



Infestation induced biochemical reactions on papaya by mealybug, *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae)

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ABSTRACT: *Paracoccus marginatus* Williams and Granara de Willink, (Hemiptera: Pseudococcidae) is an invasive alien sucking pest that attacks several genera of economically important tropical and subtropical plants. Biochemical study revealed that total protein content was very low (1.88mg/g) in plants inoculated with third instar nymphs of papaya mealybug, whereas the plants inoculated with adult mealybugs recorded a higher protein concentration of 4.60 mg/g. Similarly, plants inoculated with third instar nymph of papaya mealybug had a decreased mean concentration (0.019 mg/g) of Indole Acetic Acid (IAA) whereas plant inoculated with adult mealybugs recorded the highest mean IAA concentration was 0.070 mg/g. The papaya seedlings uninfested with papaya mealybug recorded the highest protein (5.34 mg/g) and IAA (0.185 mg/g) content. However, the gibberellic acid content of 5µg/g was estimated from the leaves of plants showing crinkling symptom infested with third instar nymphs and the uninfested papaya seedlings recorded lower levels of gibberellic acid content of 1 µg/g. ©2014 Association for Advancement of Entomology

KEYWORDS: Papaya mealybug, *Paracoccus marginatus*, total protein, Indole-3-Acetic Acid, Gibberellic Acid.

INTRODUCTION

Papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink, native to Mexico and Central America (Miller *et al.*, 1999), is an invasive insect pest has been first reported in India from Coimbatore (Tamil Nadu, India) on papaya, jatropha and certain other plants in 2008 (Muniappan *et al.*, 2008, Regupathy and Ayyasamy, 2011). Later it was recorded in Kerala (Krishnakumar and Rajan, 2009; Lyla and Philip, 2010), Karnataka, Andhra Pradesh, Maharashtra, Tripura and Odisha. Approximately 95 host plant species belonging to 39 families

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were recorded to be infested by *P. marginatus* in Kerala (Manichellappan *et al.*, 2013).

Immature and adult stages of *P. marginatus* suck the sap by inserting their stylets into the epidermis of the leaf resulting in curling, crinkling, rosetting, twisting and leaf distortion. The honey dew excreted by the mealybugs and the associated black sooty mould formation impairs photosynthetic efficiency of the infested 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, subsequently.

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.

MATERIALS AND METHODS

Mass culturing of *P. marginatus* was done on sprouted potatoes as standardised by Gautam (2008). Three months old healthy papaya seedlings with an average height of 15cm with 4-5 leaves were selected and ensured the seedlings free from pests, diseases and nutrient deficiency. In case of protein and IAA content estimation, total of 33 seedlings were replicated thrice at the rate of 11 seedlings per replication. Single 1st instar nymph (crawler) of papaya mealybug was transferred to group I, two crawlers to group II, three crawlers to group III and so on upto ten crawler to group X. Similarly 2nd, 3rd instar nymphs and adult mealy bug were also transferred to papaya seedlings. Uninfested papaya seedlings were maintained as untreated control. In case of GA estimation, papaya seedlings infested with five 3rd instar nymph of papaya mealy bug and uninfested plants were analysed without any change in the number/ stage of insect, owing to the large sample size required for the analysis.

The healthy and infested leaves from the growing point were collected individually and subjected to the analyses of total protein and IAA.

Total protein

Leaf samples (500mg) were taken from the growing point of plant and were grounded well in 10ml cold distilled water using mortar and pestle. The ground sample was spun at 5000 rpm for 10 minutes in a refrigerated centrifuge (REMI[®], CFC free, C-24). The supernatant was collected and 0.1ml aliquot was taken for analysis. Total protein content in healthy and infested leaves of papaya was estimated as per Lowry *et al.* (1951).

Bovine serum albumin (BSA) was used as standard. Folin – phenol reagent (ready mix; 0.5 ml) was added to the sample tubes and incubated at room temperature for 30 minutes. After

incubation, absorbance was measured at 660nm by UV spectrophotometer (ELICO®). 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.

Indole Acetic Acid (IAA)

IAA present in healthy and infested leaves of papaya were estimated using spectrophotometric method standardised to suit the present investigation.

Indole acetic acid (10 mg) weighed and dissolved in 100 ml of 0.1M Na₂CO₃ 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.

Leaf sample (500 mg) 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 each of phosphate buffer (68 ml of 0.2 M monobasic-NaH₂PO₄ + 32 ml of 0.2M dibasic-Na₂HPO₄ and made up to 200 ml with cold distilled water) and distilled water was added. Distilled water alone (2 ml) served as blank and to which 1ml of phosphate buffer was added. To stop the reaction, the first set of tubes (control) was 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 at 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.

Gibberellic acid (GA)

The method of extraction and purification of endogenous level of gibberellic acid (GA) in plant samples was extensively modified from those described by House (1961). 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. As the procedure involved large quantity of the growing tissues, infested plant parts that exhibit greater extent of damage due to third instar nymph were used.

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 are pooled and concentrated to a water residue in vacuum (30-40°C) by rotary evaporator (Superfit®). The volume was adjusted to 10 ml with 0.2M PO₄ 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 NaHCO₃. 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.

Sample (1.5 ml) containing GA extracted as per the above 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.

Analysis of data

Protein, IAA and GA concentrations in plants under each experiment were tabulated and analysed statistically by one sample t-test using the statistical package, SPSS Version 16.

RESULTS AND DISCUSSION

Mealy bug infestation caused a marked variation in the total protein, indole acetic acid (IAA) and giberellic acid (GA) content of the host plants.

Total protein

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 3.16 mg/g with the release of ten crawlers per plant. Whereas, a total protein concentration of 4.57 mg/g was estimated from the plants inoculated with one second instar nymph; whereas plants with ten second instar nymphs recorded a protein concentration of 2.73. mg/g. Papaya plants inoculated with third instars showed a decrease in total protein content compared to those 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 1.88 mg/g in plants inoculated with ten third instar nymphs. When the plants were inoculated with adult mealybugs there was no noticeable variation was observed in total protein concentration. As the number of mealybugs per plant was increased from one to ten, the total protein concentration decreased from 4.96 to 4.17 mg/g. 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 4.66 mg/g (Table 1).

There was a consistent inverse relationship between the level of damage by the insect and protein content in host plants. 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, Eid *et al.* (2011) studied the impact of

Table 1. Effect of infestation of *P. marginatus* on the total protein content of papaya seedling

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

pink mealy bug, *Saccharococcus sacchari* infestation on chemicals and allelo chemicals of some sugar cane cultivars, among them the crude protein content in the infested cane was significantly lower than the un infested cane cultivars. Pitan *et al.* (2011) found that protein, fat, carbohydrate, ash, crude fibre and moisture contents were depleted with increase in mealybug, *Rastrococcus invadens* William 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).

Indole -3 Acetic Acid

With the infestation of one first instar crawler, the IAA content was 0.131 mg/g. It was reduced to 0.022 mg/g when the pest load increased to ten crawlers of first instar on the plants. 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 0.023 mg/g with increase in pest load to ten second instar nymphs per plant. 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 for one and ten crawlers, respectively. When plants were inoculated with one adult mealybug, the IAA content was 0.127 mg/g and it was reduced to 0.070 mg/g with increase in population to ten adults per plant (Table 2).

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. 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 load.

Infestation by insects brings about a change in level of endogenous growth regulators that result in abnormal development and growth forms. These include epinasty (leaf and stem distortion), hypertrophy (cell enlargement), hyperplastic (cell proliferation), internodal shortening, proliferation of adventitious buds, unusual rooting, flowering or fruiting pattern, abnormal organ development and irregular bud accession (Allen, 1947; Carter, 1973).

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 its 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 meristematic region and thereby caused a decrease in IAA content in infested plants.

Table 2. Effect of infestation of *P. marginatus* on the IAA content of papaya seedling

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

The IAA content in the papaya mealy bug infested leaves was less as compared to uninfested leaves. The IAA content showed a decreasing trend from first group to tenth group in all the stages of infestation. The per cent decrease of IAA which is very high in papaya plants infested with third instars of papaya mealybug. Similarly, aphids substantially reduced the plant growth by their feeding. Honeydew from aphids contains a wide variety of chemicals ingested from the host, including auxins, gibberellins, and cytokinins (Phillips and Cleland 1972; Hussain *et al.* 1973). Marked changes in the normal hormone balance occur within 10 days of infestation by aphids, with growth inhibitors increasing and growth promoters decreasing (Hussain *et al.* 1973). These authors suggested that the large drain on the plant food and hormone resources might be sufficient to account for the growth reduction observed. Saikia *et al.* (2011) found that red spider mite infested plum tree had lower level of auxin than the non-infested one. Bari and Jones (2009) found that blocking of auxin responses had been shown to increase resistance in plants. The tea mosquito bug, *Helopeltis* sp. attack on the axillary vegetative buds and young leaves of tea resulted in decrease in auxin content.

Gibberellic Acid

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 GA content of 1.0 µg/g was recorded from uninfested leaves of papaya (Table 3).

Table 3. Effect of *P. marginatus* on the gibberellic acid content in papaya seedling

Stage of mealybugs released	Gibberellic acid content in leaves (µg/g)	
	Infested	Uninfested
III instar	5.0	1.0

Mean of 3 replications

The third instar mealybug infested papaya plants showed a fivefold increase in the GA content. 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 GA₃ 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 gibberellic acids (GAs), a group of diterpenoid compounds with phytohormone activity, affect various stages of plant development, including seed germination, stem elongation, root growth, flowering and pollen tube elongation (Davies, 2004; Swain and Singh, 2005). 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.

In the present study it was inferred that third instar papaya mealy bug infestation on papaya significantly reduced the total protein and indole acetic acid content in the plants whereas gibberellic acid content increased significantly. It would help us to know the hypersensitivity of plant species to the infestation of papaya mealy bug, the pest load that a host plant could bear and to evolve appropriate management practices to reduce such reactions.

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