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Effects of field attack by *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) on the morphology and nutritional quality fresh fruit of *Citrus sinensis* L.

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The sweet orange, *Citrus sinensis* L. is an important fruit crop in Nigeria and its production is seriously constrained at maturity by attack of the fruit fly, *Ceratitis capitata* which results in severe yield loss. Effects of attack by *C. capitata* on fruit morphology and nutritional characteristics of *C. sinensis* from selected sites in southwest Nigeria were investigated. Twenty five mother citrus trees with >45 cm diameter at breast height (DBH) were randomly selected at two plantation sites: Ede (Osun state) and Olodo (Ogun state) in southwestern Nigeria. Ten mature fruits, plucked randomly from four cardinal sections of each tree were classified based on levels of infestation. Effects of attack on fruit morphometrics were assessed by standard measurements while the effects on fruit quality were determined by proximate and mineral analyses. Results indicated that attack by *C. capitata* was characterized by oviposition punctures which predisposed the fruit to secondary infection by *Penicillium notatum*. Fruits were attacked at any point on the surface but significantly more at about 2 cm from the fruit stalk scar. Multiple attacks did not follow a regular pattern. Fruit attack by *C. capitata* significantly decreased moisture content of fruit by 79% and mineral content; calcium, phosphorous, copper, zinc and iron while percentage dry matter, crude protein and crude fiber decreased significantly (P<0.50) with increased infestation. Fruit infestation also caused significant decrease in the vitamin C content by about 50% but increased saccharose, maltose and glucose content of attacked fruits by about 10%.

Key words: *Citrus sinensis,* multiple infestations, *Ceratitis capitata,* fruit morphometrics, *Penicillium notatum*

INTRODUCTION

Sweet orange (*Citrus sinensis* L.) is the most widely grown fruit crop in Nigeria and throughout the world (Morton, 1987; Yang et al., 2000) for its richness in essential minerals and vitamins (Olaniyan, 2001; Onibon

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Table 1. Agro-ecological characteristics of the survey sites.

Location	Lat. °E	Long. °N	Altitude (m)	Rainfall (mm)	Temperature (°C)		Relative humidity (%)
					Min.	Max.	
Ede, Osun State	4.25	7.43	300	2000	22	27	50-80
Olodo, Ogun State	3.37	7.20	200	2500	21	27	60-80

et al., 2007; Janati et al., 2012; Ekpete et al., 2013). However, production of citrus fruits is constrained at fruiting by pests; causing partial or total fruit loss (Parra et al., 2004). The Mediterranean fruit fly, *Ceratitis capitata* is a major fruit insect pest of sweet orange and the symptoms of its attack include different kinds of morphological distortions on the fruit surface which could predispose the mature fruit to infections by pathogens (Weems, 1981; Helle, 1989). Christenson and Foote (1960) had reported that the genus *Ceratitis* originated in Africa. It is well known that *C. capitata* has the most extensive geographical distribution among the pests of citrus. However, due to the devastating effects of this pest as an invasive species, citrus fruit consuming countries have imposed strict quarantine regulations on importation from endemic countries (Okwu and Emelike, 2006).

Citruses are attacked by a complex of many pests and diseases which often demand expenditures in the order of 160 million US dollars to initiate and effect control in order to avoid what could develop into total loss (Parra et al., 2004). These species which constitute the harmful entomofauna of this crop include the fruit flies, scale insects and mite species among others (Parra et al., 2004).

The fruit flies constitute a complex group of dipteran insects known to be characteristically polyphagous; attacking different kinds of crops. The extent of damage by the pest depends on the host crop, the agro-ecological characteristics of the location where the host crop is established as well as the fruit fly species; thus it becomes important to identify, classify and characterize the damage caused by *C. capitata* to *C. sinensis* in the southwest Nigeria.

This paper reports the effects of infestation by this insect pest on some morphological characteristics and nutritional quality of fresh fruits of sweet orange attacked by this insect pest and its implication for secondary infection by fungal pathogens.

MATERIALS AND METHODS

The study was carried out in two years on two farmers' fields in two states of southwestern Nigeria. Fresh fruits of citrus were sampled from mixed plantations of citrus and lemon from two sites located in Ede (Osun state) and Olodo (Ogun State). The samples were sorted and assessed for attack by *C. capitata.* Field collected samples of the insect were identified at the Insect Reference Collection and Identification Centre of the Department of Crop Protection and Environmental Biology (CPEB), University of Ibadan, Nigeria and by comparison with published samples (Alford, 1984). Detailed observation and fruit classification were carried out at the Entomology Research Laboratory, Department of CPEB, UI, Ibadan, Nigeria. Biochemical analyses for effects of attack on the nutritional characteristics of the samples were conducted at the Central Laboratory, Institute of Agricultural Research and Training, Moor Plantation, Ibadan, Nigeria.

Agro-ecological characteristics of the study sites and confirmation of pest's identity

The agro-ecological characteristics of the two survey sites are presented in Table 1. The first site: Ede is located in Osun State, Nigeria; with a bimodal annual rainfall of 2000 mm which peaks in June and September. The field was a mixed orchard of C. *sinensis* and C. *limon.* The stands of *C. sinensis* were distributed at 15 stands/50 m^2. The second site: Olodo village is located in Ogun State; also with a bimodal annual rainfall of 2500 mm which peaks in June and September. This field was also a mixed orchard with *C. sinensis* as the predominant species occurring at 18 stands/50 m^2. The agro-ecological characteristics of these sites were conducive to field infestation of Citrus fruits by *C. capitata*.

Pest sampling

Fifty mother citrus trees with ≥ 75 cm Girth at Breast Height (GBH) were randomly selected per study site. Five fruits were carefully harvested randomly per tree and thereafter sorted, examined and classified based on morpho-physical characteristics of damage on the attacked fruit into four groups (Table 1). Each fruit was particularly examined for oviposition puncture that was characteristic of female *C. capitata* on the outer surface of the fruit epicarp.

Characterization and classification of severity of field infestation by *C. capitata* on *C. sinensis*

Ten fruits were randomly selected from each class of attack (Table 2) and examined for the following morpho-physical parameters.

1. Fresh fruit weight (g) using a Chemical balance
2. Fresh fruit diameter (cm) via a transverse cut through the mid section of fruit
3. Fresh fruit diameter through a longitudinal section across the two fruit scars
4. Distance of oviposition puncture to the fruit stalk

The fruits were further examined to detect qualitative alteration of colour, secondary attack by other arthropods, opportunistic infections, surface damages and internal morphological changes. Field infestation of *C. sinensis* by *C. capitata* was classified based on the percentage of fruits attacked per tree (Table 3).

Table 2. Classification of field infestation of citrus fruit by *Ceratitis capitata.*

Classification	Description
Unattacked	No observable oviposition puncture and therefore used as the control
Single attack	One point of oviposition puncture
Double attack	Two points of oviposition punctures
Multiple attack	More than two oviposition punctures

Table 3. Classification of severity of field infestation by *C. capitata* in sampled trees of *C.sinensis.*

Class of infestation	Description of severity of field infestation
Unattacked	0% infestation
Mild infestation	1-5% of fresh fruit samples with at least one. Oviposition puncture/larval exit hole
Severe infestation	6-25% of randomly selected fresh fruits with at least one oviposition mark/larval exit hole
Very severe infestation	> 25% of randomly harvested fresh fruit with at least one oviposition mark/larval exit hole

Table 4. Morpho-physical characteristics of infested and uninfected fruits of *citrus sinensis* by *C. capitata.*

Class of infestation	Fresh weight of citrus			Description of physical distortion
	Weight (g)	Length (cm)	Width (cm)	
Unattacked	170.5^a	7.2^a	7.26^a	None
Single attack	198.5^b	7.31^a	7.32^a	Fruit Surface with one exit hole, surface hard; discolored brown
Double attack	198.5^b	7.36^a	7.35^a	Surface around exit widens, becomes hard and appeared/ compressed; about 3 .5mm radius (coloured brown and dry)
Multiple attack	194^b	7.4^a	7.45^a	Fruit surface with three or more larval exit holes; up to 4mm radius around exit hole; appeared compressed, discoloured brown, hard and dry; sells rottenness

Means followed by same letter in the same column are not significantly different (Tukey HSD, P>0.05); site.

Isolation and identification of field pathogens associated with oviposition puncture of *C. capitata* on *C. sinensis*

Ten diseased samples of fresh fruits of citrus collected from the field were used for this study in the laboratory. All samples were initially surface sterilized with sodium hypochlorite to remove external infections and thereafter rinsed several times with distilled water before blotting to dry on filter paper. A flamed wire loop was used to pick a little part of the discolored fruit at the oviposition puncture area created by *C. capitata.* All the scooped samples including an uninfested were streaked and thereafter plated on potato dextrose agar (PDA) in sterilized plastic Petri-dishes and incubated at room temperature control in three replications. The set up was observed for outgrowth of pathogens on the scooped skins for 48 to 72 h.

The emerged pathogens were sub-cultured in fresh PDA to obtain a pure culture of each pathogen sample. Each preparation was flamed gently on a Methylated spirit lamp to remove air bubbles before examination under microscope at increased magnification.

Effects of attack by *C. capitata* on proximate and other metabolites of citrus fruits

Samples were randomly selected from each class of infestation from each site in three replicates and analyzed for proximate, mineral and vitamin components at the Institute of Agricultural Research and Training, Moor Plantation, Ibadan using the official methods of AOAC (1990).

Statistical analysis

All the data were analyzed using descriptive statistics and analysis of variance and where significant, the means were separated using Tukey HSD test at 5% level of probability (SAS, 1990).

RESULTS AND DISCUSSION

Morpho-physical damage to infested fresh fruits of *C. sinensis* by *C. capitata*

The Morpho-physical characteristics of fruits of *C. sinensis* attacked by *C. capitata* and the subsequent distortions caused by the feeding activities of the developing larva inside the hesperidium are presented in Table 4. Infestations by *C. capitata* caused significant increase in the fresh fruit size of *C. sinensis*. Although the

Table 5. Morphometrics of fruits of *C. sinensis* attacked by *C. capitata* in two locations in southwest Nigeria.

Degree of attack	Fruit length (cm)		Fruit diameter (cm)		Fruit weight (g)	
	Ede	Olodo	Ede	Olodo	Ede	Olodo
Control	22.7[a]	24.3[a]	7.3[a]	7.7[a]	178.8[a]	219.0[a]
Single	23.2[a]	22.7[a]	7.7[a]	7.2[a]	213.8[a]	189.0[a]
Double	26.8[a]	23.7[a]	7.5[a]	7.6[a]	196.0[a]	206.0[a]
Multiple	23.3[a]	23.2[a]	7.4[a]	7.4[a]	188.0[a]	194.0[a]

Means followed by same letter in the same column are not significantly different (Tukey HSD, P > 0.05); site A = Ede (Osun state); site B = Olodo (Ogun state).

Table 6. Percentage field infestation of fruits of *Citrus sinensis* by *Ceratitis capitata* at two sites in the Southwest Nigeria.

Classification of attack	Mean percentage of fruit attacked/ site	
	Ede	Olodo
No attack	72 ± 4.32[a]	74 ± 2.83[a]
Single point attack	14 ± 1.63[b]	16 ± 1.63[b]
Double point attack	8 ± 1.63[b]	6 ± 2.83[b]
Multiple point attack	6 ± 2.31[b]	4 ± 1.63[b]

Means followed by same letter in the same column are not significantly different (Tukey HSD, P > 0.05); site.

fruit length and width were not significantly affected by different levels of attack by the pest; yet, attack by this pest caused different levels of severity of physical distortions on the surface of attacked fresh fruits with increasing number of attacks per fruit. For example, the surface of fruits attacked with one exit hole just hardens and gets discoloured while those with two exit holes or more had the fruit surface discoloured at about 3.5 mm radius around the exit hole which widens and become brown and dry, hardened and appeared compressed. Similarly, the fruit surface with three or more larval exit holes which occurred usually up to around 4.0 mm radius around the exit hole; appeared compressed, discoloured brown, hard and dry with foul smell of rottenness (Table 4).

Thus, field attack by *C. capitata* caused a characteristic water-soaked area with rancid odour oozing out of the infested fruit. Although there were visible physical distortions of the original spherical shape on the surface of the citrus fruit, there was no significant (P>0.05) difference between the diameter of attacked fruits and the control (Table 5). Each fly-exit hole on the fruit surface was 1.3 to 2.4 mm in diameter and the area around the puncture appeared hard and dehydrated.

There was however a significant increase (P<0.05) in fruit weight with increase in the number of oviposition punctures compared to the control (Table 5). Although, another very important fruit fly, *Bactrocera dorsalis* appears to compete and displace this pest in some orchards in Nigeria (Sapkota et al., 2010); *C. capitata* is a very important pest of agriculture that attacks several fruit crops like citrus, mango and apples (White and Elson-Harris, 1992). Damage to fruits is done first by the adult; which deposits its eggs by piercing the fruit with the ovipositor and also by the feeding larvae within the damaged fruit. These inflict heavy losses on the fruit yield of the citrus tree. This pest is economically important as it causes direct loss of yield and increased control costs (Bateman, 1972; Dowell and Wange, 1986; Okwu and Emelike, 2006).

It also leads to a great reduction and loss of export markets and/or the cost of constructing and maintaining fruit treatment and eradication facilities (Okwu and Emelike, 2006; Khamis et al., 2012). The fruit fly is considered a quarantine pest in many countries and this affects the export value of citrus fruits. The perception of farmers interviewed in this study agreed with Dowell and Wange (1986) that field infestation by *C. capitata* causes a lot of setbacks to citrus yield.

Severity of field infestation of *C. sinensis* by *C. capitata*

Field infestation of fruits of *C. sinensis* by *C. capitata* varied significantly (Table 3). Severity of fruit attack at the two sites ranged from 26 to 28% in the field. Of these, 14 to 16% were single point attack; 6 to 8% of the fruits had double points attack while 4 to 6% had multiple points attack (Table 6). Crown infestations of all mother Citrus trees at the two sites also varied significantly (Table 7). Whereas, 72 to 74% of the sampled citrus trees were

Table 7. Citrus crown infestation by *C. capitata* in two locations in the southwest Nigeria.

Class of infestation	Percent crown infestation/site	
	Ede	Olodo
Unattacked	0	0
Mild infestation	72	74
Severe infestation	16	18
Very severe infestation	12	8

Table 8. Effects of field infestation by *C. capitata* on the proximate composition of attacked and unattacked fresh fruits of *citrus sinensis* from two sites in the southwest Nigeria.

Class of infestation	Dry matter (mg)		Crude protein (mg/100 g)		Fibre (mg/100 g)		Vit.C (mg/100 g)		M.C. (%)	
	Ede	Olodo	Ede	Olodo	Ede	Olodo	Ede	Olodo	Ede	Olodo
Unattacked (Control)	20.00[a]	20.37[a]	0.41[a]	0.46[a]	0.84[a]	0.85[a]	48.47[a]	46.67[a]	79.00[a]	79.23[a]
Single	18.57[b]	18.81[b]	0.33[b]	0.35[b]	0.67[b]	0.68[b]	37.44[b]	33.52[b]	64.13[b]	61.27[b]
Double	16.50[c]	16.40[c]	0.22[c]	0.24[c]	0.44[c]	0.45[c]	23.95[c]	25.83[c]	40.57[c]	47.80[c]
Multiple	13.25[d]	13.30[d]	0.13[d]	0.11[d]	0.21[d]	0.28[d]	18.18[d]	20.29[c]	27.70[d]	29.7[d]
$LSD_{(0.05)}$	0.75	0.85	0.06	0.05	0.06	0.06	3.38	6.17	1.60	1.15

Mean followed by the same letter in the same column are not significantly different (P>0.05).

classed as mild infestation, 16 to 18% of the trees had severe infestations while 8 to 12% had very severe infestations. Damage to fruits of citrus and other host plants by fruit fly species is directly related to severity of field infestation (Nasiruddin et al., 2002) and so; it is particularly important to distinguish between attacks of different Tephritid species. Further characterization of the nature and specificity of physical damage to citrus by *C. capitata* is underway; and this would help field characterization of damage by specific fruit fly species to different host crops.

Morphophysiology and microbial spoilage of citrus fruit at oviposition punctures created by *C. capitata*

Morphological assessment of the surface around each point of attack up to 3.5 cm radius showed a somewhat compressed epicarp with a characteristic dehydrated and hardened surface. The original spherical shape of the orange fruit was distorted. The 3 to 5 mm surface on the fruit; around the exit hole turned brownish to black with a characteristic foul odour; indicating decay and collapse of fruit cells within the hesperidium as the deposited eggs begin to hatch and the larvae feed. This indicates that the factors of damage is the larvae developing inside the fruit and the associated pathogens that attack as consequence of attack by the flies (Okwu and Emelike, 2006; Khamis et al., 2012).

Initially, the oviposition puncture on the fruit surface was difficult to spot by an untrained and unaided eye but this point later became visible as a needle point with a characteristic black dot which spreads with time on the fruit surface. After pathogenicity test, the ‘black points’ on the oviposition puncture/exit hole were confirmed to be colonies of *Aspergillus* sp. and *Penicillium* sp. The extra cellular digestive activities of the pathogens degraded the nutritional components of the attacked fruits; thus the surface around the infected puncture/exit hole became hardened with time. The degree of surface distortion was directly related to the number of exit holes appearing as dent patches. It is believed that the holes created at oviposition had provided opening for opportunistic infection by pathogens particularly *Penicillium notatum.* Characteristically, the openings became rusty, hard and the spoilage spread from the locus.

Attacked fruits also had a characteristic rancid smell which resulted from the feeding activities of the larvae and the decay organisms encountered. From this study also, it is evident that the growth, development and feeding activities of the larvae of *C. capitata* caused extensive damage to the juicy endocarp. This was characterized by an offensive odour while the juice also tasted bitter. The deterioration and shrinking of the infected fruit is believed to have been increased by the fungus *P. notatum* encountered.

Effects of infestation by *C. capitata* on the nutritional composition of citrus

Infestation caused very significant depletion of the nutritional content of the attacked fruits especially in the carbohydrate and minerals (Tables 8 to 10). Similarly,

Table 9. Effects of field infestation by *C.capitata* on different types of carbohydrate in fresh fruits of *C. sinensis.*

Class of infestation	Saccharose (mg)		Lactose (mg)		Maltose (mg)		Glucose (mg)	
	Ede	Olodo	Ede	Olodo	Ede	Olodo	Ede	Olodo
Unattacked (Control)	6.26[a]	9.36[ab]	12.00[a]	9.98[a]	4.03[b]	7.50[a]	3.82[b]	5.93[ab]
Single	12.53[b]	7.91[b]	11.21[a]	8.70[a]	8.33[a]	5.21 [b]	7.00[a]	5.06[ab]
Double	12.61 [b]	1O.79[a]	4.82[b]	6.82[ab]	9.28[a]	7.20[a]	8.02[a]	6.01[a]
Multiple	6.31[a]	6.67[bc]	4.70[b]	5.13[b]	2.96[b]	4.27[b]	2.34[b]	3.55[b]
$LSD_{(0.05)}$	1.86	1.85	2.67	2.38	1.78	1.48	1.84	2.44

Mean followed by the same letter in the same column are not significantly different (P>0.05).

Table 10. Effects of field infestation by *C. capitata* on the mineral composition of fresh fruits of C. *sinensis.*

Class of infestation	Calcium		Phosphorus (mg)		Copper		Zinc		Iron	
	Ede	Olodo	Ede	Olodo	Ede	Olodo	Ede	Olodo	Ede	Olodo
Unattacked (Control)	67.60	69.33	51.63	49.05	0.007	0.003	0.65	0.67	0.09	0.07
Single	40.95	42.89	31.27	31.18	0	0	0.44	0.45	0.05	0.02
Double	21.28	20.84	16.89	16.32	0	0	0.23	0.21	0	0
Multiple	9.47	0.65	9.52	9.48	0	0	0.11	0.11	0	0

infestation caused significant reduction in the crude protein from 0.33 to 0.11 mg. This suggests that field infestation is serious and efforts should be directed at effective control of this pest. Observations on the distance of first point of attack indicated by the oviposition puncture created by *C. capitata* from the fruit stalk did not show a particular trend or pattern in relation to the point of second and third attacks respectively. The preference for the distance between the first point of oviposition and the fruit scar is worthy of note.

However, carbohydrate content of attacked fruits showed a peculiar trend which was different from the other parameters and metabolites under study (Table 9). From the result, the Saccharose, Lactose, Maltose and Glucose values increased in value with single and double point attack per fruit except for Lactose which significantly dropped to 4.82% (P<0.5). There was no significant difference between the values of Saccharose, Maltose and Glucose in the single and double point attack per fruit. The quantitative sugar values recorded for the multiple point attacks were not significant compared to the control except for lactose (P<0.05). The trend observed for the sugars could have been as a result of *C. capitata* attack on the oranges which resulted in deterioration of the fruit. Similarly, the level of disaccharide sugars reduced due to their conversion to monosaccharide. The drop in quantitative values generally observed at the multiple point attack stage could have been due to the stress caused by the feeding activities of the larva of *Ceratitis* growing inside the fruit.

The result of the microbiological investigation of fungal growth on the rind of the sweet orange confirmed earlier studies by Adegoke (2000) which identified *P. notatum* as an invading and colonizing organism around the slit created by *C. capitata* at oviposition. The *Penicillium* derives its metabolites from the orange fruit. This could have been responsible for accelerated sweet orange fruit damage. It may also be partly responsible for the significant drop of the quantitative values of the sugar observed on the fruits with multiple point attack in this study.

This is because the damage to the rind and endocarp was severe and larval/microbial activities increased at the multiple attack stage. The vitamin C content of unattacked sweet orange (control) in this study agreed with earlier reports by Morley (1987) which suggested that *C. capitata* attack and activities on the sweet orange fruit caused decreased values of vitamin C progressively as the severity of attack increased. The fruit dry matter content decreased significantly (P<0.05) with increased attack. This may be attributed to the growth and development activities of the larvae of *C. capitata* on the sweet oranges (Tables 8, 9 and 10).

The saccharose content of attacked and unattacked citrus fruits reduced significantly with infestation at the two sites. Although, the differences between the saccharose content of fruits from the two sites with single attack and those with double attack were not significant (P>0.05), yet the saccharose content of fruits with single and double attack were reduced by as much as 50% compared to unttacked fruits.

Whereas there was no significant difference between lactose and maltose content of single and non attacked fruits from both sites; the difference between the double and multiple attacked fruit were significantly different (P<0.05) compared to the non attacked fruits (Table 9). At Ede, infestation caused a reduction of lactose content of attacked citrus fruit from about 12.0 to 4.7 mg; and at Olodo site, from about 10.0 mg to about 5.0 mg which translated to more than 50% loss. Similarly infestation caused a reduction of the maltose and glucose by more than 50%.

The most abundant mineral element in citrus fruit was calcium (67.6-69 mg) followed by Phosphorus (49.0-51.0 mg). Infestation caused significant reduction of the calcium from about 38% at single attack to about 86 to 99% when the fruit suffered multiple attacks. Similarly, infestation reduced the phosphorus content by about 39% at single attack to about 86% when the fruit suffered multiple attacks. Infestation has a significantly high impact on the trace elements of copper, zinc and iron in which attacked fruits were depleted to zero levels.

Conclusion

This study had shown the oviposition activities of *C. capitata* and the subsequent development of the larvae caused extensive damage to the juicy endocarp and this reduced the quality of attacked fruits, making the juice to taste bitter and created site for and aggravate opportunistic infection by pathogens on the fruits especially by *P. notatum.* It also created rusty openings which become hard, spreading the spoilage from the locus and accelerating fruit deterioration and the shrinking of infected fruit.

Conflict of Interests

The authors have not declared any conflict of interests.

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