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Research Article

Morphometry of the Citrus Mealybug *Planococcus citri* (Risso) and its Feeding Impact on the Proximate Composition of Citrus Species Leaves in Makurdi Southern Guinea Savanna, Nigeria

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ABSTRACT

The study assessed the feeding impact of citrus mealybug *Planococcus citri* (Risso) and its morphometry on (*Citrus* species L.) at the Teaching and Research Farm, Joseph Sarwuan Tarka University, Makurdi. A 2x5 factorial experiment comprising five species of two-year-old citrus seedlings (*C. sinensis*, *C. nobilis*, *C. reticulata*, *C. limon,* and *C. aurantium*) each replicated three times were planted on the field under screen cages in a Randomized Complete Block Design (RCBD). Fifteen stands were infested with 20 young adult female mealybugs each while 15 were un-infested to serve as the control. Data collected was analysed using GenStat Release 2007 (7.2) and means were separated by Duncan's Multiple Range Test (DMRT) at P = 0.05. Results showed that the percentage of dry matter content was highest on the control compared with the infested plants. The proximate content of the host plant leaves indicated that all the control plants had more sugar than infested plants. Crude fibre and Ash content of infested host plants were lower than that of un-infested. The impact of mealybug feeding was most pronounced on *C. sinensis* and *C. reticulata.* Morphometrically, males of *P. citri* found on *C. nobilis* were in body length, width, and weight. Adult females, however, showed no significant variation in length and width across all citrus species. *C. aurantium* produced the heaviest females while *C. reticulata* had females with the lowest weight. In conclusion, *C. nobilis* is suitable for commercial production of seedlings. Routine inspection for early detection of mealybug infestation is recommended.

Keywords: Citrus, Morphometry, Proximate composition, *Planococcus citri*, Southern Guinea

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INTRODUCTION

The citrus mealybug *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae) is morphologically highly variable under different environmental conditions and on different host plants (Kerns *et al*., 2001). The adult female mealybug which is wingless is about 3 mm long with a white, brownish, or pink body covered in white wax (Kerns *et al*., 2001; Gill *et al*., 2012). The edges of the body are lined with waxy filaments. It has a light grey longitudinal line down on its back. Its legs and antennae are brown. The adult male has narrower and slightly longer body which has long filaments on the posterior end of the body and a pair of functional wings. The citrus mealybug is a polyphagous species known throughout the world. It attacks a wide range of crop plants, ornamentals and wild flora (Gill *et al*., 2012). The citrus mealybug can live and feed on almost any flowering plant (Von Ellenrieder, 2003; ARS-USDA, 2007). It feeds on newly expanded shoots, leaves, flower buds and twigs of its host plants. Colonies of citrus mealybug are gregarious and tend to congregate on the underside of young leaves or around the porous glands of the fruit (Kerns *et al*., 2001). Mealybugs feed on phloem vessels of plants with their piercing/sucking mouths known as stylets (Pitino *et al*., 2014). This penetrative feeding habit harm the plant by draining its sap which creates a lack of vigour in the plant. *P. citri* has also been reported to transmit disease causing organisms like plant viruses to its hosts (Kubiriba *et al*., 2001; Watson and Kubiriba, 2005; Goldasteh *et al*., 2009). During feeding, *P. citri* secretes honey dew on the leaves which attract sooty mould which blackens the leaves, delay fruit setting and can reduce the ability of the leaves to photosynthesize (Gill *et al.,* 2012). Severe mealybug infestations can result in 80% defoliation and 100% fruit loss (Gill *et al*., 2012). However, little or no assessments appeared to have been made on the direct effects of its feeding on citrus plant within this zone hence this research was carried out to investigate the impact of mealybug feeding on the proximate composition of citrus leaves and its morphometry on different species of citrus.

MATERIALS AND METHODS

Location: The study was carried out at the Teaching and Research Farm of the Joseph Sarwuan Tarka University Makurdi (Latitude 7°45'N and Longitude 8°37'E with an altitude of 97 m above sea level) Southern Guinea Savanna. The research lasted for 12 months (January-December, 2015).

Treatments and Sources

Two-year-old potted seedlings of five different species of citrus were used for this study. Sweet orange (*Citrus sinensis* L.), Sour orange (*C. aurantium* L.), Tangerine (*C. reticulata* Blanco), King's orange (*C. nobilis* Lour) and Lemon (*C. limon* L.)]. The seedlings were obtained from the nursery farm of Akperan Orshi College of Agriculture, Yandev in Gboko Local Government Area, Benue State.

Establishment of *P. citri* Culture

Citrus mealybugs were collected from infested citrus trees from the Research farm of the University and released on citrus seedlings raised in a net enclave under shade. The mealybugs were allowed to reproduce for a period of two months to serve as culture. Progenies from the culture were used for subsequent experiments.

Treatments and Experimental Design

A 2x5 factorial experiment consisting of five infested and un-infested species of citrus with three replications were arranged in a randomized complete block design (RCBD). This was set up in the field to investigate the feeding impact of *P. citri* on the host plants as well as the influence of the host plants on the morphometry of *P. citri*. The field, measuring 14m x 12m, was cleared of existing vegetation, tilled and demarcated into 30 plots each 2m long by 2m wide. Planting was done during the cool evening hours. Wooden screen cages of size 50x62x106 cm (LxWxH) were put in place over the seedlings to protect them.

Parameters investigated

Experiment 1a: Comparison of biomass weight of the leaves of five citrus species infested and not infested with citrus mealybug

After 12 months of being established in the field, 20 leaves were randomly picked from each of the infested and un-infested plots of the citrus species to determine and compare the effect of mealybug infestation on dry matter.

Wet leaf biomass weight

A random sample of 20 leaves was taken from each of the infested and un-infested plots, bagged and immediately brought to the laboratory. An electronic weighing balance (Model CB 125) was used. An empty bag was placed on the weighing plate and manipulated to have a zero scale. The paper bags containing samples were placed on the weighing plate and the scale readings were recorded for every treatment.

Dry leaf biomass weight

After taking the fresh weight of the leaves, the samples were dried at 60°C in a Gallenkamp Hot box oven (Model: Size 1) for 48 hours and transferred to new paper bags into which a small amount of silica gel was placed to absorb traces of moisture. The percentage of dry matter in the samples was determined by dividing the oven dry weight by the fresh weight of samples multiplied by 100.

Experiment 1b: Comparison of chlorophyll content and proximate composition of the leaves of five citrus species infested and uninfested with citrus mealybugs

Leaf chlorophyll content: After 12 months of infestation, random sample of 5 leaves were taken from each of the infested and un-infested plots for chlorophyll content analysis. Mean values of six readings were taken from each leaf using a digital chlorophyll meter (Model SPAD 502 Plus).

Proximate analysis: Another batch of 20 leaves from each citrus stand was obtained and air dried for 14 days in the laboratory after which it was subjected to proximate analysis to determine sugar, crude protein, ash and crude fibre content following AOAC (1995) procedure.

Experiment 2:

a. **Morphometry**: For the morphological measurements, live specimens (nymphs, adults) were collected on designated leaves from all the infested plants and dropped in a killing jar containing 80% ethanol. Each of the specimens was carefully observed under a calibrated *Peak Scale Lube* magnifying field glass of x7 magnification. The body length (in millimeters) was measured along each insect's dorsal midline from the vertex of the head to the tip of the abdomen. The width (in millimeters) was measured at the widest point across the middle portion of the insect. The weight of the insects specimens (30 bulk) was measured (in milligrams) using a precision electronic weighing balance (Model CB 125) before manipulation to get individual weight.

b. **Leaf chlorosis:**

All leaves of plants infested with mealybugs were counted and the proportion that was chlorotic was determined for each citrus species as follows:

$$\text{\% of leaves with chlorosids} = \frac{\text{No. of chlorotic leaves}}{\text{Total no. of leaves}} \text{ x } 100$$

Data Analysis

The mean values of the above parameters from infested plants and un-infested control as well as morphometrical parameters of the citrus mealybug were determined along with their standard errors for the host plant species. Data collected was subjected to analysis of variance (ANOVA) using GenStat Release 2007 (7.2) and the means were separated by Duncan's New Multiple Range Test (DNMRT) at 5% level of probability.

RESULTS

Effect of *P. citri* Infestation on the Fresh and Dry Weight, Percentage Moisture and Dry Matter Contents of Leaves of Citrus Species

In Table 1, main effect of infestation on the fresh and dry weight, percentage moisture and dry matter content showed that leaves of infested host plants recorded significantly (P<0.05) higher fresh weight compared to the un-infested control. No significant differences were obtained for dry weight between the infested and un-infested plants. Percentage moisture content of leaves from infested plants was significantly higher (P<0.05) as compared to the control. On the other hand, the infested host leaves contained significantly lower dry matter content than the un-infested leaves.

Chlorophyll Content and Proximate Composition of the Leaves of Five Species of Citrus Infested with *P. citri*

In Table 2, main effect of *P. citri* infestation on the host plants showed that infested plants were not significantly different (P>0.05) from the untreated plants in terms of chlorophyll content. On the other hand, all infested citrus species contained significantly (P<0.05) lower amounts of sugar, crude fibre and ash contents than the un-infested plants. Crude protein content of treated plants however deviated as it was significantly higher than that of the insect-free plants. Among the citrus species, the results showed that chlorophyll content which was highest in *C. sinensis* (76.20) followed by *C. nobilis* (73.05) and *C. aurantium* (71.90) leaves differed significantly (P<0.05) from *C. limon* and *C. reticulata*. The proximate content of the leaves of different citrus species showed that sugar content of the leaves differed significantly (P<0.05) amongst the species with *C. sinensis* leaves having the highest sugar content (34.82) and *C. aurantium* having the lowest (18.63). The crude protein content of leaves was also significantly different across all the species with *C. sinensis* having the highest (15.83) and *C. nobilis* having the lowest (10.92).

Table 1: Effect of *P. citri* infestation on the fresh and dry weight (g), percentage moisture (%) and dry matter (g) contents of leaves of citrus species

	Mean ± S.E*			
Status	**Fresh weight**	**Oven dry weight**	**% Moisture**	**% Dry matter**
Infested	23.67 ± 1.28^{a}	6.54 ± 0.46^{a}	72.63 ± 0.67^{a}	27.37 ± 0.67^{b}
Un-infested	18.42 ± 0.95^{b}	6.61 ± 0.34^{a}	64.09 ± 0.54^{b}	35.91 ± 0.54^{a}
Citrus species				
C. sinensis	24.88 ± 2.56^{a}	8.03 ± 0.56^{a}	66.95 ± 1.66^{b}	33.05 ± 1.66^{a}
C. nobilis	22.63 ± 2.03^{a}	6.92 ± 0.53^{a}	68.60 ± 2.77^{ab}	31.40 ± 2.77^{ab}
C. reticulata	18.80 ± 0.98^{b}	5.45 ± 0.18^{b}	70.67 ± 1.64^{a}	29.33 ± 1.64^{b}
C. limon	15.80 ± 0.59^{b}	4.93 ± 0.34^{b}	68.57 ± 2.44^{ab}	31.43 ± 2.44^{ab}
C. aurantium	23.12 ± 1.33^{a}	7.53 ± 0.30^{a}	67.03 ± 1.58^{b}	32.97 ± 1.58^{a}

*Means of three replications; means followed by the same letter(s) in a column are not significantly different P= 0.05 using DMRT

Table 2: Effect of mealybug infestation on the chlorophyll content and proximate composition of leaves of citrus species

	Mean ± S.E*				
Status	**Chlorophyll**	**Sugar (g)**	**Crude protein (%)**	**Crude fibre (%)**	**Ash (%)**
Infested	65.73 ± 2.59^{a}	27.53 ± 1.43^{b}	15.57 ± 0.77^{a}	19.20 ± 0.56^{b}	7.47 ± 0.15^{b}
Un-Infested	68.97 ± 2.27^{a}	32.94 ± 1.80^{a}	11.99 ± 0.26^{b}	22.53 ± 0.16^{a}	10.93 ±0.32^{a}
Citrus species					
C. sinensis	76.20 ± 1.35^{a}	34.82 ± 1.26^{a}	15.83 ± 1.67^{a}	22.78 ± 0.30^{a}	7.73 ± 0.43^{e}
C. nobilis	73.05 ± 2.17^{a}	33.60 ± 1.30^{c}	10.92 ± 0.14^{e}	20.68 ± 1.11^{b}	9.57 ± 1.12^{c}
C. reticulata	56.10 ± 2.31^{b}	34.18 ± 1.66^{b}	13.65 ± 0.10^{d}	20.63 ± 1.19^{b}	9.82 ± 0.77^{b}
C. limon	59.57 ± 2.25^{b}	29.93 ± 1.22^{d}	14.58 ± 1.16^{b}	19.93 ± 0.82^{d}	9.88 ± 0.77^{a}
C. aurantium	71.90 ± 2.18^{a}	18.63 ± 0.61^{e}	13.90 ± 1.03^{c}	20.28 ± 0.86^{c}	9.02 ± 0.78^{d}

*Means of three replications; means followed by the same letter in a column are not significantly different P= 0.05 using DMRT.

Influence of Five Species of Citrus on the Morphometrics of *P. citri*

The 1st instar nymphs reared on *C. nobilis* were significantly (P<0.05) longer than those reared on *C. sinensis* and *C. limon* (Table 3). The 2nd instars on *C. sinensis* were significantly P<0.05) shorter than those reared on *C. nobilis*, *C. reticulata* and *C. aurantium*. The 3rd nymphal instars reared on *C. reticulata* were significantly longer (P<0.05) than those reared on other citrus species. The 4th nymphal instar's mean length varied from 2.8-3.1 mm but the differences were not significant. Adult males reared on *C. nobilis* were significantly (P< 0.05) shorter and narrower than those reared on all other host plants. In contrast, the adult female showed no significant variation in length and width across all citrus species.

In terms of weight, *C. sinensis* and *C. aurantium* produced heavier males which were significantly different (P<0.05) from *C. nobilis*, *C. reticulata* and *C. limon*. *C. aurantium* produced heavier females which were significantly (P< 0.05) different from females reared on *C. reticulata*, *C. nobilis* and *C. sinensis*. The weights of females reared on *C. limon* were also significantly different (P<0.05) from those of females reared on *C. nobilis* and *C. reticulata*.

Leaf Chlorosis

Species variation in mean number of leaves per plant (367.33-461.00) was due to random variation; order of leaf production was *C. sinensis*>*C. limon*>*C. nobilis*>*C. aurantium*>*C. reticulata*. Leaves of *C. nobilis* were the least chlorotic which differed significantly (P<0.05) from other species excluding *C. aurantium* (Table 4). Chlorosis was 2-3 times more pronounced on *C. limon*, *C. sinensis* and *C. reticulata* than on *C. nobilis*.

Table 3: Effect of citrus species host on the morphometrics and weight of *P. citri*

Citrus species	Nymphal instar (Means ±S.E)*				Adult Male (Mean ±S.E)*			Adult Female (Mean ±S.E)*		
	1st	2nd	3rd	4th	Length (mm)	Width (mm)	Weight (mg)	Length (mm)	Width (mm)	Weight (mg)
C. sinensis	0.80±0.10[b]	.27±0.03[b]	2.17±0.19[b]	3.10±0.06	3.70±0.06[a]	1.50±0.06[a]	0.28±0.01[a]	4.23±0.03[a]	1.87±0.03[a]	3.35±0.03[b]
C. nobilis	1.07±0.07[a]	.47±0.03[a]	1.97±0.03[b]	2.80±0.10	3.30±0.10[b]	1.17±0.03[b]	0.17±0.02[c]	3.90±0.38[a]	1.93±0.13[a]	3.12±0.07[c]
C. reticulata	1.03±0.09[ab]	.53±0.09[a]	2.67±0.03[a]	3.07±0.03	3.70±0.10[a]	1.63±0.09[a]	0.20±0.01[bc]	3.90±0.06[a]	1.77±0.03[a]	2.65±0.19[d]
C. limon	0.80±0.06[b]	.43±0.03[ab]	1.97±0.17[b]	2.80±0.15	3.67±0.09[a]	1.63±0.09[a]	0.22±0.01[b]	3.83±0.03[a]	1.77±0.03[a]	3.68±0.18[ab]
C. aurantium	1.03±0.07[ab]	.53±0.07[a]	2.10±0.06[b]	3.00±0.15	3.73±0.18[a]	1.50±0.00[a]	0.27±0.02[a]	3.90±0.06[a]	1.80±0.00[a]	3.76±0.05[a]

*Means followed by the same letter(s) in a column are not significantly different P= 0.05using DMRT

Table 4: Proportion of chlorotic leaves in citrus species infested with *P. citri*

Citrus species	Mean no. of leaves/plant*	% chlorosis
C. sinensis	461.00[a]	24.40[a]
C. nobilis	437.33[a]	7.33[c]
C. reticulata	367.33[a]	25.80[a]
C. limon	441.33[a]	15.20[b]
C. aurantium	435.67[a]	9.70[bc]

Means of three replications; Means followed by the same letter(s) in a column are not significantly different P =0.05using DMRT

DISCUSSION

The result on biomass weight of the host plant leaves shows that fresh leaves obtained from all the infested plants had more weight, with *C. sinensis* leaves being the heaviest and *C. limon* with the lowest weight. The high values of fresh biomass weight of infested plant leaves compared to un-infested host plants could be due to the host plant's ability to produce larger leaves as a defensive strategy aimed at compensating losses from the pest activity. This result is in line with the findings of Koch *et al*., 2016 who reported that plants possess the ability to withstand or recover from herbivore injury through growth and compensatory processes. This takes place in the form of increased branching or tillering, production of larger leaves, and re-allocation of carbon to the shoots.

Percentage (%) dry matter which represents the actual mineral or nutrient content of a sample indicates that despite the lower fresh weight and percentage moisture content of the un-infested plant leaves, their percentage dry matter content was higher with *C. nobilis* being the highest as compared with the infested plants. The high percentage dry matter content of the un-infested leaves is an indication that the nutrient status of the insect-free leaves was not tampered with by the pest. The difference in the values of the percentage dry matter of un-infested and infested plant leaves may probably be on account of feeding by the bugs. Similarly, the low percentage dry matter content of *C. reticulata* may be responsible for the production of adult females with the lowest body weight. In the same vein, high percentage dry matter content of un-infested *C. sinensis*, *C. nobilis*, *C. limon* and *C. aurantium* may have accounted for the production of heavier females on the infested host plants. This is obvious as variations in the quantity or quality of an acceptable diet can have profound effects on insect development as reported by Chapman (1998). The quantity of protein and amino acid ingested by an insect is important for its optimal growth and reproduction (Chapman, 1998). In separate studies, Scriber and Slansky (1981), and Ridsdill-Smith (1991) gave many examples of insects feeding on natural foods in which growth and reproduction were positively correlated with nitrogen content of the food which is a constituent of amino acids.

The result on proximate contents of the host plant leaves shows that all un-infested plants had higher sugar content than the infested plants. This is an indication that the feeding activity of the mealybug has probably depleted the sugar present in the leaves harboring the insects. Also, the genetic makeup of these species of plants responsible for the high sugar content of *C. sinensis*, *C. nobilis,* and *C. reticulata* could probably be the reason for the large numbers of mealybugs found on them. Similarly, the low sugar content of *C. aurantium* and *C. limon* may also likely relate to the low numbers of the pest. Furthermore, the taste of juice extracted from *C. aurantium* and *C. limon* fruits may provide further proof.

Probably due to honeydew secretion, the leaves of all the infested host plants contained higher crude protein (non-protein nitrogenous substances inclusive) than the un-infested plants. This finding is in agreement with Chan *et al*. (2010) and Wilson *et al* (2011) who reported higher nitrogen metabolism in aphid infested plants relative to un-infested plants. The high crude protein content of *C. sinensis* may also be responsible for the production of high numbers of crawlers since protein is vital in the stimulation of ovarian development. This result agrees with Clements (1992) who reported that egg production in mosquitoes was proportional to the amount of nitrogen ingested with blood meal. All the un-infested plants had high ash content which is the inorganic residue remaining after the organic matter has been burnt. The difference in the values of the ash content of infested and un-infested plant leaves could possibly be the representative fraction of what the insects sucked away during feeding. The high values of crude fibre of the plants may also give credence to why homopterans (mealybugs and aphids) prefer younger leaves which are less fibrous since the digestibility of a feeding stuff varies inversely with its fibre content. Although there was heavy secretion of honey dew by the mealybug on all the infested plants with its attendant presence of black sooty moulds, its effect on the host plants was not assessed or quantified in this study. Neuenschwander (1989) and Atusiuba (1990) reported that the photosynthetic surface area of leaves is greatly reduced by moulds during high infestation. This may subsequently lead to decrease in the level of carbohydrate manufactured with effect on growth as well as fruit yield in the host plant.

The result of morphometrics has shown that the 2nd instar nymphs found on *C. aurantium* and *C. reticulata* were the longest and those on *C. sinensis* were the shortest. The 3rd instars on *C. reticulata* were, however, longer than the others. In terms of the adult male length, *C. nobilis* produced males that had shorter and narrower bodies than males reared on other citrus species. This implies that there may be some growth regulatory components in *C. nobilis*. This result agrees with the findings of Tunaz and Uygun, 2004 who reported that the presence of some substances known as insect growth regulators in the diet of insects can affect certain physiological regulatory processes essential to the normal development of the insects and their progeny. The longer, wider, and heavier males reared on *C. sinensis*, *C. reticulata*, *C. limon* and *C. aurantium* may be the result of presence of male specific growth boosters in these hosts. *C. aurantium* produced the heaviest females while *C. reticulata* produced the least in weight. This may also not be unconnected with the nutrient content of these host plants.

Leaf chlorosis, which is the appearance of yellow spots, and a visible manifestation of mealybug feeding, shows that 25.80 % of *C. reticulata* and 24.40 % of *C. sinensis* leaves had chlorosis, thus making them the most affected host plants. The high percentage of chlorosis observed on *C. reticulata* is also reflected in its reduced chlorophyll content. Edmond *et al*. (1988) pointed out that the level of chlorophyll production is reduced when mealybugs pierce the epidermal layer and suck the tiny chloroplasts, soluble foods and vitamins from the leaves. This reduction in chlorophyll content may be due to death of palisade cells associated with chlorophyll pigments consequent to attack by the mealybug as reported by Ukwela and Liman (2002). *C. nobilis* with only 7.33 % chlorosis may likely be on account of its hardy status. *C. aurantium* also exhibited same trait with 9.70 % of its leaves being chlorotic. This is also reflected in the high chlorophyll content of the infested leaves of *C. nobilis* and *C. aurantium*. On the contrary, *C. sinensis*, despite having many of its leaves chlorotic, still maintained a high level of chlorophyll content for photosynthesis. This may be as a result of its ability to produce a large number of leaves to serve as a mechanism of survival strategy. This reasoning agrees with Letchamo and Gosselin (1995) that increased chlorophyll content also ensure increased photosynthesis. It therefore means that a reduction in chlorophyll content could lead to a reduction in carbohydrate (or sugar) production in the affected plant. Generally, leaves of all un-infested plants were higher in chlorophyll, sugar, crude fibre and ash contents than the infested plants. This result corroborates the findings of Tobih *et al*. (2002) who reported lower sugar, crude fibre and ash contents of mango fruits infested by the mango mealybug *Rastrococcus invadens* (Williams) compared to un-infested fruits. In another research, Zakka (2019) reported lower nutritional composition of infested date palms compared to those that were not infested. This difference in proximate composition between infested citrus leaves and the control is a clear manifestation of the effect of mealybug feeding on the infested host plants.

CONCLUSION

Morphologically, adult males were completely different from adult females on all the host plants just as pupal instar (males) compared to other instar stages. Males of *P. citri* were shorter in body length, narrower in width and lighter in weight. On the other hand, females were longer in length, wider in width and heavier in weight. The percentage dry matter content representing the actual nutrient content of the host plants was highest on the insect-free plants compared with the infested plants. The proximate content of the host plants indicates that all the insect-free plants had more sugar, ash content and crude fibre than the plants harbouring the insects. Tangerine and sweet orange species were mostly affected by mealybug feeding.

This study recommends the use of *C. nobilis* (King orange), a hybrid of *C. sinensis* (sweet orange) and *C. reticulata* (tangerine) for commercial scale production of seedlings. Routine inspection should be carried out for early detection of mealybug infestation. More research should be directed towards the development of citrus cultivars that could be resistant to mealybug.

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