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HOST PLANT PREFERENCE BY THE PAPAYA MEALY BUG, *Paracoccus marginatus* (HEMIPTERA : PSEUDOCOCCIDAE) ON SELECTED HOST PLANTS UNDER LABORATORY CONDITION

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ABSTRACT

The papaya mealy bug, *Paracoccus marginatus* has invaded Sri Lanka in 2008 that attacks many host plants, including economically important tropical fruits, vegetables and ornamentals. None of the insecticides has given complete control of the pest and hence, *Acerophagus papayae* an endo-parasite of *P. marginatus* was introduced to Sri Lanka in 2009 from Puerto Rico. The parasitoid has recorded more than 90% control of the pest in the locations where the parasitoid was released. This pest has established as a major pest of papaya in the Island at present. Out-breaks of the pest has been reported time to time in certain locations. Therefore, need has aroused for mass rearing and release of the parasitoid to the infested areas during pest out-breaks. Present studies aimed at selecting a more economical and effective host plant than potato for mass rearing of *P. marginatus* under laboratory conditions. Development time, survival percentage, preference of the different developmental stages of the pest on four different host plants, *Hibiscus rosasinensis, Jatropa curcas, Codiaeum* sp. and *Ipomoea batatas* were studied. Lowest developmental time of different nymphal stages, was on *Jatropha curcas* and *Hibiscus rosa-sinensis* plants. Highest survival percentage and highest preference observed from the nymphs reared on Jatropha and Hibiscus plants.

Key words: ***Host plant preference, Mass rearing, Paracoccus marginatus parasitoids***

INTRODUCTION

The papaya mealy bug (PMB), *Paracoccus marginatus* is a polyphagous insect (Hemiptera: Pseudococcidae) (Borror *et al*., 1992). It is a major pest of papaya while many other economically important tropical fruits, vegetables and ornamentals are attacked by the pest. Papaya mealy bug is considered as an invasive pest and has invaded into many countries around the world. The PMB was first reported in Mexico in 1955 and believed to be native to Mexico and/or Central America and has never attained status of the serious pest due to the presence of an endemic natural enemy complex in the region (Williams and Willink, 1992). As shown in Figure 1, the female PMB life cycle comprises of 3 stages (egg, nymph and adult) while male life cycle has 5 stages (egg, nymph, pre-pupa, pupa and adult) (Tanwar *et al*., 2010).

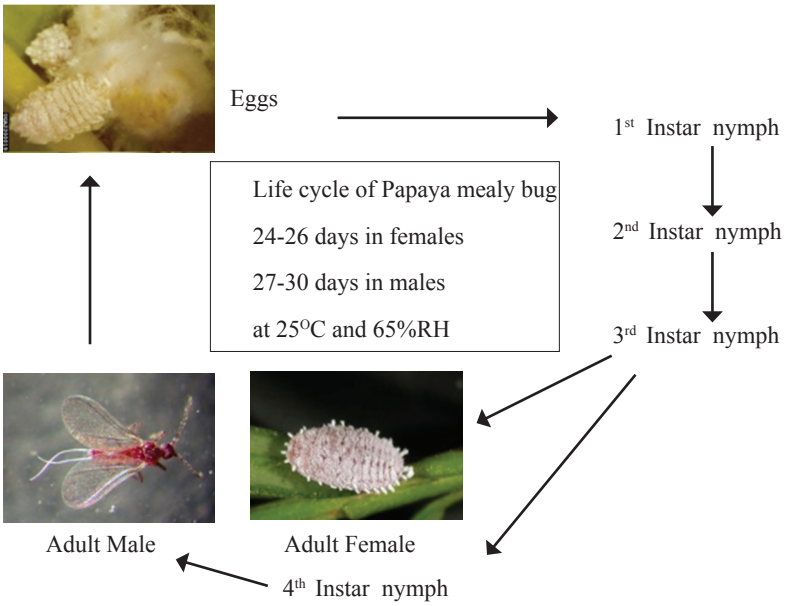

Figure 1. Life cycle of papaya mealy bug

In Sri Lanka, occurrence of the PMB was first recorded during 2008 from Colombo and Gampaha districts and observed spreading rapidly with large colonies on aerial parts of papaya and *Plumeria* (Galanihe *et al*.,2010). The pest had spread further to the other districts in the Western, Sabaragamuwa, Southern, North Central, Central provinces and to the Eastern province, indicating its potential to spread very rapidly.

Since a newly emerged pest and the absence of natural enemies, the PBM has multiplied rapidly and developed to epidemic levels mainly in papaya growing regions. Although a number of insecticides were evaluated none of them were effective (Galanihe *et al*., 2010). Thick waxy, cottony sacs of the pest prevent effective management using pesticides and thus, chemical control was partially effective and required frequent multiple applications. Furthermore, this led to problems with insecticide resistance development and destroying the native natural enemy complex in the ecosystem.

Therefore, classical biological control was identified as an important component in the management of *P. marginatus* in the country. There are quite a few local natural enemies that feeding on the PMB such as several coccinellids, lacewings, wasps, spiders and parasitoids are available in the natural ecosystem. However, these natural enemies were not efficient in controlling the pest at the epidemic condition. Majority of the countries affected with the PMB invasion have successfully controlled the PMB outbreaks through the inundated release of the highly efficient and prominent exotic parasitoid, *Acerophagus papayae*. Therefore, Department of Agriculture, Sri Lanka has introduced the highly successful mealy bug parasitoid, *A.papayae*, in 2008 from Puerto Rico (Galanihe *et al.*, 2010). More than 90% success was achieved from the introduction of the parasitoids to the selected locations. Within five years of the introduction of the parasitoid, *A. papayae,* several out breaks of the PMB from certain locations were reported time to time. Therefore, need has aroused for mass rearing of the mealy bugs for mass production of parasitoids under laboratory conditions. The standard technique for mass rearing of the pest is done using sprouted potatoes, which involves high cost for the mass production program of the parasitoids due to higher market price of the potato in Sri Lanka. Hence, present study was initiated with the objective to find out a suitable alternative host plant for the mass rearing of PMB at a cheaper cost under laboratory conditions.

Materials and methods

The experiments were conducted in the laboratory and in the plant house of the Division of Entomology at Horticultural Crops Research and Development Institute, Gannoruwa, Peradeniya for 4 months from September to December, 2014.

Maintenance of Host plants

Stem cuttings of four host plant species: Hibiscus (*Hibiscus rosa-sinensis*;) Jatropha (*Jatropa curcas*;) Croton (*Codiaeum variegatum*) and Sweet Potato (*Ipomoea batatas*) were planted in 15 cm diameter polythene pots and maintained under plant house conditions until rooting and new shoot development. Four to six months old plants were used for the experiments.

Maintenance of sprouted Potato cultures

Insecticide free potatoes cleaned with water and 1% sodium hypochlorite solution were kept for drying and these potatoes were placed on plastic trays (60 x 60 x 60 cm) containing fine sand and kept in dark for sprouting (Plate 1). Water was sprayed daily on tubers to maintain the moisture content on media and humidity of the chamber.

Plate 1. Sprouted potatoes on racks

Collection and maintenance of *Paracoccus marginatus* cultures

Affected leaf, shoot and fruit samples of papaya were collected from various locations (Manikhinna, Gannoruwa, Kalpitiya, Kurunegala, Suriyawewa) and kept in insect proof cages for emergence of nymphs (Crawler stage one) of the PMB. Emerged crawlers and egg masses were introduced to the trays containing sprouted potatoes.

Mean developmental time and percentage survival of different nymphal stages on four different host plants

Well-developed leaf with plant intact of each host plant was individually placed inside 13 cm diameter plastic petri dishes and ten eggs of PMB were introduced on leaf terminal of each leaf using a hairbrush. Position of the eggs on each leaf was mapped on to paper. Two halves of the petri dishes were sealed with gum tape and a small cotton piece was attached to the petri dish to prevent damage to the leaf stalk and allow ventilation. End of the petiole was covered with wetted cotton plug and moistened daily.

The treatments were replicated 10 times (40 plants x 10 eggs = 400 eggs from one egg mass). Observations were made through microscope and hand lens at 24 hour intervals for the development of life stages on each host plant.

Host plant preference study through choice tests

This experiment was done in two different ways. Plants raised in the greenhouse were used in the first experiment while single leaves of each host plant were used for the second experiment.

1. Choice test using plants

Each replicate consisted of a set of four different host plants that were tied together to allow the mealy bugs to move freely and there were 8 replicates in the experiment. The plants were then arranged in Randomized Complete Block Design. 25 mealy bugs of each nymphal and adult stage (total of 100) were introduced to each host plant. Numbers of mealy bugs of each developmental stage on each host plant were counted at 24, 48 and 72 hours after introduction.

2. Choice test using leaves

Leaves of the four host plant species were kept in Petri dishes were arranged in a Completely Randomized Design. Similarly as in the experiment 1, each leaf was introduced with 25 mealy bugs of each nymphal and adult stage (total of 100) and obtained same data on each host leaf.

Statistical Analysis

One-way analysis of variance (ANOVA) was performed using a general linear model (GLM) for all experiments (SAS software). Means were compared at the P=0.05 significance level using the Turkeys Honestly significant Difference (HSD) test.

RESULTS AND DISCUSSION

Developmental time on different host plant species

Mean development time of the nymphs of all the developmental stages and total development time of *P. marginatus* was significantly lower when reared on Jatropha plants and on Hibiscus plants than that of other two treatments at α=0.05. (Table 1).

Table 1. Mean development time of different nymphal stages of papaya mealy bug on four different host plant species under laboratory conditions

Host plant Species	Mean Development time (Days)			
	First instar nymph	Second instar nymph	Third instar nymph	Total Development time
Hibiscus	4.69 [b]	7.51 [b]	4.07 [b]	16.27 [b]
Jatropha	4.11 [b]	6.90 [b]	3.89 [b]	14.74 [b]
Croton	5.66 [a]	8.02 [b]	5.33 [a]	19.01 [a]
Sweet potato	5.62 [a]	9.58 [a]	5.55 [a]	20.75 [a]
SE	± 0.15	± 0.26	± 0.18	± 0.44

Means within a column followed by the same letters are not significantly different at α=0.05(Tukey's HSD test)

Amarasekare *et al*. (2008) reported differences in the developmental times of *P. marginatus* reared on four host species, *Hibiscus rosa-sinensis* (hibiscus), *Plumeria rubra* L. (plumeria), *Acalypha amentacea* Roxb. (acalypha), and one weed species, *Parthenium hysterophorus* L. (parthenium). They further explained that developments of the different stages are much faster on *A. amentacea* and *P. hysterophorus* than on *H. rosa-sinensis* and *P. rubra*. They also conclude that lower survival rate of first and second instars on *P. rubra* and *survival rate* of third-instar and the fourth-instar is not affected by the host species.

Survival of life stages on different host plant species

Mean percentage survival of the each nymphal stage on four different host plants revealed that higher survival rate was on Hibiscus and Jatropha. However, Hibiscus gave the highest survival rate of 79% from first nymphal stage, 73% from second nymphal stage and 61% from third nymphal stage. Furthermore, a clear difference was observed among all the nymphal stages for the survival rates. The number survived reduced gradually from first to third nymphal stage (Figure 1).

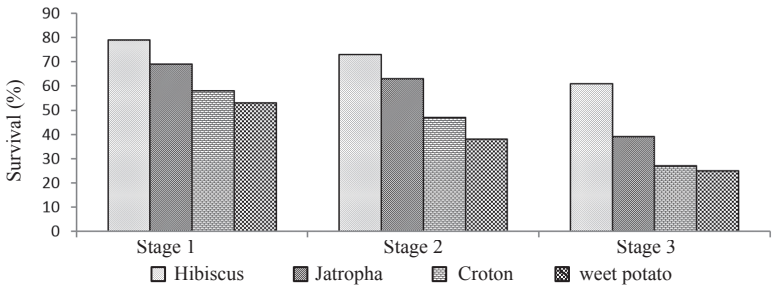

Figure 1. Mean percentage survival for each developmental stage of the papaya mealy bug reared on four different host plants under laboratory conditions

Choice test for host preference using leaves

First stage nymphs displayed varied preferences for the leaves of four host plants tested at different time intervals. All four stages showed the highest preference for Jatropha leaves at all three time intervals after introduced. A significantly higher preference was observed to Hibiscus than to the Croton and Sweet potato (Table 2).

First instar nymphs showed varied responses in their choice among the four host leaves until 72 hours after introduction to the leaves. This must be because the 1st instar has higher mobility than other developmental stages and that is the stage which looking for best suitable host for their establishment and nourishment during that period. By 24 hours after introduction, 2nd and 3rd instar nymphs showed the highest choice towards Jatropha while by 72 hours both the 2nd and 3rd instars showed highest preference for Jatropha and second highest preference for Hibiscus. Both Croton and Sweet potato showed lowest preference compared to other two host plants. This is because 1st nymphal stage of papaya mealy bug is more active and searching for more preferable host plant and place for their development while second and third stages are mostly less mobile and once they have found a suitable host they may probably get settled on it in less time than the 1st instar nymphs. Highest mean number of the 4th instar nymphs have selected and settled on Jatropha leaves by 48 hours after introduction, while second highest mean number have settled on Hibiscus leaves.The lowest mean number of nymphs except 1st nymphal stage was found on Croton and Sweet potato while highest mean number was found on Jatropha. These results clearly showed that except 1st nymphal stage all other stages have significantly higher preference for Jatropha and Hibiscus than for Croton and sweet potato (Table 2).

Table 2. Host preference of the development stages of papaya mealy bug for leaves of four different host plants species observed at 24, 48 and 72 hours after released

Host plant species	Mean number of Developmental Stages of papaya mealy bug on leaves											
	At 24 hours				At 48 hours				At 72 hours			
	First	Second	Third	Fourth	First	Second	Third	Fourth	First	Second	Third	Fourth
Hibiscus	1.70 ^{a} (2.63)	1.71 ^{ab} (2.67)	1.29 ^{b} (1.37)	1.49 ^{a} (2.00)	1.63 ^{a} (2.50)	1.65 ^{ab} (2.43)	1.76 ^{a} (2.90)	1.74 ^{b} (2.80)	1.68 ^{a} (2.73)	1.69 ^{b} (2.67)	1.58 ^{b} (2.27)	1.73 ^{a} (2.80)
Jatropha	1.76 ^{a} (2.90)	1.80 ^{a} (3.07)	1.90 ^{a} (3.43)	1.68 ^{a} (2.47)	1.73 ^{a} (2.93)	1.93 ^{a} (3.60)	1.96 ^{a} (3.70)	2.11 ^{a} (4.33)	1.66 ^{ab} (2.73)	2.10 ^{a} (4.33)	2.14 ^{a} (4.37)	2.11 ^{a} (4.57)
Croton	1.31 ^{b} (1.43)	1.27 ^{c} (1.37)	1.15 ^{b} (1.00)	1.68 ^{b} (1.03)	1.37 ^{a} (1.73)	1.34 ^{b} (1.60)	1.32 ^{b} (1.57)	1.20 ^{c} (1.20)	1.25 ^{b} (1.33)	1.21 ^{c} (1.20)	1.32 ^{b} (1.53)	1.15 ^{b} (1.00)
Sweet potato	1.32 ^{b} (1.43)	1.37 ^{bc} (1.60)	1.29 ^{b} (1.33)	1.68 ^{a} (1.90)	1.42 ^{a} (1.93)	1.35 ^{b} (1.63)	1.36 ^{b} (1.57)	1.32 ^{c} (1.43)	1.41 ^{ab} (1.80)	1.23 ^{c} (1.20)	1.30 ^{b} (1.37)	1.31 ^{b} (1.43)
SE	±0.20	±0.21	±0.20	±0.19	±0.26	±0.22	±0.23	±0.22	±0.25	±0.22	±0.21	±0.24

Means within a column followed by the same letters are not significantly different at α =0.05 Tukey's HSD test Figures in parentheses are transformed values √ (x+0.5).

At 24 hours after introduction to different host plant species, 1st and 2nd stage nymphs of PMB has not shown any significant preference for the different host plants, but 3rd and 4th stage nymphs have shown significantly more preference levels for Jatropha and Hibiscus (Table 3).

Same results were observed for 1st nymphal stage at 48 hours after introduction also. Higher preference was shown by 2nd, 3rd and 4th stage nymphs for Jatropha, hibiscus and sweet potato plants over Croton while the highest numbers of nymphs were found on Jatropha plants.

Results observed at 72 hours after release were not consistent with 24 hours and 48 hours results. According to these results There were no significant choice for different host plants by 1st, 2nd and 3rd nymph. This may be due to time spending in the plant itself and hence they may pass through one stage to another. But 4th stage showed significantly higher preference to Jatropha and Hibiscus. This difference in choice may be due to low mobility after settling for feeding of 4th nymphal stage of papaya mealy bug.

In this study, movement of crawlers (first instars) away from the leaf tissues and they falling off the plants and misplacing leaves were commonly observed on all plant species. Crawlers shown more preference to light and may have caused them to move toward light and dislodge from the leaves or the shoots. Finally, the low percentage survival of different instars was reflected in the low number to survival of *P. marginatus*.

Determining the life history of a polyphagous insect is vital factor to recognize its development, distribution and abundance. Their life cycle can vary with the plant species that is used for nutrient requirement. Different plant species make available different nutrients and chemical composition for their reproduction, development and survival. The differences observed in this study on life history of *P. marginatus* may be due to nutritive factors, allelo-chemical compounds, and physical differences in leaf structures and plant tissues.

In the Republic of Palau, *P. marginatus* has caused serious damage to plumeria (Muniappan *et al*., 2006) and found to be the most common homopteran found on *Plumeria alba* in Puerto Rico (Sloan *et al*., 2007) showing its ability to develop well on this plant species. A loss of 17 - 18% of the first instars was also observed on *H. rosa-sinensis, A. amentacea*, and *P. hysterophorus*.

Choice test for host preference using plants

Table 3. Mean number of papaya mealy bugs of different development stages reared on plants of four different host plant species observed at 24, 48 and 72 hours after released

Host plant species	Development Stages											
	At 24 hours				At 48 hours				At 72 hours			
	First	Second	Third	Fourth	First	Second	Third	Fourth	First	Second	Third	Fourth
Hibiscus	1.98 ^{a} (4.00)	2.36 ^{a} (6.25)	2.66 ^{ab} (7.00)	2.70 ^{a} (7.00)	1.77 ^{a} (3.38)	2.31 ^{ab} (5.38)	2.58 ^{ab} (6.75)	2.50 ^{ab} (6.38)	1.93 ^{a} (3.75)	2.25 ^{a} (5.25)	2.60 ^{a} (6.75)	2.28 ^{ab} (5.50)
Jatropha	2.48 ^{a} (6.25)	2.87 ^{a} (8.63)	2.93 ^{a} (8.38)	2.81 ^{a} (7.63)	2.30 ^{a} (5.38)	2.74 ^{a} (7.50)	2.94 ^{a} (8.50)	3.08 ^{a} (9.63)	2.12 ^{a} (4.25)	2.32 ^{a} (5.88)	2.42 ^{a} (6.38)	3.24 ^{a} (10.63)
Croton	1.85 ^{a} (3.25)	1.68 ^{a} (2.75)	1.83 ^{b} (3.25)	1.70 ^{b} (3.00)	1.80 ^{a} (3.00)	1.65 ^{b} (2.63)	1.92 ^{b} (3.88)	1.70 ^{b} (3.25)	1.93 ^{a} (3.88)	1.78 ^{a} (2.88)	1.88 ^{a} (3.88)	1.69 ^{b} (3.00)
Sweet potato	1.91 ^{a} (3.63)	1.77 ^{a} (2.88)	1.92 ^{b} (3.50)	2.27 ^{ab} (4.88)	1.91 ^{a} (3.63)	1.73 ^{ab} (3.13)	2.49 ^{ab} (5.88)	2.10 ^{ab} (4.63)	2.09 ^{a} (4.50)	1.65 ^{a} (2.50)	2.28 ^{a} (5.25)	1.68 ^{b} (2.88)
SE	±0.27	±0.31	±0.23	±0.22	±0.28	±0.27	±0.26	±0.32	±0.27	±0.28	±0.32	±0.31

Means within a column followed by the same letters are not significantly different at α =0.05 Tukey's HSD test Figures in parentheses are transformed values √ (x+0.5)

In addition, considering other mealy bug species, Serrano and Lapointe (2002) recorded suitability of mass rearing of pink hibiscus mealy bug, *Maconellicoccus hirsutus* (Green), was able to develop equally well on *Cucurbita pepo* L. There was no difference in survival, development, and fecundity of cohorts of the mealy bug, *Phenacoccus parvus* Morrison when reared on *Lantana camara* L. *Lycopersicone sculentum* Miller, and *Solanum melongena* L. (Marohasy 1997). However, *Gossypium hirsutum* L., *Ageratum houstonianum* Miller and *Clerodendrum cunninghamii* Benth were identified as less suitable host plants for the development of *P. parvus* compared to *L. camara* (Marohasy 1997).

The ability of *P. marginatus* to develop on these plant species reveals the possibility of movement, distribution, and establishment of *P. marginatus* in different host plant species. Therefore, it demonstrates that this information will support to select new host plant for mass rearing programme of *P. marginatus*. However, further studies may be required to confirm the selection of suitable host plant by the investigating series of another set of precise experiments with many details on time taken to complete the life cycle and the suitable maturity stage of the plant.

CONCLUSION

The life history of *P. marginatus* is affected by host plant and it has the ability to develop and survive on variety of host plant species. This study revealed Jatropha and Hibiscus plants can be used in the maintenance of mealy bug cultures under laboratory conditions as alternative to sprouted potatoes. Similarly, confirmations on low development time of its life cycle and its higher ability to survive on these two host plants supports further developments of the mass rearing programme of the *P. marginatus*. However, additional studies may be required to carry out in future for the fine tuning of the mass rearing programme using these alternate host plants under laboratory conditions.

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