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Infestation of host plants by the African mistletoe, *Tapinanthus bangwensis* [Engl. and K. Krause] Danser: Implications for the leaf and fruit production of *citrus sinensis* and *irvingia gabonensis* hosts

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Abstract
Mistletoe infests susceptible host plants, cohabits and survives on such hosts deriving nourishment and support. The sustenance and survival of the parasitic plant on its hosts is achieved at the cost of shared resources resulting in suboptimum production in physiological outputs which include features like leaf size and weight and also the quantity and quality of fruits realized among others. The African Mistletoe, *Tapinanthus bangwensis* on *Citrus sinensis* and *Irvingia gabonensis* hosts were studied for the leaves and fruits product for which the infested and uninfested hosts were investigated. Measurements for leaf size revealed that the uninfested hosts had larger leaf sizes (*Citrus* 77.43; *Irvingia* 51.77cm^2) and weight (*Citrus* 2.17; *Irvingia* 0.76 g) including higher number of fruits (*Citrus* 238; *Irvingia* 87) relative to the infested (*Citrus* 67.75, 1.69, 124; *Irvingia* 47.52cm^2, 0.70 g, 69). The presence and growth of mistletoe on the hosts therefore depletes resources which culminate in reduced physiological outputs in the infested hosts.

Keywords: Leaf size, leaf weight, infested hosts, uninfested hosts

Introduction

Mistletoes are hemiparasites and they depend largely on their host tree for water and nutrient supply (Watson 2001; Zuber, 2004) [17, 18]. Mistletoes though are capable of fixing atmospheric carbon still extract carbon from the xylem sap of the host which results in partial heterotrophy. This might additionally contribute to a weakening of the host under limiting growing conditions (Escher *et al*., 2004; Rigling *et al*., 2010) [4, 15].

The infestation of mistletoes on host branches often brings about changes in host leaf area, leaf number, reduction in growth performance and biomass which are outcomes of the disruption of the physiological and metabolic processes in the hosts caused by the parasite (Karunaichamy *et al.,* 1999) [9]. Competition for water, inorganic ions and metabolites has been adduced for loss in host production and consequently area and weight of leaves (Hosseini *et al*., 2008) [8]. The depth to which mistletoe-host association thrives and survives may be determined by the amount of nutrient resources available within the host and the quantity diverted by the parasite. Some leafy mistletoes are closely attached and lived on their hosts for decades with little noticeable damage while others inflict severe damage within a short period of cohabitation. As parts of a host plant are exposed to heavy invasion by mistletoe, the potential for photosynthesis and reproduction of such parts diminish which ultimately lead to death of the parts. The damage that can be done to host depends on density of the parasitic plant, extent of dependence on host resources, stage of growth and development of the parasite as well as host.

This work seeks to investigate the implication of the infestation of the African mistletoe, *Tapinanthus bangwensis* on the leaf and fruit production of susceptible *Citrus* and *Irvingia* hosts.

Materials and Methods

Site of the study

Leaves of the infested and uninfested *Citrus* and *Irvingia* plants were collected and processed as appropriate from the *Citrus* orchard and *Irvingia* plantation at Moor Plantation Apata, Ibadan, Nigeria. Random collections of sample materials were carried out on the selected and marked plants in both rainy and dry seasons.

The annual rainfall ranged from 750 to 1557 mm and temperature range was 23/34 ^{0}C (Minimum/maximum). Relative humidity was between 45 and 89% throughout the year.

Evaluation of the leaf area and fresh leaf weight of host trees

An investigation to assess effect of the presence of mistletoe on leaf area and fresh leaf weight of susceptible hosts was conducted by selecting ten infested hosts and ten uninfested hosts on the field. The selected trees were such that they possessed approximately similar Diameter at Breast Height (DBH), height and crown figure. The parasitized and unparasitized branches of selected trees have similar lengths, diameter and sunlight direction. Nine largest leaves from stem branches of selected samples were collected, for measurement of leaf area and for determination of fresh weight.

Evaluation of the fruit production capacity of host trees

The impact of mistletoe presence on host tree productivity with respect to the number of fruits produced was evaluated by selecting ten infested and ten uninfested trees with approximately similar DBH, height and crown figure. Fruit counts of the number of matured fruits produced by each selected tree were carried out and the mean productivity calculated. This experiment was conducted for three years at two sites each for the *Citrus* and *Irvingia* hosts.

Proximate Analysis Tests

Crude protein content estimation

Test for crude protein was conducted using the Kjeldahl method according to the standard methods of Association of Official Analytical Chemists (AOAC, 2005) [1]. Two (2) grammes each of samples of fruits from the ten selected infested host trees and similar uninfested hosts of both *Citrus* and *Irvingia* was placed in the Kjeldahl flask with 5 g of Kjeldahl catalyst. Also, 25 ml Conc. H_2SO_4 was added with few boiling chips inside the flask. The flask was then heated in the fume chamber until a clear mixture was obtained. The solution which was allowed to cool under room temperature and then emptied into a 250 ml volumetric flask was marked up to volume with distilled water to obtain the sample digest.

The apparatus which made up the distillation equipment was set up and properly cleaned. Five (5) millilitres solution of 2% Boric acid was introduced with the addition of a few drops of methyl red indicator into the distillate collector which was a 100 ml conical flask with the flask placed under a condenser. A 5 ml measure of the prepared sample digest was pipetted into the apparatus and washed down with distilled water. Further added to the digest was 5 ml of 60% Sodium hydroxide solution. Heating of the sample was maintained until a distillate of 100 ml has accumulated in the receiving flask. Titration of content of the receiving flask to a pink coloured end point using 0.049 M H_2SO_4 was carried out. A blank with filter paper was subjected to the same procedure.

Calculation:

$$\% \text{ Total Nitrogen} = \frac{(\text{titer} - \text{Blank}) \text{ x } N_2 \text{ x Normality of acid}}{\text{Weight of sample}}$$

Nitrogen factor = 6.25

Crude protein = % total N x 6.25

Ether extract (Crude Fat) determination

Soxhlet extraction method according to AOAC (2005) [1] was used to determine the fat content. Ethyl acetate of 300 ml was measured into a pre-weight 500 ml capacity round bottom flask connected to soxhlet extractor. Two grammes of the fruit sample obtained (From infested and uninfested *Citrus* and *Irvingia* hosts) was transferred into the extraction thimble and the thimble was placed in soxhlet apparatus. The apparatus was refluxed for six hours under heat and the thimble was then removed with care. Ethyl acetate was recovered for reuse. The ether-free flask was removed and dried at 105^{0}C in an oven for 1 hour after which it was cooled in a desiccator and weighed.

Total lipid content was calculated thus:

$$\text{Crude fat (\%)} = \frac{(\text{extracted lipid + weight of flask}) - (\text{weightof empty flask})}{\text{Weight of sample}} \text{ X } 100$$

Crude fibre content estimation

The crude fibre was determined in accordance with the methods of AOAC (2005) [1]. Sample extract of the fruits of *Citrus sinensis* and *Irvingia gabonensis* (both from infested and uninfested host sources) were weighed (two grammes each) and 150 ml of 0.1275 M H_2SO_4 was used to digest them for the duration of 30 minutes. The content was filtered over Buchner funnel using filter paper No. 51 and rinsed with hot water for the removal of acid. The residue obtained was further subjected to the addition of 150 ml of 0.313 M KOH and boiled for 30 minutes then rinsed with boiling water and acetone. The residue was oven dried at 105 °C for 12 hours and weighed. The resultant residue was transferred to muffle furnace at 520 °C for 3 hours. The loss in weight represented the crude fibre. The calculation for the crude fibre was expressed as below.

$$\text{Crude fat (\%)} = \frac{(\text{extracted lipid + weight of flask}) - (\text{weight of empty flask})}{\text{Weight of sample}} \text{ X } 100$$

Determination of ash content

Assessment for ash content was performed in accordance with AOAC (2005) [1] procedure. Two gramme of sample extract of each fruit from infested and uninfested *Citrus* and *Irvingia* hosts placed in silica dish was ignited, allowed to cool and the weight was thereafter taken. To achieve a white or grey ash, the dish and sample were initially heated gently and then progressed to 550^{0}C in a muffle furnace for 3 hours. Desiccator was used to cool the dish and content was weighed.

$$\% \text{ Ash} = \frac{(W_3 - W_1)}{(W_2 - W_1)} \text{ x } 100$$

Where

W_1 = dish weight

W_2 = dish weight + sample before ashing

W_3 = dish weight + sample after ashing

Determination of moisture content

Assessment for moisture content was performed in accordance with the standard methods of AOAC (2005) [1]. To achieve a constant weight for the work, stainless steel oven dishes were cleaned and dried in oven for 1 hour at 100^0C. They were cooled in desiccators and then weighed. Placed in each dish were two grammes (2 g) of sample extract from infested and uninfested *Citrus* and *Irvingia* hosts dried at 100^0C in oven for the attainment of constant weight. The samples and dishes were together cooled in desiccators and weighed.

$$\% \text{ moisture content} = \frac{(W_2 - W_3)}{(W_2 - W_1)} \times 100$$

Where
W_1 = dish weight
W_2 = dish weight + sample before drying
W_3 = dish weight + sample after drying

Statistical Analysis
Data obtained were subjected to statistical analysis using the SPSS 21 Statistics Program. Statistical analysis was performed using one-way analysis of variance with Duncan's Multiple Range Test.

Results
1. Leaf area and leaf weight of infested and uninfested *Citrus sinensis* and *Irvingia gabonensis* hosts in the dry season
The leaf area and fresh leaf weight of the infested and uninfested *Citrus sinensis* and *Irvingia gabonensis* hosts are presented in Table 1 The leaf area cover of the uninfested *C. sinensis* (77.43 cm^2) was significantly higher ($\alpha = 0.05$) than the infested plants. Similarly, the leaf fresh weight of the uninfested *Citrus* had significantly higher value (2.17 g) than the infested. The same pattern was observed in the leaf area of *I. gabonensis* with the uninfested having a higher leaf area (51.77 cm^2) than the infested, however, there was no variation between the infested and uninfested *Irvingia* fresh leaf weight. The leaves of the infested and uninfested *Citrus* had higher mean weights than *Irvingia* under similar conditions.

2. Leaf area and leaf weight of infested and uninfested *Citrus sinensis* and *Irvingia gabonensis* hosts in the rainy season
Table 2 shows the results of the leaf area and leaf weight of infested and uninfested *Citrus* and *Irvingia* hosts in the rainy season. The leaf area cover for the uninfested *Citrus* (76.55 cm^2) host was significantly higher (α=0.05) than the infested (46.82 cm^2) and similar trend was observed for the *Irvingia* host (infested 31.09 cm^2 and uninfested 67.85 cm^2). The fresh leaf weight was significantly higher in the uninfested *Citrus* leaf (1.48 g) compared to the infested (0.85 g). A similar observation was noted with the uninfested *Irvingia* (0.77 g) leaf which had a higher weight than its infested (0.39 g) counterpart.

3. Number of fruit production capacity of the infested and uninfested host trees
Table 3 shows the fruit production capacity of the infested and uninfested *Citrus sinensis* and *Irvingia gabonensis* host trees. The fruit production output of the uninfested *Citrus* at the two sites in the first year ($FP1_i$ - 238 counts; $FP1_{ii}$ - 158 counts) was significantly higher ($\alpha = 0.05$) than the infested during the period of assessment. The pattern of fruit production output of the *Irvingia gabonensis* trees was similar to that of *Citrus* as the fruit production of the uninfested host ($FP1_i$ - 87 counts; $FP1_{ii}$ - 122 counts), was significantly higher ($\alpha = 0.05$) than the infested. In the second year of observation (FP2) for the *Citrus* fruit production, the uninfested ($FP2_i$ - 210 counts; $FP2_{ii}$ - 139 counts) had significantly higher fruit output than the infested at the two sites. The uninfested *Irvingia* ($FP2_i$ - 232 counts; $FP2_{ii}$ - 88 counts) also produced significantly higher number of fruits than what was obtained in the infested stands. In the third-year observation, the fruit production output for the uninfested *Citrus* ($FP3_i$ - 151 counts; $FP3_{ii}$ - 165 counts) was higher than the infested while the uninfested *Irvingia* ($FP3_i$ - 264 counts; $FP3_{ii}$ - 144 counts) also produced fruits that was significantly higher than the infested at the different sites.

4. Proximate composition of the infested and uninfested fruits of the host trees
The proximate composition of the infested and uninfested fruits of *Citrus* and *Irvingia* host plants is shown in Table 4. The crude proteins of the infested and uninfested *Citrus* fruits were similar but the crude protein of the uninfested *Irvingia* fruit (2.21%) was significantly higher than the infested. The crude fat in the fruits of uninfested *Citrus* (0.70%) was significantly higher ($\alpha = 0.05$) than that of the infested and the uninfested *Irvingia* fruits (0.76%) had significantly higher fat content than the infested. The crude fibre of the infested and uninfested *Citrus* fruits was similar while the uninfested *Irvingia* fruit (1.55%) had significantly higher ($\alpha = 0.05$) crude fibre than the infested. The percentage ash content of the infested and uninfested *Citrus* was similar and so also for the infested and uninfested *Irvingia* fruits. The percentage moisture content of the uninfested *Citrus* fruit (57.04%) was significantly higher ($\alpha = 0.05$) than that in the infested *Citrus*. Also, the uninfested *Irvingia* fruits (71.72%) had significantly higher moisture content than the infested.

Discussion
The comparatively smaller sizes in leaf area and lesser quantity in leaf weight and lower number of fruits produced in the infested *Citrus sinensis* and *Irvingia gabonensis* relative to the uninfested was a pointer to the influence and effect which the presence of mistletoe exerts on the host trees. Undoubtedly, this could mainly result from competition for water, inorganic ions, and other important solutes necessary for growth and reproduction (Glatzel and Geils, 2009) [6]. The incidences of infestation of *Mimosa clerodendron* by *Struthanthus flexicaulis* as studied by Mourão *et al*., (2009) [13] implicated the parasitic plant for the loss of leaf area of host tree which was in correlation with reduction of fruit number as well as seed weight. This work bore similarities to the output resulting from relationship between the mistletoe and the *Citrus* and *Irvingia* host plants. Sinha and Bawa, (2002) [16] also found out that the presence of hemiparasite of the Loranthaceae family brought about a drain in its host resources. Thus, the growth rates in trees which were free of parasitic infestations when compared with the infested were significantly different. They noted a negative correlation for parasitic load and fruit production in the *Phyllanthus* species, (*Phyllanthus emblica* and *Phyllanthus indofischer*i). Lei (2001) [10] equally noted that mistletoe-infested *Senegalia greggii* experiencing moderate to massive

infestation had significant reduction in canopy volume, flower and fruit production. Hence, it would not be inappropriate to support the postulation by scholars such as Karunaichamy *et al.,* (1999) [9] and Hosseini *et al*., (2008) [8] which emphasized that the presence of parasites like mistletoe on host tree branches induce physiological and metabolic perturbation which often leads to loss in host tree productivity in aspects such as leaf area, leaf weight or even quantity and quality of fruits produced.

The proximate composition of the fruits of both infested and uninfested *Citrus* and *Irvingia* host plants showed that the presence of mistletoe on susceptible hosts could exert some level of influence on the reproductive capacity and the reactive tendency of such hosts. The overall diminished nutrient status of the infested fruits of both the *Citrus* and *Irvingia* host plants are affirmations to the several proclamations of competitiveness for limited resources provided by the host plants. Infested trees are often affected by the mistletoe parasite in several ways which include loss of water and nutrient, including reduction in photosynthetic organelles which would bring about poor assimilation of carbon (Meinzer *et al*., 2004; Rigling *et al*., 2010; Galiano *et al*., 2011) [12, 15, 5]. These biological processes so described ultimately cause noticeable growth reduction, change in nutrient composition and loss of vigour in the infested host plants under increased parasitic activities. Research findings from other scholars revealed that the degree to which the impact of the mistletoe's infestation could manifest is dependent on the peculiarity and nature of the host-parasite interaction (Lei, 2001; Raftoyannis *et al*., 2015) [10, 14]. For instance, it was observed that there were differences in the proximate composition between the infested and uninfested *Citrus* and *Irvingia* host plants; but while these differences (reduced content in the infested hosts) were largely inconsequential in the *Citrus* the differences were of marginal significance in the *Irvingia* host plant. This could probably occur due to one or more of factors such as degree of infestation on host plant, accessible nutrients within host plant and/from environment, stage of growth/age either of the host plant or the mistletoe and the prospective interplay of some other emergent factors relative to the host-parasite association (Graves, 1995; Aukema and Del Rio, 2002; Li *et al*., 2015) [7, 2, 11].

Table 1: Fresh leaf area and leaf weight of infested and uninfested *Citrus sinensis* and *Irvingia gabonensis* hosts in the dry season

Leaf parameter	Area (cm^2)	Fresh leaf weight (g)
Infested Branch of *Citrus sinensis*	67.75^b	1.69^b
Uninfested Branch of *Citrus sinensis*	77.43^a	2.17^a
Infested Branch of *Irvingia gabonensis*	47.52^d	0.70^c
Uninfested Branch of *Irvingia gabonensis*	51.77^c	0.76^c

Means with the same letter were not significantly different in each column according to Duncan Multiple Range Test at α = 0.05

Table 2: Fresh leaf area and leaf weight of infested and uninfested *Citrus sinensis* and *Irvingia gabonensis* hosts in the rainy season

Leaf parameter	Area (cm^2)	Fresh leaf weight (g)
Infested Branch of *Citrus sinensis*	46.82^{ab}	0.85^{ab}
Uninfested Branch of *Citrus sinensis*	76.55^a	1.48^a
Infested Branch of *Irvingia gabonensis*	31.09^b	0.39^b
Uninfested Branch of *Irvingia gabonensis*	67.85^{ab}	0.77^{ab}

Means with the same letter were not significantly different in each column according to Duncan Multiple Range Test at α = 0.05

Table 3: Number of fruit production capacity of the infested and uninfested host trees

Sample	*FP1$_i$	*FP1$_{ii}$	*FP2$_i$	*FP2$_{ii}$	*FP3$_i$	*FP3$_{ii}$
Infested *Citrus sinensis*	124.00^b	135.00^b	146.00^b	115.00^b	67.00^c	140.00^b
Uninfested *Citrus sinensis*	238.00^a	158.00^a	210.00^a	139.00^a	151.00^b	165.00^a
Infested *Irvingia gabonensis*	69.00^d	107.00^d	99.00^c	72.00^d	142.00^b	120.00^c
Uninfested *Irvingia gabonensis*	87.00^c	122.00^c	232.00^a	88.00^c	264.00^a	144.00^b

Means with the same letter were not significantly different in each column according to Duncan Multiple Range Test at α = 0.05

*Fruit Production assessed as per Host Tree Grouping for *Citrus* @ 56 – 60cm, *Irvingia* @ 41- 50cm mean trunk diameter range into:
1st year observation: site i - FP1$_i$; site ii - FP1$_{ii}$
2nd year observation: site i – FP2$_i$; site ii – FP2$_{ii}$
3rd year observation: site i – FP3$_i$; site ii – FP3$_{ii}$

Table 4: Proximate composition of the infested and uninfested fruits of the host trees

Sample	Crude Protein	Crude Fat %	Crude Fibre	Ash	Moisture Content
Infested *Citrus sinensis*	1.48^b	0.57^b	1.12^b	2.30^b	55.86^c
Uninfested *Citrus sinensis*	1.74^b	0.70^a	1.22^b	2.42^a	57.04^c
Infested *Irvingia gabonensis*	1.73^b	0.51^b	1.23^b	1.18^d	63.76^b
Uninfested *Irvingia gabonensis*	2.21^a	0.76^a	1.55^a	1.38^c	71.72^a

Means with the same letter were not significantly different in each column according to Duncan Multiple Range Test at α = 0.05

Conclusion

The infestation of susceptible host plants by mistletoe leads to suboptimum output in the hosts. Infested host plants give rise to leaves of comparative smaller size and lesser weight as well as fewer quantity and reduced quality of fruits relative to uninfested hosts.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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