Nigerian Journal of Ecology (2014) 13:54-62.

ISSN: 1116-753X

Varietal differences in the cowpea calyx morphology: implications for abundance of *Megalurothrips sjostedti* larvae

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(Accepted 23 September 2014)

ABSTRACT
Investigations on the underlying biophysical basis of resistance in selected resistant cowpea cultivars were conducted to elucidate the cowpea calyx internal tissue arrangements and its role in the abundance of larval populations on cowpea under field conditions. Across all the cultivars, Sanzisabinli and Sewe cultivars had lower larval populations (15.50 and 9.13, respectively) which were significantly different from TVu 1509 (29.13), the resistant check, during the early season. However in the late season there were no significant differences in the larval populations across all the cultivars. IT90K-277-2 and Sanzisabinli had reduced air spaces (0.063 µm and 0.054 µm) in the calyx tissues which were significantly different ($P < 0.001$) from the resistant check (0.124 µm). Interestingly Sewe had no air space in the calyx tissues (0.00 µm). The positive highly significant correlation between the larval populations and calyx air space in the early season ($r = 0.56^{***}$) and the non-significant low correlation ($r = 0.18^{NS}$) during the late season suggest that Megalurothrips sjostedti uses other parts of the cowpea plant as oviposition substrate apart from the calyx tissues. Therefore, reduced air spaces in calyces of the resistant cultivars may not play any role as a biophysical factor in their underlying basis of resistance to the Flower bud thrips.

Keywords: *Megalurothrips sjostedti*, Calyx anatomy, resistance, *Vigna unguiculata*

INTRODUCTION

Cowpea, *Vigna unguiculata* (L.) Walp. is a major source of cheap protein in the humid tropics (Steel, *et al.*, 1985). A variety of dishes is prepared from the seeds (Dolvo, *et al.*, 1975). Leaves and pods are also consumed as vegetables in some parts of Africa (Singh, *et al.*, 1983). It is useful for making hay, silage and green manure as a cover crop (Oghiakhe, 1995). However despite its importance in farming systems and diets, insect pests poses great threats and challenges to actualising the full yield potentials of cowpea since they can reduce yield to virtually 300 Kg/ha and are responsible for up to 100 per cent yield loss (Jackai, 1995; Singh and Allen, 1980; Singh and Jackai, 1985).

The flower bud thrips (FBT), *Megalurothrips sjostedti* (Trybom) is a major pest of cowpea that causes abscission of flower buds, flowers and peduncles, leading to seed yield losses of up to 100 per cent (Taylor, 1969). Synthetic insecticides have been the commonly used method of controlling

thrips and other cowpea pests (Singh and Jackai, 1985). However due to the undesirable side effects of pesticides on humans and the environment, the focus has shifted to an integrated pest management scheme that reduces absolute reliance on insecticides and encourages the use of combination of management options such as habitat management, biological control, host plant resistance and a host of others.

Varying degrees of successes have been achieved in cowpea resistance research in Africa (Singh and Jackai, 1985, Oghiakhe, 1995). Apart from the renown TVu 1509 and TVx 3236 varieties reported to have moderate levels of resistance to *M. sjostedti* (Singh, 1977), four cultivars, IT90K-277-2, KVx404-8-1, Sanzisabinli and Sewe have been added to the list of resistant varieties (Alabi *et. al.,* 2011). These cultivars harbour low thrips populations under replicated field trials (Alabi *et al.,* 2003; IITA, 2005) and their mechanisms of resistance and biochemical basis of resistance have been determined (Alabi, *et al.,* 2004; 2011). *Megalurothrips sjostedti* adult females and some other thrips in the Suborder Terebrantia lay their eggs inside plant tissues (Tamò et al. 1993; Terry, 1997). The eggs are inserted into the plant tissues with a saw-like ovipositor. Thrips larvae emerging from the eggs usually cause more damage than adults because of their greater abundance, the gregariousness of some species and commitment to feeding (Ananthakrishnan, 1971; Wiesenborn and Morse, 1986). Studies on oviposition sites of *M. sjostedti* on cowpea revealed a very high percentage (94.2%) of eggs in calyx tube than in the petals (5.8%) (Okwakpam (1978)). Petals are not the usual oviposition sites whereas the high percentage of eggs in the calyx tube indicated that the calyx tissue is the preferred oviposition site for the eggs of *M. sjostedti* (Okwakpam, 1978). Furthermore Salifu *et al.* (1988) reported that *M. sjostedti* ovipositional nonpreference on TVx3236 was associated with reduced air spaces in the calyx tissue of TVx3236, a thrips-resistant cowpea genotype.

In other to understand the underlying basis of cowpea resistance to thrips, the biochemical bases of resistance was investigated (Alabi, *et al.,* 2006; 2011), however information on possible biophysical basis of resistance is lacking for the resistant cultivars investigated in this study. To what extent an insect population can establish on a particular plant is dependent on its ability to oviposit on different plants, amongst other factors. Therefore the research sought to elucidate the anatomy (structure and dimensions) of the calyces of the cowpea cultivars in other to establish the relationship between calyx anatomy and larvae populations encountered on the resistant cultivars under field conditions.

MATERIALS AND METHODS

Field evaluation of cowpea cultivars for resistance to *Megalurothrips sjostedti* in early and late season.

Six cowpea cultivars, IT90K-277-2, TVu1509, KVx404-8-1, Sanzisabinli, Sewe and Vita7, were obtained from International Institute of Tropical Agriculture, Ibadan, and screened for resistance to *Megalurothrips sjostedti* during the early and late cowpea planting seasons. TVu1509 is known to be resistant to thrips and Vita7 is susceptible (Jackai and Singh, 1988; Singh et al., 1983) therefore these two varieties were used as control standards throughout the experiment.

Experimental layout and procedure

Each cultivar was planted in five row plots of 5m long with an inter-row spacing of 1m. Distance between plants was 0.2 m and a distance of 1.5 m was left between adjacent plots following the design reported by Jackai and Singh (1988). Varieties were planted in a way to synchronize flowering since IT90K-277-2, KVx404-8-1 and Vita7 were medium maturing varieties and Sanzisabinli, Sewe and TVu1509 were early maturing varieties.

The test varieties were planted to fit a randomised complete block design with four replicates. An increase in thrips population was achieved by planting a susceptible variety (Vita7) as spreader rows in a checkerboard design two weeks prior to planting the experimental materials. At the raceme stage of the test plants, the spreader rows were uprooted and plants laid between rows of the test plants. This caused the thrips to move away from the drying plants to those of the test rows. Oviposition was assessed indirectly throughout the field experiment using larval counts on racemes and flowers of the different cowpea cultivars during the field experiments.

Calyxes morphological studies

Planting materials

Seeds of the six cowpea cultivars used for the field evaluation were sown in 18cm diameter pots in the screenhouse and the plants were kept insect-free throughout the period of the experiment. At flowering, 10 flowers were collected from each cultivar plants at similar physiological developmental stages, specifically 30 days after planting for sectioning.

Preparation and Sectioning of calyces

Preparation and sectioning of calyces of the different cowpea cultivars to investigate the underlying physical basis of cowpea resistance to thrips oviposition was achieved by adopting the method of Sass (1958).

Calyces of test cultivars were harvested and fixed in Formaldehyde fixing fluid (FAA) for 48 hours. Fixed calyces were then washed in series of graded Tertiary Butyl Alcohol solution (TBA) at an interval of 1 hour and after then infiltrated with paraffin wax. Thereafter, specimens were cut out into 0.5cm squares and fastened to a mounting block for sectioning on a microtome. Paraffin sections of 2 – 3 µm thick per specimen were cut for light microscopy and affixed to slides. After which they were stained with aqueous Safranin and Fast green, dried and 2 drops of resin (DPX) was placed on the sections and a cover glass lowered obliquely onto the resin. Calyx sections mounted on slides were viewed and photographed under light microscope to measure the internal diameter of the air space in the spongy layer using a micrometer scale affixed to the microscope eyepiece. Means of internal diameter were obtained from 10 replicated readings for each cultivar.

Data analyses

Population densities of *M. sjostedti* larvae were estimated by randomly picking 20 racemes and 20 flowers of cowpea per plot in the field trial. The racemes or flowers were placed separately in labelled glass vials containing 30% ethanol solution. Later, larvae of *M. sjostedti* were separated from the plant parts and counted under a macroprojector. The late season larval population data were subjected to log transformation so that the data can conform to the assumptions of a normal distribution. Subsequently all data were subjected to ANOVA and significant means were separated using Students New Kuels Test. Correlation analysis

was carried out to assess the degree of association between larval populations for each season and calyces air space measurements.

RESULTS

Larval populations on cowpea varieties in the early and late season

Larval populations in the early season were lower than the population in the late season (Table 1). The early season was the period of rains while the late season was the dry season period in Ibadan. In the early season, the cultivars were grouped into three based on the larval populations in the different cultivars: IT90K-277-2, Vita 7 and KVx404-8-1 in the first group; TVu 1509 in the second group; and Sewe in the third group. Sanzisabinli overlapped between the two groups of TVu1509 and Sewe. The highest larval population was observed on IT90K-277-2 however it was not significantly different from Vita7, the susceptible cultivar. Sewe had the lowest larval population that was not significantly different from Sanzisabinli.

In the late season due to the high larval population on the cultivars, there was no significant difference in the larval populations among the cultivars. However Vita 7 had the highest larval population among all the cultivars.

Table 1: Larval populations on cowpea cultivars in the first and second seasons

Cultivars	First Season	Second Season
IT90K-277-2	57.00 ± 4.86 a	111.33 ± 39.04 a
Vita 7	56.63 ± 6.92 a	191.33 ± 51.07 a
KVx404-8-1	46.50 ± 5.28 a	114.50 ± 37.43 a
TVu1509	29.13 ±3.50 b	187.33 ± 103.07 a
Sanzisabinli	15.50 ± 2.42 bc	123.17 ± 52.22 a
Sewe	9.13 ± 2.18 c	139.67 ±58.65 a

Means in each column followed by the same letter(s) are not significantly different at $P < 0.01$ using Student–Newman Kuels test. Values are means of 4 replications.

Sectioning of calyxes to reveal internal cell organization

Transverse sections of Vita 7 revealed the internal structures of the calyx under light microscopy (Plate 1). Within the sections there are parenchyma cells (a) and empty air spaces (b) where the eggs are laid. The plate shows two eggs embedded in the air space (c). Compared with the other cultivars whose calyxes were sectioned, the spongy layer of Vita 7 consists entirely of air spaces. The converse is true for the spongy layers of Sanzisabinli (Plate 2) and IT90K-277-2 (Plate 3). These cultivars have smaller spongy layers with a large area of tightly packed parenchymatous cells. Sections from calyces of KVx404-8-1 (Plate 4) revealed a larger amount of spongy layer than the calyces of Sanzisabinli and Sewe (Plates 2 and 5). Visually the diameter of air spaces in the calyces of KVx404-8-1 is almost similar to the spaces in Vita 7 calyxes. Though the diameter of air spaces in TVu 1509 (Plate 6) is large this is not uniform throughout the whole length of the calyx. Along the transverse section there are some portions where there was little or no space.

It was observed from the sections of Sewe calyces that there was no air space, that is, no spongy layer, whatsoever (Plate 5). Also the ground parenchyma cells of Sewe calyx were closely packed than in all the other cultivars. Hence no intercellular space measurements could be taken from the calyces of Sewe (Table 2).

Cultivars were conveniently grouped into four based on the calyces air space measurements (Table 2). Vita 7 calyces had the highest internal air space (0.162 µm) and it was significantly higher than all the other cultivars at $P < 0.001$. TVu 1509 (0.124 µm) was next to Vita 7 in ranking but it was not significantly different from KVx404-8-1 (0.118 µm). Down the list of measurements, the diameter of air spaces in the following cultivars decreased in the order: IT90K-277-2 > Sanzisabinli > Sewe. The diameters of air spaces in these cultivars were significantly lower as compared with the diameter of air spaces in TVu 1509. Furthermore, Sewe calyces had no air spaces within the calyx tissues.

Correlation analysis

Correlation analysis was conducted between larval populations and calyx air spaces in the early and late planting seasons (Table 3). A highly significant positive correlation was obtained during the early planting season ($r = 0.56$) at $p < 0.001$. However a non-significant, low correlation was obtained between these two parameters in the late season.

Table 2: Measurements of relative amount of air spaces present in the spongy layer of calyxes of cowpea cultivars.

Cultivars	Diameter of air spaces (µm)
Vita 7 (SC)	0.162 ± 0.006a
KVx404-8-1	0.118 ± 0.011b
TVu 1509	0.124 ± 0.013b
IT90K-277-2	0.063 ± 0.004c
Sanzisabinli	0.054 ± 0.005c
Sewe	0.000 ± 0.000d

Means followed by the same letter are not significantly different at $P < 0.001$ using Students Newman Keuls Test. Values are means ± SE of 10 replications. SC = Susceptible control

Table 3: Correlations between larval populations and Calyx air space in the early and late planting seasons

	Calyces air spaces	
	Early season	Late season
Larval populations	0.56***	0.18 NS

P < 0.001***

NS – Not significant

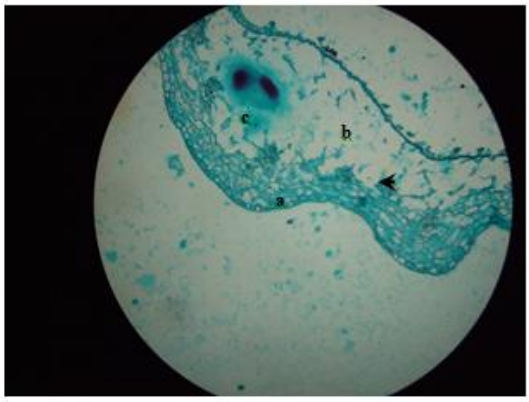

Plate 1: Transverse section of calyx of cowpea cultivar Vita 7, a = parenchyma; b = spongy layer; c =
ruptured egg of thrips. Arrowhead shows the air space. Magnification X 100

a

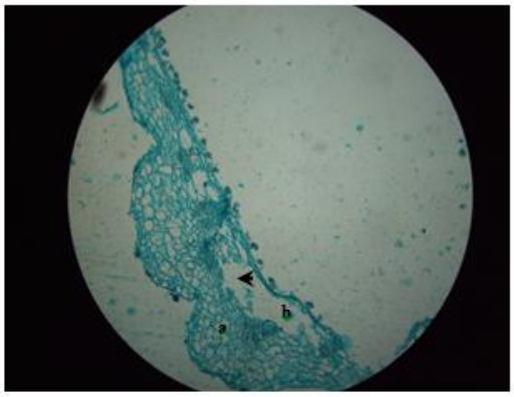

Plate 2: Transverse section of calyx of cowpea cultivar Sanzisabinli, a = parenchyma; b = spongy layer; Arrowhead shows the air space. Magnification X 100

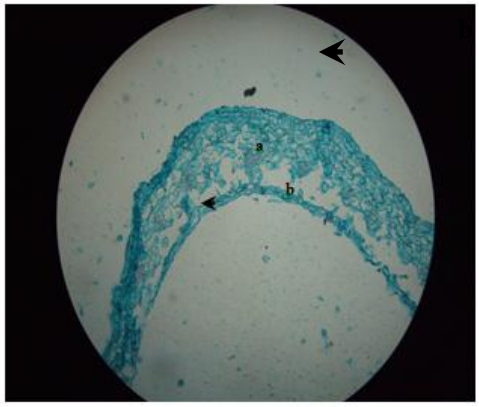

Plate 3: Transverse section of calyx of cowpea cultivar IT90K-277-2, a = parenchyma layer; b = spongy layer. Arrowhead shows air space Magnification X 100

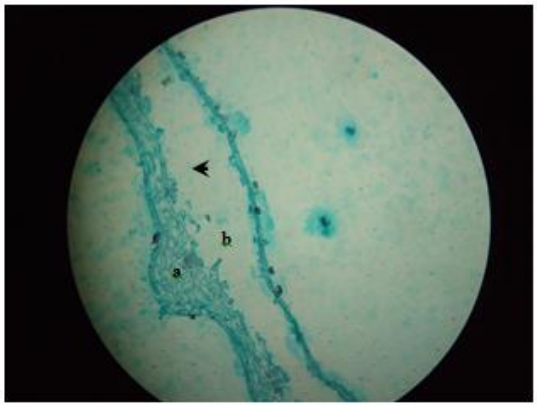

Plate 4: Transverse section of calyx of cowpea cultivar KVx404-8-1, a = parenchyma layer; b = spongy layer. Arrowhead shows air space Magnification X 100

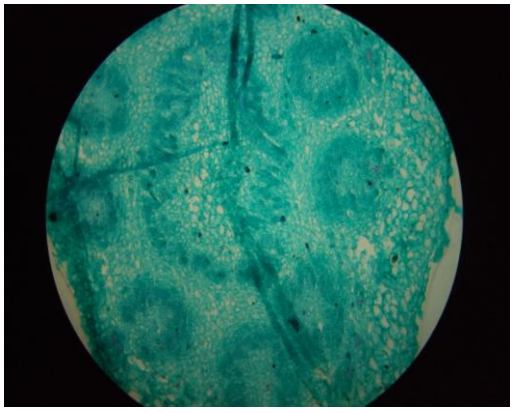

Plate 5: Transverse section of calyx of cowpea cultivar Sewe, a = parenchyma layer, Magnification X 100

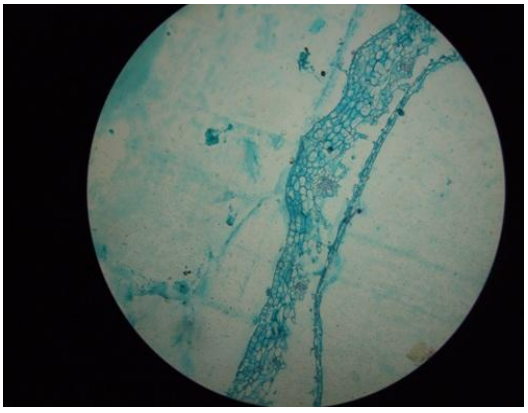

Plate 6: Transverse section of calyx of cowpea cultivar TVu 1509, a = parenchyma layer; b = spongy layer. Arrowhead shows air space Magnification X 100

DISCUSSION

Terry (1997) stated that the number of immatures provides a measure of the acceptability of the plant as a reproductive host. The results obtained in the early season agrees with Terry (1997) however this was not the case in the late season because the larval population on the resistant check, TVu 1509 was not significantly different from the susceptible check, Vita 7. Also, Irvin and Hoddle (2004) reported that there were no significant differences in the total number of *Homalodiaca coagulata* nymphs and adults, and the number of leaves with *H. coagulata* egg masses between Citrus limon cultivars 'Eureka' and 'Lisbon' under field trials. The lack of distinction between the cowpea resistant and susceptible checks could be as a result of high thrips population pressure in the late season. However, Irvin and Hoddle (2004) attributed the non-significant differences to differences in environmental factors and tree age between field-planted (~17 years of age) and small containerized trees (~2 years of age). However in the laboratory experiments *H. coagulata* preferred Eureka cultivar as an oviposition substrate compared with Lisbon. Therefore when screening cowpea cultivars for resistance to thrips in the field, screening should be conducted in the early and late seasons so as to ensure that high thrips population does not obscure good sources of resistance.

Tamo *et al.,* (1993) had earlier investigated the distribution of *M. sjostedti* eggs within-plant in field plants over time and found that changes in preference occurred over phenological stages. In the early season, leaf petioles are preferred to young inflorescences because of their small size, however after peduncle elongation, inflorescences are preferred, especially the older ones (Salifu, 1986; Tamo, *et al.,* 1993). In the case of Sewe cultivar where there are no air spaces inside the calyx, it further suggests that the female thrips utilized other parts of the plant like the leaf petiole, peduncles, or flower petals as its oviposition sites. In view of this, it is possible to deduce that during the late season, female thrips utilized other parts of the cowpea plants as oviposition sites due to the high number of thrips present during this season. This can be ascertained from the non-significant low correlation value between the larval population and calyces air spaces obtained during the late season. In addition, cowpea peduncles and pods have been used as oviposition structures and source of food to main *M. sjostedti* culture over a long period of time under laboratory conditions (Alabi, 2006; M. Tamo, personal communication).

CONCLUSION

From previous investigations conducted on the underlying basis of cowpea resistance to *Megalurothrips sjostedti*, biochemical factors in the resistant cowpea cultivars played a major role in the resistance of cowpea to *Megaluothrips sjostedti* (Alabi, *et. al.*, 2006; Alabi, *et al.,* 2011). Further investigations in this study to establish if reduced air spaces in the calyx tissues of resistant cultivars could be a biophysical factor of resistance in resistant cowpea cultivars was not confirmed. Therefore air spaces in calyx tissues of resistant cowpea cultivars was not responsible for *M. sjostedti* oviposition preference on the cultivars, since Sewe had no air space in its calyx tissues and it still harboured high larval populations, especially during the late season. This further confirms that *M. sjostedti* can utilize other parts of the plants as oviposition sites apart from the cowpea

calyx tissues. Therefore the size of air spaces in the cowpea calyces may not be one of the underlying bases of Sewe, Sanzisabinli, KVx404-8-1 and TVu1509 resistance to *Megalurothrips sjostedti*.

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