	Entire plant

(Mani Chellapan, 2010)

2.3. Biology of mealybug

Miller and Miller (2002) had given description of *P. marginatus* including descriptions of the immature stages and adult male. The authors provided illustrations and keys for all stages of the insect including first, second and female third instars, male prepupa, female fourth instar, male pupa, adult female and fifth instar male or the adult male.

Amarasekare *et al*. (2008a) studied the effect of temperature on the life history of the mealybug *Paracoccus marginatus* in the laboratory. *P. marginatus* was able to develop and complete its life cycle at 18, 20, 25 and 30 ± 1°C.

Amarasekare *et al*. (2008b) studied the life history of papaya mealybug, *P. marginatus* on three ornamental plants *viz. Hibiscus rosa-sinensis* L., *Acalypha wilkesiana* (Muell.-Arg.), and *Plumeria rubra* L. and one weed species (*Parthenium hysterophorus* L.) under laboratory conditions. There were differences in the life history parameters of mealybug on these host plants. Adult females that developed on *Acalypha* and *Parthenium* emerged approximately 1 day earlier than those that developed on *Hibiscus* and *Plumeria*. Adult males had a longer developmental time on *Plumeria* than on the other hosts. Pre reproductive and reproductive periods of the females were not affected by hosts (averaged 6.3 ± 0.1 and 11.2 ± 0.1 days respectively). Mean fecundity of 186.3 ±1.8 eggs on *Plumeria* was lower than on the other three plant species.

Ghose (1972) made a detailed study on the biology of *M. hirsutus* on hibiscus *var*. roselle (*Hibiscus sabdariffa L. var. altessima*). The mealybug normally completed its life cycle in 23 to 29 days on Roselle, but it was prolonged during winter. The females underwent three moulting during their nymphal period of 11 to 17 days. The males completed their nymphal development in 10 to 19 days with four moultings. The difference in fecundity of the insect on different species of host plant was also reported.

In countries with a cool winter, *Maconellicoccus hirsutus* (Green) survived cold conditions as eggs (Bartlett, 1978) or other stages both on the host plants and in the soil (Pollard, 1995a). There might be as many as 15 generations per year (Pollard, 1995b). Each adult female of *M. hirsutus* lays 150-600 eggs over a period of one week (Bartlett, 1978; Mani and Thontadarya, 1989). One generation was completed in about 5 weeks in warm conditions. Reproduction was mostly parthenogenetic (Singh and Ghosh, 1970) but there was also a report of biparental nature in *M. hirsutus* (Ghose, 1971 & 1972).

Sinacori (1995) studied the life history of *Phenacoccus madeirensis* (Green) under laboratory conditions and reported that it completed 5-6 generations per year. According to Chong *et al*. (2003) *P. madeirensis* was oviparous and reproduced bisexually whereas *Phenacoccus solani* showed thelytokous parthenogenesis (Lloyd, 1952). These two species showed large differences in reproductive performance and nymphal survival under certain conditions (Nakahira and Arakawa, 2006). Nakahira and Arakawa, (2006) observed that the development and reproduction of an exotic mealybug, *Phenacoccus solani* (Ferris) at three constant temperatures (20, 25 & 30°C) and found that optimum temperature for increased development was at 25°C. Survival rates of immature stages were high at all temperatures.

Awadallah *et al*. (2008) reported that *Ferrisia virgata* completed five generations when reared singly on sprouting potato tubers. Rate of mortality of nymphal instars in the autumn/ winter generations was higher than that in the spring/ summer (being 14.3 – 100 and 1-23% respectively).

Laboratory studies on mealybug biology have been accomplished using plant leaves containing nymphs inside test tubes (Ito, 1938), clip cages (Santa-Cecilia *et al*., 2008), potato sprouts (Ghose, 1983; Nakano, 1972), leaves in nutritive solution (Menezes, 1973), leaf sections (Colen *et al*., 2000), germinated broad bean seeds (Narai and Murai, 2002) and foliar sections in agar water (Correa *et al*., 2005). Santa-Cecilia *et al*. (2008) compared three methodologies for the biological studies of citrus mealybug, *Planococcus citri* (Risso) and observed that foliar sections maintained in agar water documented lowest mortality of mealybugs.

2.4. Mode of spread

Heu *et al*. (2007) found that first instar crawler was the dispersal stage of papaya mealybug. Small ‘crawlers’ were readily carried by wind, rain, birds, ants, clothing and vehicle and might settle in cracks and crevices, usually on new plants. Non-infested plants could be infested from infested plants as crawlers could crawl

from an infested plant to another plant. Long distance movement was through carrying infested planting material and fresh fruit and vegetables across the country or even from one end of a farm to the other. Ants also helped in the transportation of mealybugs from plant to plant (Tanwar *et al*., 2007). Due to its short life cycle, more generations per year, higher fecundity, easy dispersal, protective mealy coating, etc., helped the pest to multiply in enormous proportions. Moreover, being exotic in nature its spread and multiplication was nearly unstoppable (Mahalingam *et al*., 2010).

2.5. Symptoms

Papaya mealybugs infest leaves and fruits of host plants, feed on the sap of plants by inserting the stylets into the epidermis of the leaf, as well as into the fruit and stem. The mealybugs inject a toxin as they feed on leaves and fruit which resulted in chlorosis (yellowing), stunting, deformation, early leaf and fruit drop, and buildup of honeydew. Sooty mould developing on honeydew excreted by this mealybug cover the leaves, fruits and stems, impeding photosynthesis and gaseous exchange. On papaya, papaya mealybug (PMB) infest the veins of older leaves, which turn yellow, dry up and shed prematurely, and all parts of young leaves and fruits. Tender leaves become crinkled and curly; flowers and young fruits drop and shoots become bunchy. Papaya trees die within a few months of after infestation. Papaya mealybug infestation of *Plumeria* caused the leaves to curl and new leaves fail to expand fully. On *Hibiscus*, leaves and flowers attacked by papaya mealybug became distorted and the shoots appear bunchy (Muniappan *et al.,* 2008).

Insects might settle, lay eggs, and severely damage plant species that were suitable for development of crawlers. Papaya mealybug infestation in mulberry as well as on *jatropha*, *Lantana*, *Eupatorium* and *Parthenium* showed bunchy terminals with distorted leaves. Severely infested plants were stunted and had sooty mould that made the mulberry leaves unfit for silkworm feeding (Krishnakumar and Rajan, 2009).

2.6. Association with ants

Mealybugs known to offer ants with their sugary excretion (honeydew) and in return ants help in spreading the mealybugs and provide protection from predatory ladybird beetles, parasitoidss and other natural enemies. Species of ants such as red ant species, *Oecophylla smaragdina* (Fabricius), *Crematogaster* sp. and *Anoplolepis gracilipes* (Fr. Smith) had been found attending colonies of mealybug, *P. citri* while feeding on honeydew on Hibiscus (Tanwar *et al.*, 2007). Ants also keep the papaya mealybug colony clean from detritus that accumulate in the secreted honeydew, which might be harmful to the colony. Red ant species, *Oecophylla smaragdina* (Fabricius) had been found attending papaya mealybug, feeding on honeydew on jatropha, papaya and other plants (Tanwar *et al.*, 2010).

2.7. Biochemical changes

The nutritive value of a host plant for insects feeding on them appears to play an important role in determining the susceptibility of the plant to insect attack. Painter (1951, 1958), Lipke and Fraenkel (1956), Thorsteinson (1960), House (1961), Auclair (1963, 1964, 1965, 1967 a and b), Turner (1971) and Auclair and Srivastava (1972) had given extensive review of the literature on the importance of nutrients for insects and their possible role in host selection.

Many biochemical factors were known to be associated with insect resistance in crop plants. In many cases the biochemical factors were more important than morphological and physiological factors in conferring non-preference and antibiosis. Some biochemical constituents might act as feeding stimuli for insects. Occurrences at lower concentration or total absence of such biochemicals lead to insect resistance (Singh, 1983). These defensive chemicals were found as either constitutive components in various plant tissues or were synthesized in response to attacking pests or pathogens (Ryan, 1990).

Pare and Tumlinson (1997) found that in response to insect feeding on the leaves, cotton (*Gossypium hirsutum* L.) plants release elevated levels of volatiles, which could serve as a chemical signal that attracts natural enemies of the herbivore to the damaged plant. Analysis of volatiles from artificially damaged plants, with and without beet armyworm (*Spodoptera exigua* Hubner) oral secretions exogenously applied to the leaves, as well as volatiles from beet armyworm- damaged and – undamaged control plants, demonstrated that the application of caterpillar oral secretions increased both the production and release of several volatiles that were synthesized in response to insect feeding.

The change in biochemical parameters like total carbohydrates, total sugars, total chlorophyll, moisture, ash, total phenolic content, peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia-lyase (PAL), catalase (CAT) were compared with susceptible (CO 2 variety) and less susceptible (*Solanum viarum*) brinjal varieties to papaya mealybug by Janaki and Suresh (2012). The resistance in brinjal varieties was characterized by the presence of defense enzymes like PO, PPO, PAL, CAT and total phenols in different levels. There was a clear correlation between the levels of biochemical constituents and mealybug incidence (Janaki and Suresh, 2012).

The primary metabolites including carbohydrates and proteins were exploited by the herbivores for their growth and development (Rockstein 1978; Ananthakrishnan 1990; Jayaraj 1966 and Uthamasamy 1969). These primary metabolites also function as precursors of secondary substances, which were major elements of resistance in plants (Whittaker and Feeny 1971). Ananthakrishnan *et al*. (1992) reported that carbohydrate level in the three host plants *viz*., *Ricinus communis* L. (castor), *Eucalyptus globulus* Labill. (eucalyptus) and *Manihot utilissima* Pohl. (tapioca) was significantly higher in leaves infested with thrips *Retithrips syriacus* Mayet.

Gopalan *et al*. (1987) estimated the contents of reducing and non-reducing sugars, total phenols and amino acids in healthy rice plants and those infested with mealybug *Brevennia rehi* Lindinger. Feeding injury by mealybugs resulted in a marginal increase in total phenolic content. Total sugar, reducing sugar, non-reducing sugar and total amino acid contents increased phenomenally. Among the amino acids isoleucine and proline contents increased more.

Piercing and sucking insects utilize phloem sap as their major nutrient source. In addition to small molecules like amino acids, phloem sap of higher land plants contains proteins that could accumulated up to higher concentrations. Phloem sap proteins could potentially influence plant-insect interactions (Kehr, 2006).

Khattab (2007) studied the defense mechanism of cabbage plant against phloem-sucking aphid (*Brevicoryne brassicae* L.). A significant reduction in sugar and amino acids levels were found with aphid feeding. The levels of antioxidant compounds (glutathione, ascorbic acid, carotenoids and phenols) were changed in response to aphid feeding. Polyphenol peroxidase and oxidase activities were enhanced by insect infestation. Free proline content of infested cabbage leaves was greater than that of control ones. These findings suggest that aphid feeding probably results in oxidative stress in cabbage. Ascorbic acid, proline, phenol peroxidases and oxidases might play a role in the defense mechanism of aphid infested leaves, thereby delay their death.

Zhang Shi-ze *et al*. (2008) studied the enhancement of phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO) and peroxidase (POD) in cucumber seedlings by *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) infestation. The results indicated that herbivore infestation increased the activities of PAL, PPO and POD. The PAL activity reached the first high peak by 23.1 per cent at 6h and the highest peak by 29.1 per cent at 48h compared to the control. The PPO activity reached the first high peak by 22.7 per cent at 6h and the highest peak by 52.6 per

cent at 24h and the POD activity reached the highest peak by 213.2 per cent at 6h and another higher peak value by 135.2 per cent at 96h. The result suggested that the enhanced activities of the enzymes might contribute to bio protection of cucumber plants against *B. tabaci* infestation.

Zhe Wei *et al*. (2009) used a quantitative masspectrometry- based proteomic approach for comparative analysis of expression profiles of protein in leaf sheaths of susceptible and resistant rice lines in responses to infestation by the brown plant hopper (*Nilaparvatha lugens* Stal, BPH). Among 693 distinct proteins identified from two independent iTRAQ experiments, the expression of 293 (42%) and 258 (37%) of these proteins were changed in the susceptible and resistant rice lines, respectively, when they were infested by the BPH.

Zhang *et al*. (2011) studied that the molecular mechanism employed by cotton for defending against *Phenacoccus solenopsis* before the pest population reach epidemic levels. They examined the effects of exogenous jasmonic acid (JA), salicylic acid (SA) and herbivory treatments on feeding behavior and on development of female *P. solenopsis*. It was observed that JA treated plants slowed *P. solenopsis* development, but plants pre-infested by *P. solenopsis* accelerated its development. Also *P. solenopsis* feeding inhibited the JA-regulated gossypol production, and prevented the induction of JA-related genes. They concluded that *P. solenopsis* was able to prevent the activation of JA dependent defenses associated with basal resistance to mealybugs.

Park *et al*. (2006) observed that the phloem feeding by greenbug, *Schizaphis graminum* (Rondani) elicit unique interactions with their host plants. In addition to well-known defense related regulators such as salicylic acid, jasmonic acid, and abscisic acid, the two molecular regulators, auxin and gibberellic acid were also involved in mediation of the defense responses against greenbug phloem-feeding in sorghum.

2.7.1. Soluble protein

According to Khattab and Khattab (2005), the levels of total soluble carbohydrates, polysaccharides, free amino acids and the total soluble proteins of infested leaves were lower than those of the healthy ones. Miles (1999) found that phloem feeding insects established a sustained interaction with sieve elements (SEs). They released saliva that inhibited plant stress responses and prevented closure of pierced sieve elements by callose or polymerized proteins. Drain of assimilates towards the insect away from the other plant parts might contribute to such metabolites reduction (Miles, 1989).

2.7.2. Indole -3 Acetic Acid

Auxin is essential for growth and development and involved in the attenuation of defense responses in plants. In contrast, blocking auxin responses has been shown to increase resistance in plants (Bari and Jones, 2008). Auxin homeostasis and maintenance of capturing auxin signaling are important in mounting defense responses (Mayda *et al*., 2000). Saikia *et al*. (2011) found that red spider mite infested plum tree had lower level of auxin than the non-infested one. *Helopeltis* sp. attack on the axillary vegetative buds and young leaves of tea resulted in decrease in auxin content.

2.7.3. Gibberellic Acid (GA)

Gibberellic acids are known for their role in the developmental processes in plants. Study showed that GA treatment enhanced the germination rate of chick pea seeds, which was inhibited by salt stress by increasing amylase activity and starch translocation rate (Kaur *et al*., 1998).

The effect of gibberellic acid (GA3) on the feeding behavior of the fifth instar nymphs of *Locusta migratoria migratoria* were studied by Abdellaoui *et al*. (2009). They revealed that Gibberellic acid significantly reduced food consumption of *L. migratoria migratoria* fifth instar nymphs.

Yokomi *et al*. (1995) found that application of chlormequat chloride, a gibberellic acid biosynthesis inhibitor, induced leaf silvering symptoms similar to those induced by the silverleaf whitefly in squash plants. Saikia *et al*. (2011) observed that GA_3 content increased after infestation by *Helopeltis* sp. in tea plants and red spider mite infestation induced higher level of gibberellic acid in plum trees.

2.8. Mass culturing of mealybugs

To maintain uniform aged mealybug stages, various host materials were used. Sprouted potatoes (Tsugawa, 1972), bleached potatoes (Murakami, 1965), Japanese pumpkin fruits (Murakami, 1965; Ueno, 1977; Izawa and Utida, 1991) could be used as an alternative food source for rearing mealybugs.

Stall *et al*. (1973) reported grape mealybug could be maintained at 17-18°C on sprouts of *Solanum tuberosum* L. Coppel and Mertins (1977) used *Solanum tuberosum* var. Bliss Triumph, a red skinned seed potato variety as the host for breeding the citrus mealybug, *Planococcus citri*. Branigan (1916) observed that most of the mealybugs of economic importance could be propagated on etiolated potato sprouts.

Potatoes were treated with potassium thiocyanide (1.0%) for one hour to break the dormancy and then allowed to sprout in trays filled with moist sand. *M. hirsutus* was reared on the fruits of cucurbits (Babu and Azam, 1987a, b; Babu and Azam 1989; Mani and Thontadarya, 1989) or on potatoes (Sagarra and Vincent, 1999). Of these, the preferred host had been Japanese pumpkin (*Cucurbita moschata* Duchesne) due to its ribbed rinds and characteristic warted surface, which provided larger areas for settling of mealybugs (Meyerdirk and Newell, 1979).

Serrano and Laponite (2002) maintained mealybugs (*M. hirsutus*) on sprouted potatoes by placing 20 medium size seed potatoes on a tray for several days in a dark room at 25 ± 2°C and 80 ± 10% RH with a layer of Pro-Mix PGX® to induce

etiolation. Once sprouts reached about 5cm in length, potatoes were cleaned and transferred to trays for infestation with *M. hirsutus*.

Rao and Srinivasan (1987) found that red pumpkin fruit was suitable for mass rearing the vine mealybug, *M. hirsutus* in the laboratory due to its easy handling, dense settlement of mealybug colonies, long standing culture and proliferation of mealybug population.

With the use of lemon (*Citrus media var. limon*) and butternut (*Juglans cinerea*) less problems were encountered than culturing on potato sprouts (Samways and Mapp, 1983). Broad bean seeds were used as an alternative food source for some polyphagous insects such as aphids, thrips and predatory bugs (Murai, 1991; Murai and Loomans, 2001; and Murai *et al* 2001). Narai and Murai (2002) successfully cultured Japanese mealybug, *Planococcus kraunhiae* (Kuwana) on germinated broad bean seeds at 24°C with increased egg hatchability, survival rate, adult emergence and longevity of the mealybug.

The procedure for rearing *P. solenopsis*, the dominant mealybug species in the North and Central Zones of India had been standardized with potato sprouts (NCIPM, 2008).

Materials and Methods

3. Materials and methods

The present investigation entitled "Infestation induced reactions of papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink, (Hemiptera: Pseudococcidae) on papaya and amaranthus" was carried out during 2011-2012. The experiments were conducted in the laboratories of All India Network Project on Agricultural Ornithology (AINPAO), Department of Agricultural Entomology and Department of Plant Physiology, College of Horticulture, Kerala Agricultural University, Thrissur.

3.1. Mass culturing of *Paracoccus marginatus*

The mass culturing of *Paracoccus marginatus* was initiated from healthy adult mealybugs collected from the field. Laboratory culture of *P. marginatus* was maintained on sprouted potato tubers at a temperature of 27±2°C and 69.9±5.5 per cent relative humidity.

3.1.1. Selection of potatoes

As the potatoes serve as substratum for the mealybug culture, selection of potatoes was done very carefully. Care was taken for the following characters while selecting the potatoes.

a) Potato tubers had a minimum of one month storage for easy sprouting
b) Medium sized potato (150 - 200g)
c) Raised eye sprouts without any blackening on the sprouts
d) Injury or disease free tubers

3.1.2. Preparation of potatoes for mass culturing

Potato tubers were thoroughly washed with clean water to remove adhered dirt and soil particles to avoid contamination by microorganism. These potatoes were air dried. An incision (7mm depth; 2cm length) was made on the tubers, opposite to the eye sprouts using a sterilized sharp blade. The potatoes were then treated with gibberellic acid (GA 1%) hormone for 30 minutes for promoting

rapid sprouting. The potatoes were then air dried. After GA treatment the potato tubers were transferred to trays (30 cm length; 25 cm width and 8 cm depth) containing wet sterilized sand. The sand was sterilized in hot air oven at 100^0C for 1 h to prevent infestation by pathogens which might induce rotting of tubers. The pots/ trays with potatoes were covered with a black muslin cloth to etiolate the potato tubers and kept in dark place. The sand was sprinkled with water every day to maintain moisture. The sprouted potatoes were then transferred after four days to trays lined with tissue paper to prevent rooting (Plate 1).

3.1.3. Inoculation of *Paracoccus marginatus*

To get uniform aged progenies of *P. marginatus* for further experiments sprouted potatoes were inoculated with field collected five adult female mealybugs with ovisac from the papaya plants (Plate 2 & 3).

3.2. Biology of *Paracoccus marginatus*

In order to know precisely the emergence, development, distribution and abundance of *P. marginatus*, biology of the candidate insect was studied on host plants *viz*., papaya (three months old) and amaranthus (one month old) at a monthly mean temperature of 27.62±4.2°C and relative humidity of 78.3±2.24%.

3.2.1. Raising of host plants

3.2.1.1. Amaranthus seedling

Red amaranthus (*var*. Arun) was utilized for the studies. Seeds were procured from the Department of Olericulture, KAU, Thrissur. Amaranthus seeds were sown in sowing trays. Fifteen days old amaranthus seedlings were transplanted in polythene cover (17x30 cm) filled with a mixture of soil and vermicompost (1:1 w/w). One month old seedlings having a height of 12.5cm and with 4-5 leaves were selected for papaya mealybug inoculation. The selected plants were thoroughly examined for the presence of any insect pest infestation or disease infection or nutrient deficiency symptoms. Extra care was taken to avoid any further contamination (Plate 4).

Mass Culturing of mealybug

Plate 1. Sprouted potatoes in trays

Plate 2. Mass culturing of mealybug on potatoes

Plate 3. Sprouted potatoes with mealybug

3.2.1.2. Papaya seedlings

Papaya seedlings were brought from Central Nursery, KAU, Thrissur. Three months old healthy plants with an average height of 15cm with 4-5 leaves were selected for the studies. These plants were thoroughly examined for presence of any insect pest infestation, disease infection and nutrient deficiency symptoms. The plants thus selected were then brought to the laboratory and care was given to avoid any further contamination (Plate 5).

3.2.2. Inoculation of *Paracoccus marginatus*

From the laboratory culture, five adult mealybugs with ovisac were transferred (@ 1/plant) to the seedlings maintained in three replications using a camel hair brush (series No.68). The seedlings were kept in an aluminium fabricated cage with aluminium net on left and right sides and glass covering on front and back sides. Observations were recorded daily on parameters *viz*., hatchability and the interval between moulting using a hand lens (10x magnification). The exuviae of nymphal instars were removed after each moulting. The length and width of each nymphal instar was measured under stereo binocular microscope with image analyzer facility.

3.2.3. Observations recorded

3.2.3.1. Incubation period: Time taken from oviposition to first nymphal emergence. Number of nymphs emerged was recorded regularly.

3.2.3.2. Duration of each nymphal instar: The period between two consecutive moulting was recorded and considered as duration of particular instar.

3.2.3.3. Oviposition period: The period up to which the females laid eggs.

3.2.3.4. Fecundity: Total number of eggs laid by a single adult during its life span.

3.2.3.5. Adult longevity: The period between adult emergence and the death of the insect

Biology of *P. marginatus*

Plate 4. Cage experiment on amaranthus

Plate 5. Cage experiment on papaya

3.2.3.6. Sex ratio: The total number of males and females emerged in each cage was noted and sex ratio expressed as female: male

3.3. Pest load assessment of *Paracoccus marginatus* in papaya and amaranthus

Papaya mealybugs often cause crinkling symptoms on the host plants. To understand the bare minimum number of the insects that cause the damage symptoms, pest load assessment studies were conducted. Three months old papaya seedlings and one month old amaranthus seedlings were used for this study.

3.3.1. Pest load assessment in papaya seedlings

Thirty three healthy papaya seedlings (three months old) were selected for the study. The seedlings were arranged in 11 groups with three replications. Single crawler of papaya mealybug was transferred to group I, two crawlers to group II, three crawlers to group III and so on. Three seedlings were maintained without the mealybug inoculation and served as control. Observations were taken on 24, 48, 72, and 96h after transfer of crawlers and missing crawlers were replaced. Control plants were covered with polythene covers to protect from mealybug infestation from cross inoculation.

3.3.2. Pest load assessment in amaranthus seedlings

To study the pest load assessment 33 healthy amaranthus seedlings (one month old) were selected. Out of these 33 plants, three plants were considered as control. Others were classified into 10 groups with three replications. Single crawler of papaya mealybug was transferred to group I, two to group II, three to group III and so on. Observations were taken daily (after 24h, 48h, 72h, and 96h of infestation) and missing crawlers were replaced. Control plants were covered with polythene covers to protect them from mealybugs.

3.3.3. Assessment of plant reaction by visual scoring on leaf deformities

Plant reaction was assessed based on leaf deformities (crinkling). A scale was prepared based on the per cent leaf area damaged. The plants infested with mealybug crawlers (pest load assessment studies) were examined (after 24h, 48h, 72h and 96h of infestation).

3.3.3. 1. Leaf damage severity

Papaya mealybug infestation on amaranthus and papaya produced leaf crinkling symptoms on leaves. Based on the number of leaves crinkled, the damage score was calculated using leaf damage severity scale adopted from Galanihe *et al*. (2010). Extent of leaf damage was recorded by visual scoring.

Table 2. Leaf damage severity scale

Damage score	Extent of leaf crinkling
0	Undamaged (no crinkling)
1	1-25% leaves crinkled
2	26-50% leaves crinkled
3	51-75% leaves crinkled
4	76-100% leaves crinkled

According to the leaf damage severity scale there were five damage scores starting from 0 - 4.

Leaf crinkling per cent (Crinkling %) was calculated using the given formula,

$$\text{Crinkling \%} = \left[\frac{\text{No. of crinkled leaves}}{\text{Total No. of leaves (crinkled \& non- crinkled)/plant}} \times 100 \right] \%$$

3.4. Dispersion of *P. marginatus* on host plants

Specified numbers (1, 2, 3, 4, 5, 6, 7, 8, 9 and 10) of mealybugs were released on two host plants (three months old papaya and one month old amaranthus). In each morning, the number of mealybugs on each plant was counted. Observations were taken after 24, 48, 72 and 96 h after infestation. From the observation the mean dispersion in each group was calculated. The same procedure was repeated for first, second, third instar and adults of papaya mealybug.

$$\text{Dispersion \%} = \left[\frac{(\text{No. of mealybugs released} - \text{No. of mealybugs retained}) \times 100}{\text{Total No. of mealybugs released}} \right]$$

3.5. Biochemical analysis

Plant reaction to *P. marginatus* infestation might have links with the biochemical constituents of the host plants. To find out the variation in the biochemical constituents of the healthy and infested leaves of papaya and amaranthus, biochemical analysis was carried out. The healthy and infested leaves from the growing point were collected individually and subjected to different biochemical analysis.

3.5.1. Total protein

Total protein content in healthy and infested leaves of both papaya and amaranthus was estimated as per Lowry *et al*. (1951). Standard curve was drawn using bovine serum albumin fraction 5 (BSA fraction V).

3.5.1.1. Plant sample

3.5.1.1.1. Papaya

Three months old infested (plants infested with all stages of mealybugs were considered) and uninfested papaya plants were selected. Leaf samples (500mg) were taken from the growing point of plant for further analysis.

3.5.1.1.2. Amaranthus

One month old infested (plants infested with all stages of mealybugs were considered) and uninfested amaranthus plants were selected. Leaf samples (500 mg) were taken from the growing point of plant for analysis.

3.5.1.2. Preparation of standard

Bovine serum albumin (50mg) was mixed in distilled water and made up to 50 ml in a standard flask. The mixture was kept overnight (stock solution). From the stock, 10ml of the solution was drawn and made up to 50 ml with distilled water in another standard flask. One ml of this solution contained 200 µg proteins. In order to get required concentrations, from the stock solution different aliquots (100µl, 200µl, 300µl, 400µl, 500µl, 600µl, 700µl, 800µl and 900µl) were drawn and pipetted out in different test tubes. The volume was made up to one ml with distilled water. A test tube with distilled water (1ml) alone served as blank.

3.5.1.3. Sample preparation

Leaf sample (500mg) was taken and it was ground well in 10ml cold distilled water using mortar and pestle. The ground sample was spinned at 5000 rpm for 10 minutes in a cooling centrifuge (REMI, CFC free, C-24). The supernatant was collected and 0.1ml aliquot was taken for analysis.

3.5.1.4. Procedure

Standard samples were prepared by taking 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0 ml of working standard. The volume was made up to 1ml in all

the test tubes including the sample tubes by adding distilled water. Alkaline copper (5ml) reagent (1% $CuSO_4$ + 1% Na-K-tartarate + 2% Na_2Co_3 in 1N NaOH) was added and incubated at room temperature for 10 minutes. Folin – phenol reagent (ready mix; 0.5 ml) was added to the tubes and incubated at room temperature for 30 minutes. After incubation, absorbance was measured at 660nm by UV spectrophotometer. A standard graph was drawn by plotting concentration (mg) of protein on the X-axis and absorbance (nm) on the Y-axis. From the graph total protein content in the sample was estimated and expressed as mg/g plant tissue.

3.5.2. IAA (Indole Acetic Acid)

IAA present in the healthy and infested leaves of papaya and amaranthus was estimated using spectrophotometric method.

3.5.2.1. Plant sample

3.5.2.1.1. Papaya

Three months old infested and uninfested papaya plants were selected. Leaves were taken from the tip of the plant for analysis.

3.5.2.1.2. Amaranthus

One month old infested and uninfested amaranthus plants were selected. Leaves were taken from the tip of the plant for analysis.

3.5.2.2. Standard preparation

Indole acetic acid (10 mg) weighed and dissolved in 100 ml of 0.1M Na_2CO_3 to prepare 100 ppm stock. From the stock solution working standards were prepared *viz*., 10, 20, 30, 40, 50, 60 ppm. The absorbance was measured at 540 nm in spectrophotometer. A standard graph was drawn by plotting concentrations of IAA (mg) on X-axis and absorbance (nm) on the Y-axis.

3.5.2.3. Procedure

Leaf sample (500 mg) was taken and the sample was macerated with 10ml cold distilled water. The content was spun at 5000 rpm (10 minutes) in a refrigerated centrifuge. The supernatant was collected, filtered and the volume was made up to 25 ml with ice cold distilled water. Two sets of 1ml of aliquot in a test tube was taken and 1ml of phosphate buffer (68 ml of 0.2 M monobasic-NaH_2PO_4 + 32 ml of 0.2M dibasic-Na_2HPO_4 and made up to 200 ml with cold distilled water) and 1 ml of distilled water was added. Distilled water alone (2 ml) served as blank and to that 1ml of phosphate buffer was added. To stop the reaction the first set tubes (control) were kept in hot water bath for 10-20 seconds. The content was then cooled and 8 ml of Garden Webber reagent (mix 2 ml of 0.5M ferric chloride +100 ml of 35% perchloric acid) was added. Pink colour developed and the absorbance was measured at 540 nm. Simultaneously second set of tubes were kept in room temperature for 1h. After 1h the test tubes were placed in hot water bath (10-20 seconds) to stop the reaction. The content was cooled and Garden Webber reagent (8ml) was added. Pink colour developed was measured at 540 nm in the spectrophotometer. The absorbance value was plotted in the standard graph and the corresponding concentrations (X µg) were recorded. Using the concentration obtained from graph IAA content was calculated and expressed as mg of unoxidised auxin per gram of plant sample.

3.5.3. Gibberellic acid (GA)

The method of extraction and purification of endogenous level of gibberellic acid (GA) in plant samples was modified from those described by Holbwok *et al.* (1961) and Sunbery (1990). Gibberellic acid present in the plants was estimated based on the conversion to gibberellic acid followed by the measurement of its absorption at 254 nm.

3.5.3.1. Plant sample collection

3.5.3.1.1. Papaya

Three months old infested and uninfested papaya plants were selected. Papaya leaves from the tip of the plant (2-3 leaves) along with growing shoot portion were taken for analysis.

3.5.3.1.2. Amaranthus

One month old infested and uninfested amaranthus plants were selected. Amaranthus leaves from the top of the plant (2-3 leaves) along with growing shoot portion were taken for analysis.

3.5.3.2. Extraction of free gibberellins from plants

Gibberellins occur in plants in bound and free form. The free gibberellins from the plant samples were extracted by the following procedure.

Fresh plant sample (2g) was homogenized with 20 ml chilled methanol (80% v/v) and left overnight at 4°C. The extract was filtered through Whatman No. 40 filter paper and solid residue further isolated by centrifugation at 10000 rpm for 5 minutes with methanol. The methanolic extracts combined and concentrated to a water residue in vacuum (30-40°C) by rotary evaporator. The volume was adjusted to 10 ml with 0.2M PO_4 buffer (pH 7.5). The methanol compounds were removed by partitioning it twice with 5 ml methyl ether in a 20 ml glass vial. The ether was layered to aqueous phase and two phases system was gently stirred for three minutes in a magnetic stirrer. After discarding the ether phase, the aqueous phase was adjusted to pH 2.7 with 1M HCl. The aqueous phase partitioned thrice against 10 ml of ethyl acetate and the ethyl acetate layer was further partitioned twice against 0.4M $NaHCO_3$. The aqueous phase was adjusted to pH 2.5 with 1.6M HCl. The acidified phase was partitioned two times against 10 ml ethyl acetate. The ethyl acetate layer was dissolved in methanol and stirred in vials at 4°C.

3.5.3.3. Estimation of GA from leaf extract

The amount of GA present in the leaf extract was estimated spectrophotometrically by the following procedure.

Leaf extract (1.5 ml) containing GA was pipetted out to the test tube and 2 ml of zinc acetate was added. After 2 minutes, 2 ml of potassium ferrocyanide was added and centrifuged at low speed (3000rpm) for 15 minutes. From this, 5 ml of supernatant was taken and 5 ml of HCl (30%) was added and incubated the mixture at 20°C for 75 minutes. The blank sample was treated with HCl (5%) and the absorbance of the sample and the blank was measured at 254 nm. The sample absorbance was plotted in the standard graph and the corresponding concentrations (X µg) were recorded for calculation of GA which was expressed as µg per gram of plant tissue.

3.6. Statistical analysis

Protein, IAA and GA concentrations in plants under each experiment were tabulated and analysed statistically by one sample t-test.

Results

4. RESULTS

Results of experiments conducted in the laboratory on the study entitled "Infestation induced reactions of papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink, (Hemiptera: Pseudococcidae) on papaya and amaranthus" are presented in this chapter.

4.1. Biology of the insect

The biology of *Paracoccus marginatus* was studied on three months old papaya seedlings and one month old amaranthus seedlings.

4.1.1. Life stages of female mealybug

4.1.1.1. Egg

Eggs were greenish yellow, round or oval shaped (Plate 6). Eggs were present inside the ovisac. Incubation period ranged from 5-9 (mean 7.5±1.2) days on papaya and 5-8 (mean 6.6±1.1) days on amaranthus (Table 3a and 3b).

4.1.1.2. Nymph

Newly emerged nymphs were greenish yellow and with oval shaped body (Plate 7). Nymphs were dispersed after emergence and started feeding. The duration of first nymphal instar was 3-5 days on papaya (mean 4.2±0.87) and 3-6 days on amaranthus (mean 4.8±0.97) (Table 3a and 3b). First instar nymphs were without any mealy coating over their body and their average length and width were 0.4±0.01 mm and 0.19±0.01mm respectively (Table 6).

Second instar nymphs were also greenish yellow and started to produce white mealy coating over their body (Plate 8). The length and width of second instar nymphs were 0.54±0.03mm and 0.31±0.01mm, respectively (Table 6). Duration of

Plate 6. Eggs of *P. marginatus*

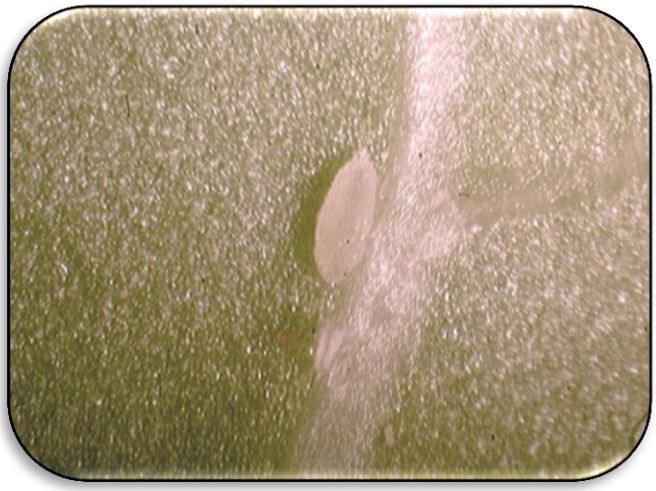

Plate 7. First instar nymph

Table 3a. Duration of life stages of female *P. marginatus* on papaya

Life stages	Duration (days)		
	Maximum	**Minimum**	***Mean ± SD**
Egg	9	5	7.5±1.20
First instar	5	3	4.2±0.87
Second instar	4	3	3.6±0.48
Third instar	5	2	4.1±0.94
Total nymphal period	**14**	**8**	**11.9±1.10**
Adult female	4	3	3.6±0.48
Oviposition period	5	3	4.0±0.77
Total life cycle	**32**	**19**	**27.0±2.44**

*mean of 10 observations

Table 3b. Duration of life stages of female *P. marginatus* on amaranthus

Life stages	Duration (days)		
	Maximum	**Minimum**	***Mean ± SD**
Egg	8	5	6.6±1.10
First instar	6	3	4.8±0.97
Second instar	4	3	3.7±0.45
Third instar	6	3	4.9±0.94
Total nymphal period	**16**	**9**	**13.4±1.42**
Adult female	5	3	4.3±0.64
Oviposition period	6	3	5.0±0.89
Total life cycle	**35**	**20**	**29.3±2.75**

*mean of 10 observations

second instar nymphs ranged from 3-4 days with a mean of 3.6±0.48 days on papaya and 3.7±0.45 days on amaranthus.

The second instar nymphs moulted into third instar nymphs. They were yellowish with white mealy coating over their body (Plate 9). The third instar nymphs moulted into adult. Third instar lasted for 4.1±0.94 days on papaya and 4.9±0.94 days on amaranthus. Total nymphal duration of female mealybug was 11.9±1.1 days on papaya seedlings and 13.4±1.42 on amaranthus seedlings (Table 3a and 3b).

4.1.1. 3. Adult female

Adult females were wingless with many lateral waxy filaments and dorsum with 14-16 pairs of cerarii. They were yellow in colour and their body was covered with white mealy coating (Plate 10). Their average size was 2.24±0.06mm length and 1.22±0.04mm width (Table 6). They lived for a mean duration of 3.6±0.48 days on papaya and 4.3±0.64 days on amaranthus (Table 3a and 3b).

4.1.1.4. Sex ratio

Sex ratio of papaya mealybug was studied on two crops *viz*., papaya and amaranthus. Sex ratios (female: male) were 3:1 and 2.5:1 on papaya and amaranthus respectively (Table 5).

4.1.1.5. Fecundity

Total number of eggs laid during life period was recorded as the fecundity of test insect. The observed fecundity of *P. marginatus* on papaya was 513.9±107.9 and on amaranthus, it was 508.1±132.5 (Table 5).

Plate 8. Second instar nymph

Plate 9. Third instar nymph

Table 4a. Duration of life stages of male *P. marginatus* on papaya

Life stages		**Duration (days)**		
		Maximum	**Minimum**	***Mean ± SD**
Egg		8	3	5.7±1.40
Nymphal instars	I	6	4	5.2±0.74
	II	5	4	4.5±0.50
Total nymphal period		**11**	**8**	**9.7±0.90**
Pre pupal period		3	2	2.6±0.48
Pupal period		5	4	4.4±0.48
Adult longevity		3	2	2.5±0.50
Total life cycle		**30**	**19**	**24.9 ± 2.30**

*mean of 10 observations

Table 4b. Duration of life stages of male *P. marginatus* on amaranthus

Life stage		**Duration (days)**		
		Maximum	**Minimum**	***Mean ± SD**
Egg		9	4	6.1±1.3
Nymphal instars	I	6	4	5.2±0.74
	II	5	4	4.5±0.50
Total nymphal period		**11**	**8**	**9.7±0.90**
Pre pupal period		3	2	2.6±0.48
Pupal period		5	4	4.4±0.48
Adult longevity		3	2	2.5±0.50
Total life cycle		**31**	**20**	**25.7±2.60**

*mean of 10 observations

Plate 10. Adult female with ovisac

4.1.2. Life stages of male mealybug

4.1.2.1 Nymphs

There were different nymphal stages for male mealybugs - first instar, second instar, pre pupa, pupa and adult.

First instar male nymphs were yellow in colour and were same as that of female nymphs. Male nymph turned into pink towards the end of second instar and pupal stage from yellow colour. The duration of second instar males was 4.5±0.5 days on both papaya and amaranthus (Table 4a and 4b).

Total male nymphal period was 8-11 days (mean 9.7±0.9 days). Pre pupal stage lasted for 2.6±0.48 days. The duration of pupal period was 4-5 days (mean 4.4±0.48 days) (Table 4a and 4b) (Plate 11). The average length of pupa was 1.78±0.12 and width 0.5±0.06 mm (Table 6).

4.1.2.2. Adult male

Males were winged (Plate 12), yellow in colour and were 1.29mm long, an elongated oval body, widest at the thorax (0.17mm). Adult males had ten segmented bristle shaped antennae, a heavily sclerotized thorax and head, and well developed wings. Adult longevity was 2.5±0.5 days (Table 4a and 4b).

Plate 11. Male Pupa

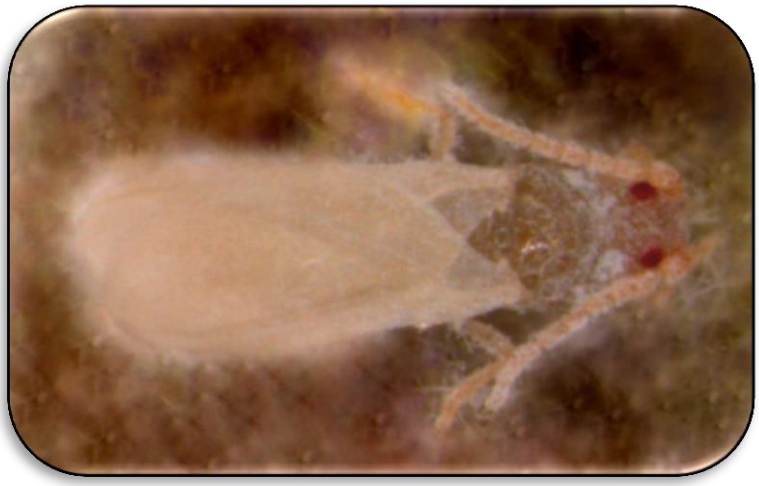

Plate 12. Adult - Male

Table 5. Sex ratio and fecundity of *P. marginatus*

S. No.	Crop	Sex Ratio (♀:♂)	Fecundity
1	Papaya	3: 1	513.9 ± 107.9
2	Amaranthus	2.5 : 1	508.1 ± 132.5

*mean of 10 observations

Table 6. Morphometric measurements of life stages of *P. marginatus*

S. No.	Insect stage	Length (mm)			Width (mm)		
		Range		*Mean±SD	Range		*Mean±SD
1	Egg	0.32	0.36	0.34±0.01	0.14	0.16	0.15±0.01
Nymph							
2	I	0.39	0.43	0.4±0.01	0.18	0.21	0.19±0.01
3	II	0.48	0.58	0.54±0.03	0.29	0.33	0.31±0.01
4	III	0.81	0.93	0.88±0.03	0.52	0.62	0.57±0.03
5	Pupa (♂)	1.52	1.88	1.78±0.12	0.42	0.62	0.5±0.06
Adult							
6	Female	2.14	2.32	2.24±0.06	1.14	1.28	1.22±0.04
7	Male	1.28	1.36	1.29±0.03	0.15	0.19	0.17±0.01

*mean of 10 observations

From the experiment (Table 3a & 3b), it was inferred that the duration of total life cycle of female *P. marginatus* on papaya and amaranthus varied slightly. On papaya the egg period (incubation period) ranged from 5-9 days whereas on amaranthus the egg period was in the range of 5-8 days. The total nymphal period of *P. marginatus* was 8-14 days on papaya; however on amaranthus it ranged from 9-16 days. *P. marginatus* female took 19-32 days to complete one life cycle on papaya, whereas the life cycle on amaranthus was 20-35 days. The female *P. marginatus* took more days (2-4 days) to complete its total life cycle on amaranthus than on papaya.

The duration of different life stages *viz*., first instar, second instar, pre pupal period, pupal period and adult longevity of male mealybug was not significantly different between papaya and amaranthus (Table 4a &4b). The incubation period of egg ranged from 3-8 days on papaya and 4-9 days on amaranthus. The total nymphal period of male *P. marginatus* was 8-11 days on papaya and on amaranthus. The total life cycle of male *P. marginatus* on papaya was 19-30 days but on amaranthus it was 20-31 days.

4.2. Pest load assessment of *Paracoccus marginatus* on papaya and amaranthus

4.2.1. Pest load assessment of *Paracoccus marginatus* on papaya plants

Pest load assessment was carried out by releasing specified numbers of nymphs and adults on three months old papaya plants as explained in 3.3.1.

Results showed that when one crawler (first instar) was released on papaya plants, no crinkling was noticed up to 96h. It was true for the treatments with 2, 3, 4 and 5 crawlers. However, when six first instar crawlers were present on the papaya plant, crinkling of the leaves (Plate 14) started at 96 hours onwards. When the pest load was increased further from seven to ten all the plants showed leaf crinkling symptoms at 96 hours onwards (Table 7a.).

From the experiment, it was observed that when the number of second instars was 1-3, there was no crinkling on papaya plants up to 72h. However, when the number of second instars increased from 4 to 10, a noticeable leaf damages appeared on the plants at 72h onwards. At 96h, all group of plants (group I –X) inoculated with second instar nymphs showed leaf crinkling symptoms (Plate 15). From the result, it was understood that at 72h, inoculation of minimum four second instar nymphs were required to produce the symptoms of leaf damage. However, at 96h even the presence of a single second instar nymph was enough to produce leaf crinkling.

No crinkling was observed up to 72h when one third instar nymph was present on the plant. Similar results were shown by the plants inoculated with two third instars of mealybug (Table 7a). When the nymphs per plant were 2-10, from 72h onwards, leaf crinkling symptoms were observed (Plate 16). At 96h, all the plants inoculated with third instar mealybug produced symptoms. At 72h, a minimum of three third instar nymphs were required to produce symptoms on plants. As in case of second instars, the presence of a single third instar nymph was enough to produce crinkling at 96h of inoculation.

Table 7a. Pest load assessment on papaya plants (leaf crinkling)

Group*/ No. of mealybugs released per plant	Hours after release of *P. marginatus*					
	I instar	II instar		III instar		Adult female
	(96 h)	72 h	96 h	72h	96 h	(96 h)
1	×	×	√	×	√	×
2	×	×	√	×	√	×
3	×	×	√	√	√	×
4	×	√	√	√	√	×
5	×	√	√	√	√	√
6	√	√	√	√	√	√
7	√	√	√	√	√	√
8	√	√	√	√	√	√
9	√	√	√	√	√	√
10	√	√	√	√	√	√
Control	×	×	×	×	×	×

√ - Crinkling of leaves

× - No crinkling

- *Grouping based on number of mealybugs released

Plate 13. Uninfested leaves of papaya

Plate 14. Papaya plant infested with *P. marginatus*

Plate 15. Infested leaves of papaya showing withering symptoms

Plate 16. Papaya plant showing severe crinkling

When the papaya plant was inoculated with 1-4 adult mealybugs per plant no leaf crinkling symptoms was produced up to 96h. However, when the pest load increased from 5 to 10 (adult mealybug per plant), a noticeable leaf crinkling symptoms were observed on plants at 96h.

4.2.2. Pest load assessment of *Paracoccus marginatus* on one month old amaranthus seedlings

Pest load assessment was carried out on one month old amaranthus seedlings as explained in 3.3.2. Observations were taken at 24, 48, 72 and 96h after inoculation.

When a single first instar nymph of papaya mealybug was released on amaranthus seedlings, it did not produce any symptoms on the leaves up to 96h (Table 7b). Similar results were obtained at 96h, with the increase in pest load from two to five crawlers per seedlings. However, six numbers of first instars were able to produce characteristic leaf crinkling symptoms on amaranthus seedlings at 96h. Similarly, amaranthus seedlings showed definite leaf crinkling symptoms with the increase in pest load from 7 to 10 crawlers per plant at 96h.

Amaranthus seedlings inoculated with the one to two second instar nymphs were unable to produce crinkling symptoms at 72h. But seedlings showed characteristics leaf crinkling with the increase in pest load from 3 to 10 second instar nymphs per seedlings. At 96h of observation, a seedling inoculated with even a single second instar nymph had produced crinkling symptoms on leaves (Plate 18). Similar results were recorded on the seedlings inoculated with 2 to 10 second instar nymphs.

At 48h of inoculation, the amaranthus seedlings with the pest load from 1 to 6 third instar nymphs were unable to produce leaf crinkling symptoms. Whereas, seedlings inoculated with 7 to 10 third instar nymph took only 48h to produce the symptoms (Plate 19). However, at 72h of inoculation, all the seedlings inoculated.

Table 7b. Pest load assessment on amaranthus seedlings (leaf crinkling)

Group*/ No. of mealybugs released per plant	Hours after release of *P. marginatus*					
	I instar	II instar		III instar		Adult female
	(96 h)	(72 h)	(96 h)	(48h)	(72 h)	(96 h)
1	×	×	√	×	√	×
2	×	×	√	×	√	×
3	×	√	√	×	√	×
4	×	√	√	×	√	×
5	×	√	√	×	√	√
6	√	√	√	×	√	√
7	√	√	√	×	√	√
8	√	√	√	√	√	√
9	√	√	√	√	√	√
10	√	√	√	√	√	√
Control	×	×	×	×	×	×

√ - Crinkling

× - No crinkling

- *Grouping based on number of mealybugs released

Plate 17. Un infested leaves of amaranthu

Plate 18. Amaranthus leaves showing crinkling symptoms

Plate 19. Leaves of amaranthus showing withering and crinkling

with third instar nymphs (1 to 10 third instar nymphs/seedlings) had shown crinkling symptoms on leaves.

It was observed that a single adult mealybug did not produce leaf crinkling symptom up to 96h of inoculation. Similarly, none of the plant produced characteristic leaf crinkling symptom when the pest load (adult/seedlings) increased from 2 to 4. However, seedlings inoculated with 5 to 10 adults showed leaf crinkling symptoms on tender leaves at 96h of inoculation.

4.3. Assessment of plant reaction by visual scoring of leaf deformities on host plants

4.3.1. Leaf damage severity on papaya

Leaf damage severity was calculated using the damage scale as explained in 3.3.3 (Table 2). The observations were taken after 96h of inoculation with mealybugs.

No leaf damage was observed on the plants inoculated with 1-5 first instar crawlers (score '0') whereas the plants inoculated with 6-10 numbers of first instar mealybug showed a leaf damage severity score of '1' (5-15 % leaf crinkling) (Table 8a). At 96h of inoculation, six first instar nymphs produced 5.5 per cent leaf area crinkling. Whereas, 6.6 per cent leaf crinkling were observed with the inoculation of seven crawlers. Leaf crinkling further increased to 11.5 per cent with a pest load of eight crawlers. However, 15 per cent leaf crinkling was recorded on papaya leaves with the inoculation of 9 to 10 crawlers.

Results showed that when one second instar nymph was released on papaya plants, it produced 5.5 per cent leaf crinkling. When the pest load was two second instars per plant, the leaf damage score was 1 and crinkling per cent was 6 per cent. The leaf damage score was '1' for plant groups with 3 to 9 second instars per plant *i.e.* the crinkling per cent was 1-25 per cent. The leaf crinkling per cent of plants inoculated with three second instars of mealybug was 11.7 per cent. However, the

plants infested with 4-7 numbers of second instars of mealybug per plant caused 17.6 per cent of leaf crinkling. When the pest load was increased to 8 per plant, 22.2 per cent leaf crinkling was observed. When the number of second instar nymphs was increased to 9 per plant the percent leaf crinkling was 23.5. A maximum crinkling of 27.7 per cent was observed when ten second instars were present on the plant with a leaf damage severity score of '2' (Table 8a).

The leaf damage severity scores of papaya plants inoculated with third instars were '1' and '2'. Score '1' was observed on plants inoculated with one to nine third instars per papaya plant. In this experiment the lowest per cent of leaf crinkling recorded was 6.6 (plants with one, third instar). Leaf crinkling per cent of 12.5 was recorded on plants inoculated with two third instars. A slight decline in leaf crinkling (11.7 per cent) was observed when the plant had three third instar nymphs. Similar to second instars, plants inoculated with third instar nymphs, 17.6 per cent of leaf crinkling was observed, when the numbers of instars were further increased from four to six per plant (Table 8a). As the numbers of instars were further increased from seven to nine, the leaf crinkling per cent also increased *i.e.* papaya plants inoculated with seven third instars recorded 21.0 per cent of crinkled area in leaf. Plants with eight and nine third instars showed leaf crinkling of 22.2 and 23.5 per cent respectively. In this experiment, the highest leaf damage score ('2') was observed on the plants inoculated with 10 third instars which produced leaf crinkling of 36.8 per cent.

Plants inoculated with 1-5 adult mealybugs per plant showed no leaf crinkling on leaves (leaf damage severity score was '0'). Plants showed 6.6 per cent crinkling with damage score of '1' when the pest load was six adult mealybugs per plant. As the numbers of adults were further increased from seven to ten, a two fold increase in leaf crinkling was observed. Leaves showed 11.7 per cent area crinkled with the pest load of seven adults per plant. Leaf crinkling of 15 per cent was recorded on plants inoculated with 8 and 9 adults per plant respectively. A slight increase in leaf crinkling

Table 8a. *Paracoccus marginatus* infestation and leaf damage severity on papaya

No. of mealybugs released	Leaf crinkling*							
	I instar		II instar		III instar		Adult	
	Crinkling (%)	Score	Crinkling (%)	Score	Crinkling (%)	Score	Crinkling (%)	Score
1	0	0	5.5	1	6.6	1	0	0
2	0	0	6.0	1	12.5	1	0	0
3	0	0	11.7	1	11.7	1	0	0
4	0	0	17.6	1	17.6	1	0	0
5	0	0	17.6	1	17.6	1	0	0
6	5.0	1	17.6	1	17.6	1	6.6	1
7	6.6	1	17.6	1	21.0	1	11.7	1
8	11.7	1	22.2	1	22.2	1	15.0	1
9	15.0	1	23.5	1	23.5	1	15.0	1
10	15.0	1	27.7	2	36.8	2	17.6	1

*mean of three observations

(17.6 per cent) was seen on plants inoculated with 10 adults (Table 8a).

From the above experiment, it was understood that at 96h of inoculation the plants treated with 10 numbers of first instar crawlers and adult insects produced 15.0 and 17.6 per cent leaf crinkling. Whereas the leaf crinkling was doubled with inoculation of second and third instars mealybugs. It showed that second and third instars were solely responsible for producing the leaf crinkling on papaya plants.

4.3.2. Leaf damage severity on amaranthus

The results were almost similar to that of papaya plants (Table 8b). All the observations were taken after 96h of inoculation.

Results showed that the amaranthus seedlings inoculated with 1 to 4 first instars per plant could not produce any leaf crinkling symptoms and damage score was '0'. Whereas damage score of '1' was recorded, with the increase in pest load from 5 to 8 first instar crawlers per plant. The plants inoculated with 5 and 6 first instar nymphs produced leaf crinkling of 11 per cent. However, the leaf crinkling of 18.7 and 23.5 per cent was recorded on plants inoculated with 7 and 8 first instar nymphs respectively. Highest leaf crinkling of 27.7 per cent with leaf damage score '2' was recorded on plants inoculated with 9 to 10 first instar nymphs per plant.

Amaranthus seedlings inoculated with 1 to 6 numbers of second instars of mealybug had the leaf damage severity score of '1'. However, the leaf damage severity score '2' was obtained with increase in pest load from 7 to 10. A single second instar nymph produced 16.6 per cent leaf crinkling in amaranthus seedlings which gradually increased to 17.64 per cent with increase in pest load (2 & 3 second instar nymphs). Leaf crinkling of 18.7 per cent recorded on plants with four number of second instar per plant. Whereas plants with 5 and 6 second instar nymph showed leaf crinkling of 22.2 and 23.5 per cent respectively (Table 8b). However, plants inoculated with 7 to 10 second instar produced leaf crinkling of more that 30 per cent, which belongs to leaf

damage severity sore ‘2’.

Amaranthus seedlings inoculated with 1 to 7 third instar mealybugs were recorded a leaf damage severity score ‘2’. A single third instar nymph produced 29.4 per cent leaf crinkling in amaranthus seedlings which gradually increased to 33.3 per cent with the increase in pest load per plant (2 and 3 third instar mealybugs per plant). Leaf crinkling of 35.2 per cent and 37.5 per cent was observed on plants inoculated with four and five numbers of third instar mealybugs per plant. Whereas a leaf crinkling of 43.75 and 44.4 per cent was recorded on plants with 6 and 7 third instar nymphs respectively. The amaranthus seedling inoculated with 8 to 10 third instar nymphs were recorded leaf damage score ‘3’ where all the plant showed a leaf crinkling of more than 50 per cent. A leaf crinkling of 52.9 per cent was recorded on plants inoculated with 8 and 9 numbers of third instar mealybug and the highest leaf crinkling of 55.5 per cent was recorded on plants inoculated with 10 third instar mealybugs per plant (Table 8b).

The experiment revealed that plants inoculated with 1 to 5 adult mealybugs were unable to produce characteristics leaf crinkling symptoms. Hence it belonged to leaf damage severity score ‘0’. When inoculated with 6 to 10 adult mealybugs per plant a leaf damage score ‘1’ was recorded. The plants inoculated with 6 and 7 adult mealybugs produced 5.5 and 5.8 per cent leaf crinkling whereas 11.1 per cent of leaf crinkling was observed with the increase in pest load from 8 to 10 adult mealybugs per plant.

Table 8b. *Paracoccus marginatus* infestation and leaf damage severity on amaranthus

No. of mealybugs released/plant	Leaf crinkling*							
	I instar		II instar		III instar		Adult	
	Crinkling (%)	Score	Crinkling (%)	Score	Crinkling (%)	Score	Crinkling (%)	Score
1	0	0	16.6	1	29.4	2	0	0
2	0	0	17.64	1	33.3	2	0	0
3	0	0	17.64	1	33.3	2	0	0
4	0	0	18.7	1	35.2	2	0	0
5	11.1	1	22.2	1	37.5	2	0	0
6	11.7	1	23.5	1	43.75	2	5.5	1
7	18.7	1	31.25	2	44.4	2	5.8	1
8	23.5	1	31.25	2	52.9	3	11.1	1
9	27.7	2	33.3	2	52.9	3	11.1	1
10	27.7	2	35.2	2	55.5	3	11.1	1

*mean of three observations

4.4. Dispersion of mealybugs

4.4.1. Dispersion of mealybugs on papaya

Dispersion of different stages of mealybug was studied on papaya plants, where 165 mealybugs were released and the numbers of mealybugs dispersed from the plants were recorded at the end of 24h. The highest dispersion tendency was recorded in first instars, followed by second and third instars and the lowest dispersion was showed by adult mealybugs (Table 9).

From the table it was understood that out of 165 crawlers released 17 crawlers were from papaya plants at the end of 24h, with the dispersion of 10.3 per cent.

In the case of second instar, at the end of 24h, 10 out of 165 nymphs were dispersed from the papaya plants with a dispersion of 6.06 per cent, whereas third instars showed a lower dispersion of 4.24 per cent and the number of mealybugs retained was 155. At 24h, adult mealybugs showed a dispersion of 3.03 per cent and the number of mealybugs dispersed from the plant was 5.

4.3.4. Dispersion of mealybugs on amaranthus

Dispersion of mealybug was studied on amaranthus seedlings also, where 165 numbers of mealybugs were released and the numbers of mealybugs dispersed from the plants were recorded at the end of 24h. The dispersion of third instars was low compared to first and second instar crawlers whereas the lowest dispersion was showed by adult mealybugs since they settled down and started producing ovisac (Table 10).

The experiment showed that highest dispersion (16.36 %) was shown by the first instars at 24h. Out of 165 first instars released, 27 crawlers were dispersed from the plant. In the case of second instar nymphs, the highest dispersion of 12.12 per

Table 9. Dispersion of *P. marginatus* on papaya

Stage of mealybug	Time for dispersion (h)	No. released	No. dispersed	Dispersion per cent*
First instar	24	165	17	10.30
Second instar	24	165	10	6.06
Third instar	24	165	7	4.24
Adult	24	165	5	3.03

*mean of 3 observations

Table 10. Dispersion of *P. marginatus* on amaranthus

Stage of mealybug	Time for dispersion (h)	No. released	No. dispersed	Dispersion per cent*
First instar	24	165	27	16.36
Second instar	24	165	20	12.12
Third instar	24	165	11	6.60
Adult	24	165	5	3.03

*mean of 3 observations

cent was observed at 24h of inoculation and the number of mealybugs dispersed from the plant was 20.

Out of 165 third instars released, at 24h, 11 nymphs were dispersed with the dispersion of 6.60 per cent. In the case of adults, at the end of 24h, 5 out of 165 were dispersed with a mean dispersion of 3.03 per cent.

4.4. Biochemical analysis

Biochemical constituents like soluble protein, indole-3-acetic acid (IAA) and gibberellic acid (GA) present in the infested and uninfested leaves of papaya and amaranthus were estimated to relate with the leaf damages on host plants.

4.4.1. Protein

4.4.1.1. Papaya

Amount of total protein present in the infested and uninfested leaves of papaya plants (three months old) with all stages of mealybug were estimated (Table 11). The total protein concentration of leaf sample was estimated after 96 hours of inoculation with mealybugs.

The concentration of total protein was 4.94 mg/g of leaf sample when the plants were inoculated with one crawler and it was reduced to 4.91 mg/g with the release of two crawlers per plant. The protein concentration on plants inoculated with three crawlers was 4.80 mg/g. Protein concentration of 4.76 mg/g was recorded on plants with 4 crawlers. Papaya plants with a pest load five crawlers resulted in 4.56mg/g of total protein. A slight decrease in total protein content (4.39 mg/g) was recorded in plants with six crawlers. The plants infested with seven crawlers showed a total protein concentration of 4.23 mg/g. A plant inoculated with eight crawlers in an indicated protein concentration of 4.17 mg/g. The papaya plants inoculated with

nine and ten crawlers showed a decrease in total protein content to the tune of 3.56 and 3.16 mg/g respectively.

A total protein concentration of 4.57 mg/g was estimated from the plants inoculated with one second instar nymph; whereas plants with two second instar nymphs showed a protein concentration of 4.51 mg/g. Plants inoculated with three second instar nymphs resulted in reduction of protein content to 4.03 mg/g. A total protein content of 3.84 mg/g was recorded on plants with four second instar nymphs. Whereas it was reduced to 3.64 mg/g on the plants inoculated with five second instar nymphs. Similar trend was noticed in plants inoculated with six second instar nymphs with 3.59 mg/g protein. A total protein of 3.48 and 3.03 mg/g was recorded on plants inoculated with seven and eight second instars nymphs respectively. Whereas plants inoculated with nine and ten second instars nymph, showed a significant decrease in total protein content to the level of 2.92 and 2.73 mg/g of leaf respectively.

Papaya plants inoculated with third instars showed a decrease in total protein content compared to plants inoculated with first, second and adult mealybugs. A total protein content of 3.10 mg/g was recorded on plants inoculated with one third instar nymph of papaya mealybug whereas the protein concentration was reduced to 3.02 mg/g in plants inoculated with two third instar nymphs. A very slight reduction in total content of protein (3.01mg/g) was recorded on plants infested with three third instar nymphs of papaya mealybug whereas a total protein content 2.96, 2.93 and 2.94 mg/g was recorded on plants inoculated with 4, 5 and 6 third instars of papaya mealybug. The plants inoculated with seven third instar nymphs showed a total protein content of 2.86 mg/g. However, papaya plant inoculated with 8 and 9 third instars gave a total protein content of 2.56 mg/g and 2.49 mg/g of plant sample. The lowest protein content (1.88 mg/g) was recorded on plants inoculated with 10 numbers of third instar nymphs.

From the result it was inferred that in the plants inoculated with adult mealybugs there was no noticeable variation in total protein concentration as the number of mealybugs per pant was increased from 1-10 *i.e.* the total protein concentration ranged from 4.96 – 4.17 mg/g. The total protein concentration of plants inoculated with one adult mealybug was 4.96 mg/g. A similar protein concentration (4.94 mg/g) was recorded when the pest load was increased to two and three adult mealybugs per plant. However, a slight decrease in total protein concentration (4.75 mg/g) was obtained when the number of adult mealybugs was increased to four. The total protein content was 4.69 mg/g when the numbers of adults released was five. A further increase in pest load (six adults /plant) results a slight reduction in protein content (4.65 mg/g). Total protein content of 4.55 mg/g was recorded on plants with seven adults per plant. Not an appreciable change in concentration was recorded as the number of adults per plant was eight (4.54 mg/g). As the number of adults per plant was further increased to nine, the total protein concentration was leveled to 4.44 mg/g. In this experiment, the lowest protein concentration (4.17 mg/g) was recorded on plants inoculated with 10 adult mealybugs (Table 11).

The analysis showed that the total protein content was very low in plants inoculated with third instar nymph (2.78mg/g), followed by second instars of mealybug (3.63 mg/g). When the plants inoculated with first instar and adult mealybug recorded a maximum protein concentration of 4.35 mg/g and 4.66 mg/g respectively. However, protein concentration of more than 5 mg/g was recorded on control plants (plants without mealybug). It was inferred that the plants inoculated with third and second instars resulted a slight reduction in protein content compared to other life stages of papaya mealybug.

4.4.1.2 Amaranthus

Amount of total protein present in the infested and uninfested leaves of amaranthus seedlings (one month old) inoculated with different stages of mealybug

Table 11. Effect of infestation of *P. marginatus* on the total protein content of papaya seedling leaves

No of mealybugs released/plant	Mean protein concentration (mg/g) of leaf sample			
	I instar	II instar	III instar	Adult
1	4.94	4.57	3.10	4.96
2	4.91	4.51	3.02	4.94
3	4.80	4.03	3.01	4.94
4	4.76	3.84	2.96	4.75
5	4.56	3.64	2.93	4.69
6	4.39	3.59	2.94	4.65
7	4.23	3.48	2.86	4.55
8	4.17	3.03	2.56	4.54
9	3.56	2.92	2.49	4.44
10	3.16	2.73	1.88	4.17
Mean	4.35	3.63	2.78	4.66
t value	4.20**	7.50**	20.10**	5.90**
Control	5.34	5.18	5.20	5.14

mean of 3 replications

** Significant at 1% level

were estimated (Table 12). The total protein concentration of leaf sample was estimated after 96 hours of inoculation with mealybugs.

The total content of protein estimated from plants inoculated with first instars of mealybug ranged from 4.95 to 2.83 mg/g. A total protein concentration of 4.95 mg/g was recorded on plants inoculated with one crawler of mealybug. It was reduced to 4.92 mg/g on plants inoculated with two crawlers. The total protein concentration of 4.84 mg/g was recorded on plants inoculated with three crawlers. As the number of crawlers was further increased to four per plant, decrease in protein concentration to 4.58 mg/g was observed. However, a plant inoculated with five crawlers per plant recorded a very slight reduction in total protein concentration (4.57 mg/g). A total protein content of 4.20 and 4.14 mg/g was recorded on plants inoculated with six and seven crawlers. It was further reduced to 3.78 mg/g on plants inoculated with eight mealybugs per plant. The amaranthus seedlings inoculated with nine first instars recorded a protein content of 3.17 mg/g, whereas an estimated protein content of 2.83 mg/g was recorded on plants inoculated with ten crawlers.

The total protein content estimated from amaranthus seedlings inoculated with second instar nymphs ranged from 4.58 mg/g to 2.79 mg/g. A total protein content of 4.58 mg/g was recorded on plant inoculated with one second instar nymph. A similar level of protein concentration was recorded on plants inoculated with two second instar nymphs (4.57 mg/g). It was further reduced to 4.28 mg/g on the plants inoculated with three second instar nymphs. An estimated total protein content of 4.24 and 4.27 was recorded on the plants with four and five second instars nymphs respectively. However, plants inoculated with 6, 7 and 8 numbers of second instars were recorded a total protein concentration of 3.70, 3.17 and 3.01 mg/g of total protein content per plant. A significantly lower level of protein contents were recorded from plants inoculated with 9 and 10 second instars nymphs, with a value of 2.82 mg/g and 2.79 mg/g respectively (Table 12).

The total protein concentration estimated from amaranthus seedlings with third instar nymphs ranged from 3.60 mg/g to 1.99 mg/g of leaf. The seedlings inoculated with one third instar nymph recorded a protein content of 3.60 mg/g, it was reduced to 3.35 mg/g on the plants inoculated with two, third instar nymphs of mealybug. The protein content was 3.33 mg/g and 3.30 mg/g on plants inoculated with 3 and 4 third instar nymphs. When a plant inoculated with five third instar nymphs, a total protein content of 2.63 mg/g was observed. A total protein content of 2.56 and 2.25 mg/g was recorded on plants with 6 and 7 third instar nymphs, which was reduced to 2.17 and 2.08 mg/g on the plants inoculated with eight and nine mealybugs per plant. However, the lowest content of total protein (1.99 mg/g) was recorded on amaranthus seedlings inoculated with 10 numbers of third instar nymphs of mealybug.

Estimation of total protein content was carried out from the leaves of amaranthus seedlings which were inoculated with adult mealybug. The protein content ranged from 5.02 to 3.56 mg/g. Total protein content of 5.02 mg/g was recorded on plants inoculated with one adult mealybug. Plants inoculated with 5 and 6 adult mealybugs showed a total protein content of 4.67 and 4.61 mg/g leaf, whereas it was further reduced to 4.02 mg/g on the plants inoculated with seven adult mealybugs. However, amaranthus seedlings inoculated with 8, 9 and 10 adult mealybugs per plant resulted in a lower protein content of 3.96, 3.59 and 3.56 mg/g respectively.

The analysis showed that the amaranthus seedlings inoculated with third instar nymphs resulted in the lowest level of mean total protein content (2.73 mg/g), followed by second instar nymphs (3.74 mg/g). However, a maximum protein content of 4.2 and 4.42 mg/g was recorded on plants inoculated with first instar and adult mealybug.

Table 12. Effect of infestation of *P. marginatus* on the total protein content of amaranthus seedling leaves

No of mealybugs released/ plant	Mean protein concentration (mg/g) of leaf sample infested by *P. marginatus*			
	I instar	II instar	III instar	Adult
1	4.95	4.58	3.60	5.02
2	4.92	4.57	3.35	4.94
3	4.84	4.28	3.33	4.93
4	4.58	4.24	3.30	4.92
5	4.57	4.27	2.63	4.67
6	4.20	3.70	2.56	4.61
7	4.14	3.17	2.25	4.02
8	3.78	3.01	2.17	3.96
9	3.17	2.82	2.08	3.59
10	2.83	2.79	1.99	3.56
Mean	4.20	3.74	2.73	4.42
t value	3.90**	5.50**	11.70**	3.90**
Control	5.12	5.04	4.99	5.15

mean of 3 replications

** Significant at 1% level

4.4.2. IAA (Indole-3-Acetic Acid)

Content of IAA from infested and uninfested papaya and amaranthus were estimated by spectrophotometric method and results are given below.

4.4.2.1. Papaya

Estimated IAA content in papaya plants inoculated with first instar nymphs ranged from 0.131 to 0.022 mg/g (Table 13). IAA content of 0.131 mg/g was recorded on the plants inoculated with one crawler of papaya mealybug and IAA content reduced to 0.123 mg/g as the pest load increased from two to five first instar crawlers on the plants. However, a higher reduction of IAA content was recorded on plants inoculated with six crawlers of papaya mealybug and which reduced to 0.022 mg/g with the increase in pest load from seven to ten crawlers per plant.

IAA content was estimated from papaya plants inoculated with second instar nymphs, which ranged from 0.035 mg/g to 0.023 mg/g. An estimated IAA content of 0.035 mg/g was recorded on papaya plants inoculated with one second instar nymph and it was reduced to 0.031 mg/g with increase in pest load from two to five second instar nymphs per plant. When papaya plants inoculated with six second instar nymphs IAA content was 0.028 mg/g and it was reduced to 0.023 mg/g with the increase in pest load from seven to ten number of second instar nymphs per plants.

Papaya plants inoculated with third instar nymphs of mealybug resulted a decreased level of IAA content which ranged from 0.022 mg/g and 0.017 mg/g. IAA level of 0.022 mg/g was estimated on the plants inoculated with one third instar nymph, which reduced to 0.021 mg/g with increase in pest load to three third instar nymphs per plants. The IAA content of 0.019 mg/g was recorded on plants inoculated with 4, 5, 6 and 7 third instar mealybugs whereas it was further reduced to 0.017 mg/g with the increase in pest load from 8 to 10 third instar nymphs per plant. When were plants inoculated with four adult mealybugs, IAA content was 0.093 mg/g,

Table13. Effect of infestation of *P. marginatus* on the IAA content of papaya seedling leaves

No of mealybugs released/plant	IAA concentration (mg/g) of leaf sample			
	I instar	II instar	III instar	Adult
1	0.131	0.035	0.022	0.127
2	0.129	0.034	0.022	0.125
3	0.127	0.032	0.021	0.124
4	0.126	0.031	0.019	0.093
5	0.123	0.031	0.019	0.091
6	0.026	0.028	0.019	0.076
7	0.025	0.026	0.019	0.062
8	0.024	0.025	0.018	0.025
9	0.022	0.024	0.017	0.025
10	0.022	0.023	0.017	0.022
Mean	0.060	0.028	0.019	0.070
t value	6.1**	50.1**	55.6**	4.3**
Control	0.169	0.099	0.185	0.135

mean of 3 replications

** Significant at 1% level

which reduced to 0.062 mg/g with the increase in pest load from five to seven adult mealybugs per plant. Hence the plant inoculated with 8 to 10 adult mealybugs showed a reduced level of IAA content in the range of 0.025 to 0.022 mg/g.

The analysis showed that the papaya plants inoculated with third instar nymph of papaya mealybug had a reduced mean level of IAA content (0.019 mg/g), followed by plants inoculated with second instar nymphs of mealybug (0.028 mg/g). Whereas estimated level of mean IAA content from the plants inoculated with first instar and adult mealybugs was 0.06 and 0.07 mg/g. The control plants showed higher IAA content of 0.099 to 0.185 mg/g of leaf sample (Table 13). From the above results, it was inferred that the auxin content recorded on papaya seedlings showed a gradual decrease in their concentration due to *P. marginatus* infestation.

4.4.1.2. Amaranthus

The IAA content in the amaranthus seedlings infested with all the stages of mealybug *i.e.* first instar, second instar, third instar nymphs and adult mealybugs (Table 14).

IAA content in amaranthus seedlings inoculated with papaya mealybug crawlers were estimated and found to be in the range from 0.099 to 0.041 mg/g. The seedling inoculated with one crawler showed IAA content of 0.099 mg/g, which was reduced to 0.070 mg/g with the increase in pest load from two to five crawlers per plant. Whereas an IAA content of 0.062 mg/g was recorded in the plants inoculated with six crawlers of mealybug, which gradually reduced and reached a level of 0.041 mg/g with the increase in pest load from seven to ten first instars per plant.

When the amaranthus seedlings inoculated with second instar nymphs the IAA content was in the range between 0.096 to 0.014 mg/g. The plants inoculated with one second instar crawler recorded an IAA content of 0.096 mg/g, which gradually reduced to 0.052 mg/g with the increase in pest load from two to five

Table14. Effect of infestation of *P. marginatus* on the IAA content of amaranthus seedling leaves

No. of mealybugs released/plant	IAA concentration (mg/g) of leaf sample			
	I instar	II instar	III instar	Adult
1	0.099	0.096	0.059	0.092
2	0.095	0.084	0.037	0.088
3	0.080	0.073	0.031	0.084
4	0.073	0.065	0.021	0.076
5	0.070	0.052	0.018	0.072
6	0.062	0.018	0.016	0.031
7	0.054	0.016	0.015	0.026
8	0.053	0.015	0.015	0.023
9	0.050	0.015	0.014	0.022
10	0.041	0.014	0.012	0.019
Mean	0.06	0.044	0.023	0.053
t value	8.5**	10.1**	14.1**	5.7**
Control	0.12	0.15	0.095	0.11

*mean of 3 replications

** Significant at 1% level

nymphs. However, a higher reduction (0.018 mg/g) in IAA content was observed plants inoculated with six second instars, which gradually decreased to 0.014 mg/g with the increase in pest load from 7 to 10 second instars per plant.

The IAA content of amaranthus seedling ranged between 0.059 to 0.012 mg/g when third instar nymphs were feeding on the plants. The plants inoculated with one third instar nymphs showed an IAA content of 0.059 mg/g which reduced to 0.012 with the increase in pest load from two to ten third instar nymphs per plants.

IAA content from amaranthus seedlings inoculated with adult mealybug was estimated, and it was found to vary from 0.092 and 0.019 mg/g. An estimated IAA content of 0.092 mg/g was recorded on plants inoculated with one adult mealybug which was decreased to 0.072 mg/g with the increase of pest load to five adult per plant. Whereas plants inoculated with six adult mealybugs recorded IAA content of 0.031 mg/g and it was reduced to 0.019 mg/g with the increase of mealybugs from seven to ten.

The analysis showed that the amaranthus seedlings inoculated with third instar nymphs recorded significantly lower mean IAA content of 0.023 mg/g, followed by second instar nymphs. Whereas the seedling inoculated with first instar and adults resulted a maximum IAA content of 0.06 and 0.053 mg/g. The estimated level of IAA in control plants was in the range from 0.095 to 0.12 mg/g.

4.4. 3.Estimation of gibberellins from plants

Gibberellic acid (GA) was estimated spectrophotometrically as explained in 3.4.3. Samples were taken from infested and uninfested leaves of papaya and amaranthus and the GA content was estimated (Table 15).

The gibberellic acid was estimated from the leaves of plants inoculated with third instar nymphs of papaya mealybug and showed a maximum level of crinkling in plants. GA content was higher in infested plants than uninfested plants. The estimated

GA content in infested leaves of papaya was 5.0µg/g whereas infested leaves of amaranthus recorded a low level of GA content (2.0 µg/g). However, same level of GA content (1.0 µg/g) was recorded from uninfested leaves of papaya and amaranthus.

Table15. Effect of *P. marginatus* on the gibberellic acid content in papaya and amaranthus

Crop	Gibberellic acid content in leaves (µg/g)	
	Infested	**Uninfested**
Papaya	5.0	1.0
Amaranthus	2.0	1.0

*mean of 3 replications

Discussion

5. Discussion

Results obtained in the laboratory study on "Infestation induced reactions of papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink, (Hemiptera: Pseudococcidae) on papaya and amaranthus" are discussed below.

5.1 Biology

Understanding the life history of an insect pest is important in predicting its development, emergence, distribution and abundance. Different plant species provide different nutritional elements and chemical constituents, which can affect the development, reproduction, and survival of an insect. The role of host plant was an important factor in regulating insect population (Umbarihowar and Hastings, 2002) as the life cycle characteristics of herbivores might be affected by variation in host plant traits, for example the life history parameters like longevity, fecundity and survival might be influenced by the variation in host plant quality (Awmack and Leather, 2002). Hence, the biology of papaya mealybug was studied under laboratory conditions on two crops *i.e.*, papaya and amaranthus during October 2011 (with a monthly mean temperature of 27.62±4.2°C and relative humidity of 78.3±2.24%). The study revealed that there were differences in the biological parameters like incubation period, duration of each nymphal instars, total nymphal period, fecundity and sex ratio of the test insect.

5.1.1. Duration of life stages of *P. marginatus*

In the present study the duration of life stages of male *P. marginatus* on two host plants varied slightly. The variation was restricted to the case of incubation period of egg only (7.5±1.2 days on papaya and 6.6±1.1 days on amaranthus) (Fig. 1). The difference in the incubation period of eggs on these two host plants is in conformity with the result obtained by Amarasekare *et al.* (2008b) in other host plants. They found that the incubation period of eggs of *P. marginatus* on acalypha was 8.6±0.1 days, on hibiscus 8.4±0.1 days, on parthenium 8.8 ± 0.1 days and 8.5 ± 0.1 days on plumeria. Amarasekare *et al.* (2008a) studied the effect of temperature on life history parameters of *P.*

marginatus and observed that at 25°C the incubation period of egg were 8.7±0.1 days. When the temperature increased to 30°C, egg took only 7.3±0.2 days to hatch out. Similarly present study was carried out in monthly mean temperature of 30°C and it was found that the incubation period of egg was 7.5±1.2 on papaya and 6.6±1.1 days on amaranthus.

P. marginatus males had five instars, the fourth of which was produced in a cocoon and referred to as the pupa. These results were in accordance with those of Walker *et al*. (2006) and Mishra (2011). First instar nymphs of male mealybugs were greenish yellow in colour. The body colour in second instar nymphs turned pink especially during the pre-pupal and pupal stages. This was in conformity with the result obtained by Walker *et al*. (2006). They observed that adult males tend to be pink, especially during the pre-pupal and pupal stages, but appeared yellow in the first and second instar. The mean length of adult male was 1.29±0.03 mm, with an elongate oval body that was widest at the thorax (0.17±0.01 mm). Miller and Miller (2002) and Walker *et al*. (2006) found that adult males were approximately 1.0 mm long and 0.3 mm wide.

The adult male of *P. marginatus* was winged and appeared yellow. The head and thorax were heavily sclerotized. These findings were in agreement with those of Miller and Miller (2002); Walker *et al*. (2006) and Mishra (2011).

However, great variation in duration could be seen in different stages of female *P. marginatus* on the two host plants (Fig. 2). In the present study, the duration of different nymphal stages was 4.2, 3.6 and 4.1 days (Table 3a) on papaya and 4.8, 3.7 and 4.9 days (Table 3b) on amaranthus. The duration of nymphal instars varied with the host plants. Similar results were also obtained by Amarasekare *et al*. (2008b) on different host plants. There was a slight reduction in the duration of nymphal stages on papaya plants *viz*., 4.2, 3.6 and 4.1 days for first, second and third instars respectively with the total nymphal duration being 11.9±1.1 days (Table 3a).

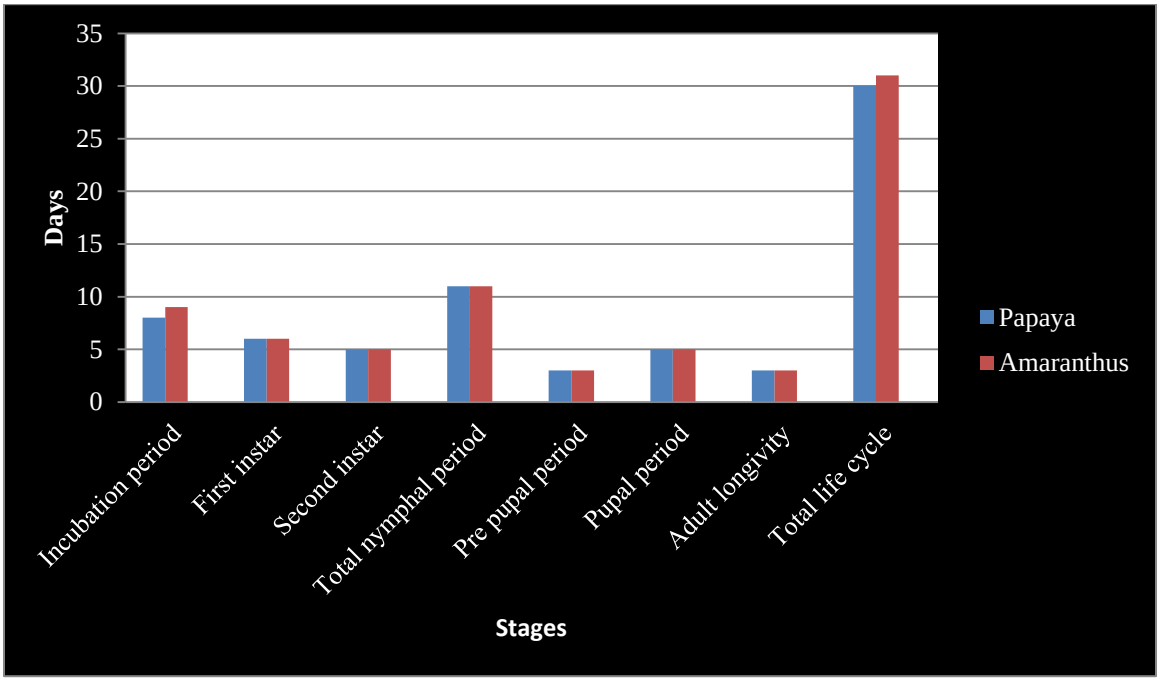

Fig. 1. Duration of life stages of male *P. marginatus* on papaya and amaranthus

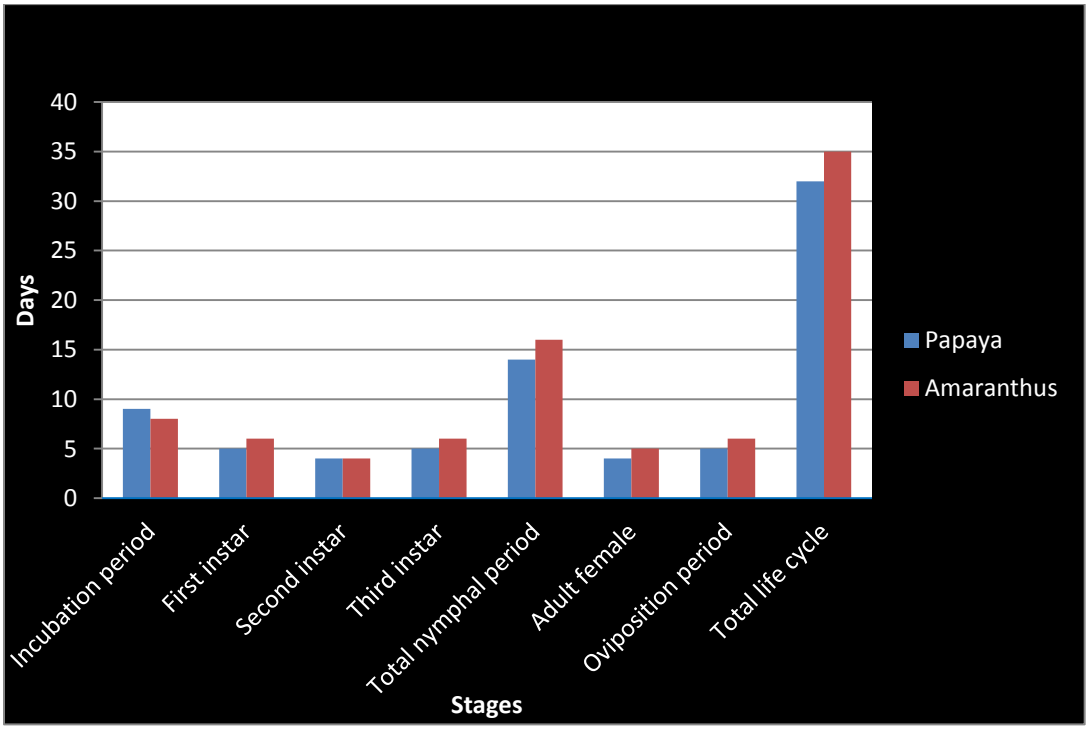

Fig. 2. Duration of life stages of female *P. marginatus* on papaya and amaranthus

Eggs were greenish yellow and were laid in an egg sac that was three to four times the body length and entirely covered with white wax. The ovisac was developed ventrally on the adult female. These results were in accordance with those of Walker *et al*. (2006).

Adult females were yellowish with short waxy filaments around the body margin. The study conducted by Heu *et al.* (2007) and Walker *et al*. (2006) supported the above result. They found that adult females were yellow and were covered with a white waxy coating. Adult females were approximately 2.24 mm long and 1.22 mm wide. Walker *et al*. (2006) observed that adult female mealybugs were approximately 2.2 mm long and 1.4 mm wide.

In the present study, there were differences in the total developmental period of male and female *P. marginatus* on papaya and amaranthus. On papaya, female *P. marginatus* took 27.0±2.44 days to complete one life cycle whereas male completed life cycle in 24.9±2.30 days. On amaranthus total developmental period of female *P. marginatus* was slightly higher than papaya (27.3±2.75 days) whereas male mealybug took 25.7±2.60 days to complete one life cycle. In polyphagous insects, life history could vary with the plant species it feed on (Amarasekare *et al*., 2008b). According to Amarasekare *et al*. (2008b) on four different plant species namely *Hibiscus rosa-sinensis* L. (male-27.6±0.1; female-25.5±0.1), *Acalypha wilkesiana* (Muell.-Arg.) (male -28.4±0.1; female-24.5±0.1), *Plumeria rubra* L. (male-30.0±0.1; female-25.5±0.1) and one weed species *Parthenium hysterophorus* L. (male-27.7±0.1; female-24.4±0.1), there were difference in the life history parameters of *P. marginatus*. The difference observed in the life history of *P. marginatus* might be due to nutritive factors, allelochemical compounds and physical properties in leaf texture and structure, although none of the factors were studied previously for *P. marginatus*.

Different host plant species had been shown to affect the life history parameters of other mealybug species. Mortality of the citrus mealybug, *Planococcus citri* (Risso) was higher on green than on red or yellow variegated *Coleus blumei* ‘Bellevue’ (Bentham) plants, and development was faster and

fecundity higher on red variegated plants. Compared with green counterparts, both red and yellow variegated plants of *C. blumei* grew more slowly, had lower rates of photosynthesis and produced more leaf area per unit of biomass (Yang and Sadof 1995). The developmental time of female *Planococcus kraunhiae* (Kuwana) was shorter when reared on germinated *Vicia faba* L. seeds than on leaves of a *Citrus* sp. L. (Narai and Murai, 2002).

5.2. Pest load assessment in papaya and amaranthus

Paracoccus marginatus could attack and damage various parts of the host plant including the leaves, stems, flowers and fruits (Williams and Willink, 1992). In order to know the pest load that could produce leaf crinkling symptoms on papaya and amaranthus, a study was conducted on three months old papaya and one month old amaranthus seedlings.

5.2.1. Pest load assessment of *Paracoccus marginatus* on papaya plants

Pest load assessment studies on papaya seedlings revealed that a single first instar of papaya mealybug could not produce leaf crinkling symptoms on papaya seedlings even after 96 h of infestation whereas in plants where the number of mealybugs were six to ten there was crinkling in the plant leaves. Williams and Willink (1992) found that *P. marginatus* could produce curling, crinkling, rosetting, twisting, reduction in plant size and surface area and general leaf distortion of leaves.

From the experiment it was observed that even a single second instar and third instar of *P. marginatus* could produce leaf crinkling symptoms on three months old papaya seedlings at 96 h of infestation (Table 7a). The papaya seedlings infested with four second instar crawlers of papaya mealybug could produce crinkling of leaves at 48 h of infestation. All the plants infested with second and third instars of *P. marginatus* produced leaf crinkling symptoms after 96 h of infestation. Williams and Willink (1992); Walker (2006) and Heu *et al*.

(2007) observed that *P. marginatus* could produce crinkling of leaves on host plants.

Some papaya seedlings showed crinkling of leaves due to feeding of adult mealybug (Table 7a). *i.e*. when six to ten adult mealybugs were present per plant, those plants showed leaf crinkling symptoms at 96 h of infestation. After 96 h of infestation all the adult mealybugs started to produce ovisac.

5.2.2. Pest load assessment of *Paracoccus marginatus* on amaranthus seedlings

Result of the experiment (Table 7b) showed that single first instar crawler of papaya mealybug, *Paracoccus marginatus* could not produce any symptoms on leaves due to the feeding. If the number of crawlers were 1-4, there was no crinkling of leaves (Table 8b). However, when the number of crawlers per amaranthus plant was 5-10, crinkling of leaves were appeared from 96h onwards. The per cent leaf crinkling on plants infested with first instar crawlers ranged from 11.1-27.7 (Table 8b).

Single second instar crawler of papaya mealybug could produce leaf crinkling symptoms in amaranthus seedlings from 96h onwards whereas the plants infested with 3-10 second instar crawlers of papaya mealybug could produce the symptoms from 72h onwards itself (Table 7b). Williams and Willink (1992); Walker (2006) and Heu *et al*. (2007) observed that *P. marginatus* could produce crinkling of leaves on host plants.

The result of the experiment showed that even a single third instar of *P. marginatus* could produce crinkling of leaves on amaranthus seedlings (one month old) after 72 h of infestation. The per cent leaf crinkling on amaranthus seedlings due to third instars of papaya mealybug ranged from 29.4 – 55.5 (Table 8b). The amaranthus seedlings infested with 5-10 adult mealybugs per plant produced leaf crinkling after 96 h of infestation. The adult mealybugs produced 5.5-11.1 per cent leaf crinkling on amaranthus leaves (Table 8b).

5.3. Assessment of plant reaction by visual scoring on leaf deformities

Damage caused to papaya and amaranthus seedlings by *P. marginatus* was assessed. The level of damage was rated using the leaf damage severity scale.

5.3.1. Leaf damage severity

The results of the experiment, pest load assessment on papaya and amaranthus showed that among the four different stages of mealybugs, third instars caused more crinkling than others (Fig 3 and Fig 4) on both papaya and amaranthus seedlings. In the case of papaya seedlings, the plants infested with ten second and third instars of mealybug produced the leaf damage score 2 *i. e*. papaya plants infested with ten second and third instars of mealybug produced leaf crinkling in the range of 26-50 per cent (Fig. 4).

However, in amaranthus seedlings (one month old) score 3 was observed on seedlings infested with 8-10 numbers of third instars of *P. marginatus* (Table 8b) (Fig. 4). The lowest crinkling was caused by the adults. New flushes of growth on damaged plants were deformed due to toxicity of the saliva injected into the plant by the mealybugs while feeding (Galanihe *et al*., 2010). These damage symptoms resembled those caused by papaya mealybug described by Walker *et al*. (2006); Heu *et al*. (2007) and Galanihe *et al*. (2010) where the tender leaves of papaya became crinkled, curled and showed symptoms of chlorosis and leaf deformation.

5.4. Dispersion of mealybugs

The highest dispersion was showed by first instar crawlers of papaya mealybug. The highest dispersion of first instars was 10.3 per cent on papaya plants and 16.36 per cent on amaranthus seedlings within 24h of inoculation. The first instars or crawlers played a key role in dispersal of mealybugs. Crawlers disperse to open feeding sites whereas the second and later instars prefered to move to more sheltered sites. The study conducted by Amarasekare *et al*. (2008b) and Furness (2010) supported the above result. Amarasekare *et al*. (2008b) found

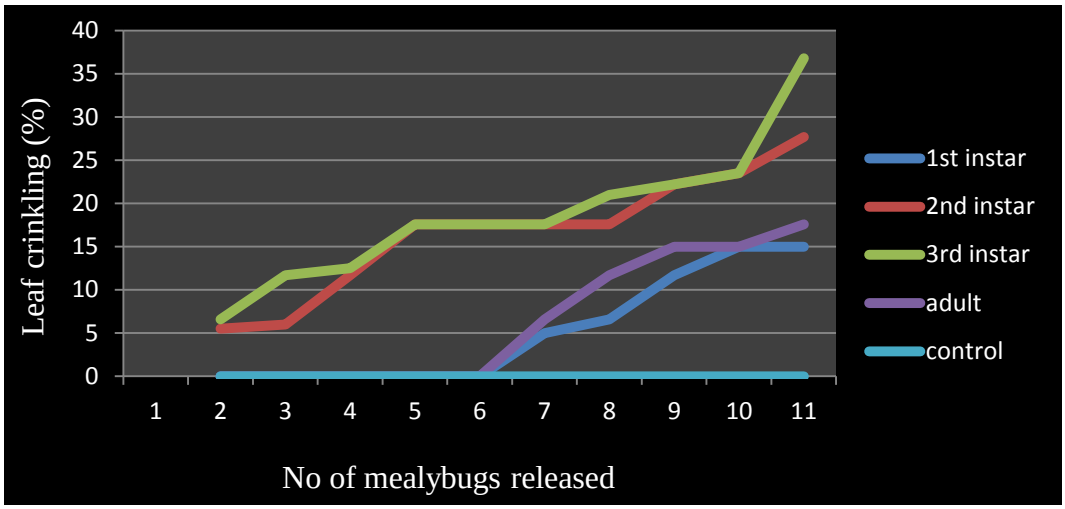

Fig.3 Leaf damage on papaya due to *P. marginatus* infestation

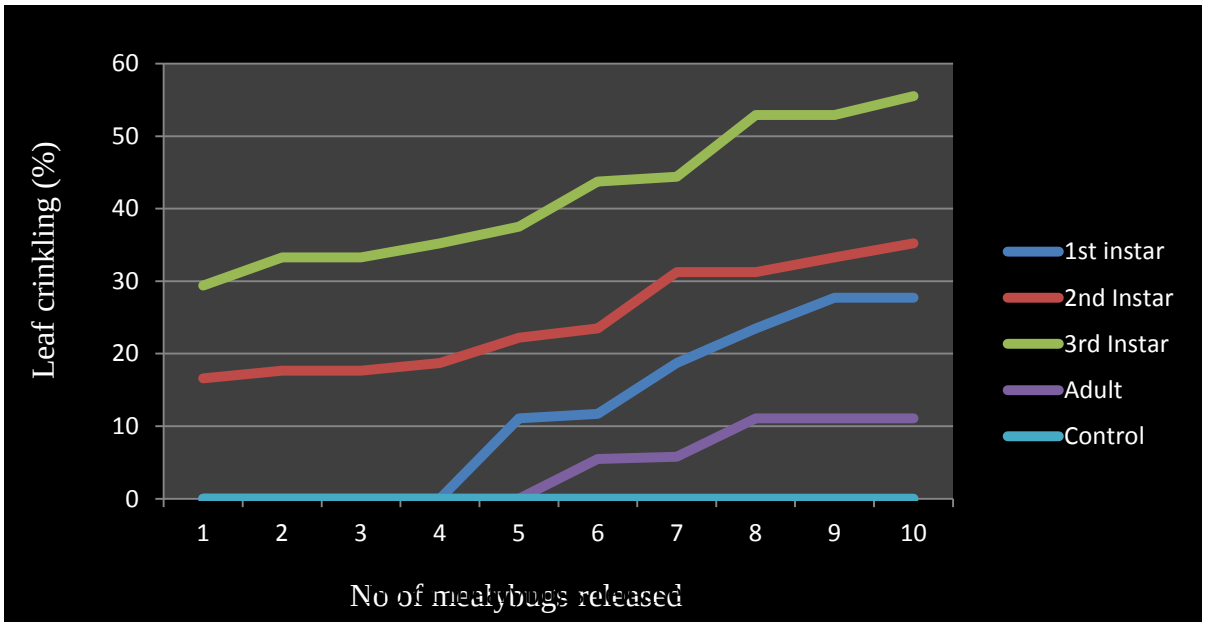

Fig.4. Leaf damage on amaranthus due to *P. marginatus* infestation

that the loss of first-instar *P. marginatus* might be due to the movement of crawlers (first instar) away from the leaf tissues and falling from the plants. Crawlers had a tendency to move toward light so the 12-h photoperiod might have caused them to move toward light and dislodged from the leaves.

5.5. Estimation of host plant constituents

Plant metabolites and macromolecules (eg. peptides, proteins, enzymes, lignin, phenolic metabolites, cuticular waxes) could serve as defense chemical against herbivores (Wink, 1997; Gutterman and Chauser-Volfson, 2002). The content of soluble protein, Indole-3-Acetic acid (IAA) and Gibberellic Acid (GA) were estimated from the infested and uninfested leaves of papaya and amaranthus.

5.5.1. Soluble protein

The result of the experiment showed that the mean concentration of total protein was very low in plants inoculated with third instar nymph (1.88mg/g) of papaya mealybug, whereas the plants inoculated with adult mealybug recorded a maximum protein concentration of 4.60 mg/g. The analysis showed that the amaranthus seedlings inoculated with third instar nymphs results in the lowest level of mean total protein content (2.73 mg/g), followed by second instar nymphs (3.74 mg/g). However, a maximum protein content of 4.42 mg/g was recorded on plants inoculated with adult mealybug.

The soluble protein in the infested and uninfested leaves was quantitatively analyzed by Lowry's method. There was a consistent inverse relationship between the level of damage by the insect and protein content. The lowest level of protein was detected in the leaves infested with ten numbers of third instars of papaya mealybug. As the number of mealybugs increased, there was a decrease in the protein content. Similarly, Pitan *et al*. (2011) found that protein, fat, carbohydrate, ash, crude fibre and moisture contents were depleted with increase in mealybug, *Rastrococcus invadens* population in mango. According to Khattab and Khattab (2005), the total soluble protein of infested

leaves of eucalyptus was lower (1.75±0.61) than those of the healthy ones (2.0±0.89 mg/g) due to feeding by gall-forming psyllid. Miles (1999) found that phloem feeding insects established a sustained interaction with sieve elements (SEs). They released saliva that inhibited plant stress responses and prevents closure of pierced SEs by callose or polymerized proteins. Drain of assimilates towards the insect away from other plant parts might contribute to such metabolites reduction (Miles, 1989). The protein content showed a decreasing trend from first group to tenth group in all the stages of insects feeding on host plants (Table 10 & 11).

5.5.2. Indole -3 Acetic Acid

The present study showed that in general the IAA content decreased in papaya and amaranthus plants infested with *P. marginatus*. The IAA content decreased sharply in the tune of 53.3 per cent to 32.14 per cent from plants inoculated with I^{st} to II^{nd} and II^{nd} to III^{rd} instars growth stages of papaya mealybug respectively. The third instar stage caused maximum decrease in IAA content when compared to all other life stages. The adult infestation caused only 58.57 per cent decrease in IAA content when compared to control, whereas first instar infestation caused 64.49 per cent reduction in IAA content.

Indole 3- acetic acid (IAA) is a naturally occurring auxin which is continuously produced in young meristematic tissues and more rapidly transported to other tissues. Any damage in apical portion can cause a change in auxin synthesis and it's concentration in plants. It is evident from the study that the mealybug infestation was concentrated to young shoots which may cause an inhibition in the synthesis of IAA at meristematis region and thereby caused a decrease in IAA content in inoculated plants.

The IAA content in the infested leaves was less as compared to uninfested leaves. Similarly, Saikia *et al*. (2011) found that red spider mite infested plum tree had lower level of auxin than the non-infested one. The tea mosquito bug, *Helopeltis* sp. attack on the axillary vegetative buds and young leaves of tea

resulted in decrease in auxin content. Bari and Jones (2009) found that blocking of auxin responses had been shown to increase resistance in plants. The IAA content showed a decreasing trend from first group to tenth group in all the stages of infestation (Table 12 & 13). The per cent decrease of IAA which is very high in papaya plants infested with third instars of papaya mealybug.

5.5.3. Gibberellic Acid (GA)

GAs are cyclic diterpenoids, transported in the entire conducting vessels i.e. both xylem and phloem. The estimated GA content in infested leaves of papaya was 5.0µg/g whereas infested leaves of amaranthus recorded a low level of GA content (2.0µg/g). However, same level of GA content was recorded from uninfested leaves of papaya and amaranthus. The present study revealed an increase in GA content after papaya mealybug infestation in both papaya and amaranthus plants.

The papaya mealybug infested papaya plants showed a five fold increase in the GA content whereas in amaranthus seedlings it was only a two fold increase. There was a direct relationship between the level of damage and GA content. This was in accordance with the result obtained by Saikia *et al*. (2011), that GA_3 content increased after infestation by *Helopeltis* in tea plants. Red spider mite infestation also induced higher level of gibberellic acid in plum trees. Yokomi *et al*. (1995) found that application of chlormequat chloride, a gibberellic acid biosynthesis inhibitor, induced leaf silvering symptoms similar to those induced by the silver leaf whitefly in squash plants.

The above reports also suggest that sucking insects can alter the quantity of GA in infested plants thereby affecting plant growth and development. An excess of GA in infested plants can be considered as a consequence of altered plant metabolism which in turn cause more damage like leaf crinkling and in later stages cause more regrowth.

Summary

6. SUMMARY

Study was carried out on "Infestation induced reactions of papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink, (Hemiptera: Pseudococcidae) on papaya and amaranthus" under laboratory conditions in the College of Horticulture, at Vellanikkara, during 2011-2012. The experiment comprised of two parts. First part comprised of the study of biology of *Paracoccus marginatus* on two host plants while the second part consisted of analysis of biochemicals *viz*., protein, IAA and GA.

The biology of *P. marginatus* was studied on two host plants *viz*., papaya and amaranthus.

Female *P. marginatus* completed its life cycle within 27.0 ± 2.44 days on papaya seedlings and 29.3 ± 2.75 days on amaranthus seedlings. The duration of different nymphal stages was 4.2, 3.6 and 4.1 days on papaya and 4.8, 3.7 and 4.9 days on amaranthus. The sex ratio exhibited difference between two host plants and it was 3:1 on papaya and 2.5:1 on amaranthus. The fecundity of the mealybug also varied on the two hosts *viz*., 513.9 ± 107.9 and 508.1 ± 132.5 respectively on papaya and amaranthus.

The duration of life stages of male *P. marginatus* on two host plants (papaya and amaranthus) vary slightly. The variation was restricted to the case of incubation period of egg only (7.5±1.2 days on papaya and 6.6±1.1 days on amaranthus). The duration of first and second instar nymphs were 5.2±0.74 and 4.5±0.50 days respectively on both papaya and amaranthus, whereas the adult longevity was 2.5±0.50 days on both papaya and amaranthus. The male *P. marginatus* completed its life cycle within 24.9 ± 2.30 days on papaya seedlings and 25.7 ± 2.60 days on amaranthus seedlings.

The results of pest load assessment studies on papaya plants revealed that at 72h of inoculation, a minimum of four second instar nymphs were required to produce the symptoms of leaf damage. However, at 96h of inoculation even the presence of a single second instar nymph was enough to produce leaf crinkling. In the case of third instars, at 72h, a minimum of three third instar nymphs were required to produce symptoms on plants. As in case of second instars, the presence of a single third instar nymph was enough to produce crinkling at 96h of inoculation. Third instar nymphs produced a maximum of 26-50 per cent leaf crinkling on papaya plants.

The pest load assessment studies on amaranthus showed that a single first instar nymph of papaya mealybug could not produce any symptoms on the leaves up to 96h of inoculation. At 96h of observation, a seedling inoculated with even a single second instar nymph had produced crinkling symptoms on leaves. Amaranthus seedlings inoculated with 7 to 10 third instar nymph took only 48h to produce the symptoms. However, at 72h of inoculation, all the seedlings inoculated with third instar nymphs (1 to 10 third instar nymphs/seedlings) had shown crinkling symptoms on leaves. However, the maximum leaf crinkling of 51-75 per cent on amaranthus plants was caused by the third instar nymphs.

In general, the nymphs tended to disperse when crowding occurred. The dispersion per cent showed a progressive reduction from a maximum of 10.3 (on papaya) and 16.36 (on amaranthus) per cent in the crawlers (first instar nymph) to the minimum of 3.03 per cent in adults on both papaya and amaranthus.

Leaf damage severity was rated using a damage scale. The per cent leaf crinkling increased when the number of mealybugs per plant increased from 1 to 10 in all instars of infestation. Per cent of leaf crinkling was highest (55.5 per cent) on the amaranthus seedlings inoculated with third instar crawlers of papaya mealybug and it was lowest (5 per cent) on the papaya plants inoculated with first instar of papaya

mealybug.

The soluble protein content was reduced due to the mealybug infestation. The infested plant samples had soluble protein at different stages. The lowest protein content (1.88 mg/g) was recorded on papaya plants inoculated with third instar crawlers of mealybug where maximum leaf crinkling was also observed. The protein content showed a decreasing trend when the number of mealybugs per plant increased from 1 to 10 in all stages of infestation.

There was reduction in the concentration of IAA in the leaves of papaya and amaranthus seedlings infested with mealybug. The highest reduction in the IAA content was shown by the plants infested with third instars of mealybug because the per cent of leaf crinkling was highest in these plants than others.

The results indicated that the IAA content was highest (0.131 mg/g) in non-infested papaya plants than non-infested amaranthus (0.099 mg/g).

Gibberellic acid content was higher in infested leaves when compared to uninfested control. Among infested samples the values were higher in papaya (5.0µg/g) than in amaranthus (2.0µg/g) where as uninfested recorded same value in both crops (1.0 µg/g). Gibberellic acid (GA) content was directly proportional to the mealybug infestation as there was an increase (5 folds in papaya and 2 folds in amaranthus) in GA concentration in infested papaya and amaranthus leaf samples as compared to the uninfested leaf.

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INFESTATION INDUCED REACTIONS OF PAPAYA MEALYBUG, *Paracoccus marginatus* WILLIAMS AND GRANARA DE WILLINK, (HEMIPTERA: PSEUDOCOCCIDAE) ON PAPAYA AND AMARANTHUS

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ABSTRACT OF THE THESIS

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ABSTRACT

Papaya mealybug *Paracoccus marginatus* Williams and Granara De Willink (Hemiptera: Pseudococcidae) is an invasive alien species introduced to India in 2008. The first record of the pest in Kerala was in 2009. Papaya mealybug is having a wide host range and has been recorded from 72 host plants in Kerala alone. However, little is understood about *P. marginatus* infestation induced reactions in plants. Studies on the infestation induced reactions in host plants will help to understand the degree of sensitivity of a host to the infestation as well as the pest load that the host plant can bear and thus help to develop threshold limits and evolve appropriate management practices. Recognizing this need, the present work has been undertaken to study the infestation by *P. marginatus* and consequent reactions in two of the most common sensitive host plants *viz*., papaya and amaranthus.

Paracoccus marginatus was mass cultured in the laboratory on potato sprouts under ambient weather conditions. From the laboratory culture, uniform stages of *P. marginatus* were released on three months old papaya seedlings as well as one month old amaranthus seedlings for further studies *viz*., biology, pest load assessment, dispersion rate and estimation of plant biochemical constituents in the infested host plants.

Paracoccus marginatus completed its life cycle within 27.0 ± 2.44 days on papaya seedlings and 29.3 ± 2.75 days on amaranthus seedlings (with a monthly mean temperature of 27.62±4.2°C and relative humidity of 78.3±2.24%). Sex ratio ranged from 3:1 on papaya to 2.5:1 on amaranthus. The fecundity of the mealybug also varied on the two hosts (513.9 ± 107.9 and 508.1 ± 132.5 respectively).

Pest load assessment studies revealed that the infestation by even a single second instar nymph of *P. marginatus* could produce leaf crinkling symptoms on papaya and amaranthus seedlings within 72 h of infestation. However, the maximum leaf crinkling of 25-50 per cent on the above host plants was caused by the third

instar nymphs. In general, the nymphs had a tendency to disperse when crowding occurred.

The dispersion of mealybug showed a progressive reduction from a maximum of 10.30 per cent in the crawlers (first instar nymph) to the minimum of 3.03 per cent in adults on papaya whereas on amaranthus the highest dispersion was 16.36 per cent (first instar nymph) and the lowest was 3.03 per cent (adult).

Estimation of plant constituents of the *P. marginatus* infested plants showed that the leaf protein and Indole -3- Acetic Acid (IAA) concentrations were inversely proportional to the degree of infestation in all life stages of the mealybug indicating increased consumption of amino acids from the phloem of the plants and disruption of translocation of the IAA within the plant system. The highest reduction in the protein and IAA content was shown by the plants infested with the third instars of mealybug because the per cent leaf crinkling was highest in these plants than others.

However, gibberellic acid (GA) content was directly proportional to the mealybug infestation as there was an increase (5 folds in papaya and 2 folds in amaranthus) in GA concentration in infested papaya (5.0 µ/g) and amaranthus (2.0 µ/g) leaf samples as compared to the uninfested leaf (1.0µg GA/g of leaf sample).