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BIOCHEMICAL CONSTITUENTS INFLUENCING THE RESISTANCE TO FLOWER BUD THRIPS IN COWPEA [*VIGNA UNGUICULATA* (L.) WALP] GERMPLASM

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ABSTRACT

The flower bud thrips, *Megalurothrips sjostedti,* is a major pest of cowpea that can cause yield losses of up to 100%. The use of cowpea cultivars resistant to thrips is among the most promising control measures. Six cultivars were evaluated in 2016 in Uganda for resistance to thrips under field conditions and analyzed for total carbon, total reducing sugar, total protein, soluble amino acid, total phenol, flavonoids, antioxidant activity and tannin contents. Data were subjected to analysis of variance, correlation and multiple linear regression analyses. The results showed that the genotypes responded differently to thrips damage and thrips counts in flowers and they presented different concentrations in total reducing sugar, total carbon, soluble amino acid, antioxidant activity, flavonoids and tannin in the plants parts. Cultivar TVU-1509 suffered the least thrips damage (1.03) while WC36 was severely damaged by thrips (6.55). A significant negative correlation was observed between thrips damage scores and total carbon concentration (r=-0.54) indicating that total carbon plays a significant role against thrips damage in cowpea. Increase in the concentration of flavonoids, total reducing sugar, total carbon in the plants contributed to the reduction of thrips damage (coefficient of regression = -1.47; -0.61 and -0.48, respectively) while the increase in the concentration of the soluble amino acid contributed to the increase of thrips damage (coefficient of regression = 2.10), suggesting that these biochemical conferred the resistance of cowpea to flower thrips damage. These biochemical compounds could be promising candidates to bolster cowpea cultivars 'resistance.

Keywords: Cowpea, flavonoids*, Megalurothrips sjostedti*, reducing sugar, total carbon, soluble amino acid.

INTRODUCTION

Cowpea, *Vigna unguiculata* (L.) Walp (Fabaceae) is an important staple food legume and cheap source of protein for many resource poor African households in the low-land humid and dry savannah tropics (Boukar *et al*., 2011). It is an excellent substitute for animal proteins (meat) in the diet of human being with its high seed protein content and rich amino acids. In some cultivars, seed protein content of about 30% has been reported (Santos *et al.*, 2012). Immature pods, immature seeds and young leaves of cowpea are also used as vegetables. Also cowpea plant residue is used as fodders and compost (Olawale and Bukola, 2016).

However, throughout the growth stages of cowpea from seedling until harvest, several important pests attack the crop, causing economic damage (Omo-Ikerodah *et al.,* 2009). The flower bud thrips, *Megalurothrips sjostedti* Trybom (Thysanoptera: Thripidae) is one of the major insect pests of cowpea. It attacks the reproductive structures of cowpea during plant development (Alabi *et al.*, 2011) and causes yield losses of up to 100% in Uganda (Karungi *et al.*, 2000c), Ghana (Abudulai *et al.*, 2006), Cameroon (Ngakou *et al.,* 2008) and Nigeria (Alabi *et al.,* 2011).

The production of cowpea based on host plant resistance to insect pests has taken a major importance in recent years (Salifu, 2001). The use of cowpea cultivars that are resistant to *M. sjostedti* is among the most promising control measures. It is economic and environmentally safe and can easily be integrated with other compatible pest management options such as habitat management and biological control (Tamo` *et al*., 2003). The less thrips damage caused on some cultivars could be due to the presence of toxic metabolites and/or to the absence or suboptimal amounts of some essential nutrients available to the insects (Saxena, 1985). A wide range of metabolic products mediate resistance in crop plants including primary (lectins, proteinase and amylase inhibitors) and secondary metabolites such as the phenolic acids, the alkaloids and the retenoids (Omitogun *et al.,* 1999). Machuka and Okeola (2000) reported that primary metabolites in seeds of African yam bean (*Sphenostylis stenocarpa* Hochst. ex A. Rich), particularly lectins and protease inhibitors, contribute more to the plant anti-nutritive properties than secondary metabolites. Smith *et al.* (1994) reported that both metabolites influenced the behavior and physiology of insects. Plant primary and secondary metabolites play a decisive role in determining host plant desirability by insects (Alabi *et al.*, 2005) and they greatly influence the

behavior and physiology of insect thereby imparting resistance or susceptibility of plants. Although the nutritional value of cowpeas has been well studied (Rivas-Vega *et al.*, 2006; Kabas *et al.*, 2007), very little is known about the existing levels of primary and secondary metabolites in reproductive structures of the test cowpea cultivars with respect to host plant resistance to *M. sjostedti*. Identification of compounds responsible for resistance can be of great value in developing resistant varieties (Snook *et al.,* 1997). Currently, some works have been done on the total protein, glucose content, terpenoids compounds, aglycones of flavones and flavonols content of cowpea floral buds in relation to resistance to *M. sjostedti* (Salifu, 2001; Alabi *et al.,* 2005; Alabi *et al*., 2011), however, little has been done on others compounds such as total phenol, tannin, amino acids, reducing sugars and antioxidant activity for all the cowpea reproductive structures (stipules, racemes, floral buds and flowers) in relation to flower bud thrips resistance since thrips damage the entire reproductive structures. Therefore, the objective of this paper is to investigate the role(s) of total proteins, amino acids, total carbon, reducing sugars, total phenol content, total tannin content, flavonoids and antioxidant activity of cowpea reproductive structures in resistance to *M. sjostedti* damage.

MATERIALS AND METHODS

Study site and plant materials: An evaluation of cowpea germplasm was conducted at Makerere University Agricultural Research Institute of Kabanyolo (MUARIK) in Uganda. MUARIK is located between longitude 32^{o} 37E, Latitude 0^{o} 28N and at 1200 m above sea level in the central region of Uganda (Sserumaga *et al.*, 2015). The average annual rainfall and temperature were 1150 mm and 21.50 ^{o}C, respectively. The soil is a sandy loam (Fungo *et al.,* 2011). Cowpea cultivars used in this study included four cultivars obtained from cowpea breeding project of MUARIK (IT2841*Brown, NE4, WC52, WC36), one resistant cultivar from International Institute of Tropical Agriculture (TVU-1509); and one resistant cultivar from Ghana (Sanzi). The cultivar TVU-1509 has been reported as highly resistant to flower bud thrips (Alabi *et al.*, 2011) and Sanzi as moderately resistant to flower bud thrips (Abudulai *et al.,* 2006). The cultivar IT2841*Brown was reported as resistant while NE4, WC52, WC36 as susceptible in some previous screening done in Uganda (Agbahoungba *et al.*, 2017). Cultivar TVU-1509 was used as resistant check and WC36 as susceptible check throughout the experiment.

Research design: The six cowpea cultivars were screened for resistance to *M. sjostedti* during the two rainy seasons of year 2016 at MUARIK under field conditions. The test cultivars were planted in a randomized complete block design with three replications. Each plot consisted of 4 rows of 5 m long and 0.75 m apart with an intra-rows space of 0.30 m. Three seeds were planted per hole and the seedlings were thinned to two plants per stand 10 days after sprouting. Regular weedings of the fields till maturity were done with hand hoe. An increase in thrips population was achieved by planting the susceptible check (WC36) as spreader rows in a checkerboard design two weeks prior to planting the experimental materials (Alabi *et al.,* 2005). 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 green rows (Alabi *et al.*, 2011). In both seasons no insecticide application was done.

In addition, seeds of the six cowpea cultivars were also planted in 18 cm diameter pots in the screen house in a completely randomized design with three replications. No insecticide application was made. Fourty-four days after planting, the reproductive structures of plant were collected for total proteins, soluble amino acids, total carbon, reducing sugar, total phenol content, total tannin content, flavonoids and antioxidant activity analyses in the biochemistry laboratory of National Crops Resources Research Institute (NaCRRI) in Uganda.

Data collection

Thrips damage rating and thrips counts in flowers: In field trial, visual rating of thrips damage was recorded during raceme and mid-podding stage (37- 55 days after planting) using the visual rating scale developed by Jackai and Singh (1988) (Table 1). The damage scores were recorded on twenty plants randomly selected within the two middle rows using a 1-9 scale from 37 days after planting and subsequently at weekly intervals during two weeks (scores were defined as: 1-3 = resistant, 4-6 = moderately resistant and 7-9 = very susceptible). Population densities of *M. sjostedti* were estimated by randomly picking 20 racemes and 20 flowers of cowpea per plot at 55 days after planting and at weekly interval for 2 weeks. The flowers were placed separately in labelled glass vials containing 70% ethanol solution. Later, nymphs and adults of *M. sjostedti* were separated from the plant parts and counted.

Plant metabolites determination: The terminal leaves, racemes, floral buds and flowers of the six cowpea genotypes were collected (44 days after planting) and dried at 32^{o} C in a hot-air Owen for 48 hours. The samples were powdered using mixer Guangzhou Co, Ltd. for 3 min (Kandakoor *et al.,* 2014). The powdered samples were sieved through a 100 mesh screen and

stored in sealed plastic containers (0.5 m diameter) at 4° C, for the biochemical analyses.

Total proteins: The determination of the total proteins was composite performed on 100 mg of terminal leaves, racemes, floral buds and flowers powder incubated in 5 ml of extraction buffer (Tris-HCl at 25 mM and pH 7.6). The mixture was kept in agitation for 2 hours, afterwards centrifuged at 2000 rpm for 10 min at 20° C and subsequently the supernatant was removed. The quantification of the total soluble proteins was carried out at 595 nm according to Bradford (1976) with albumin bovine (Sigma Chemicals) used as standard. The total protein content was calculated as percentage per 100 g dry weight from the calibration curve Y=0.0057X-0.0064; R^2=0.9935.

Soluble amino acids: The soluble amino acids were determined in 50 mg samples composed of terminal leaves, racemes, floral buds and flowers dry matter powder that were incubated in 5 ml of sterile distilled water at 100° C for 30 min. After being homogenized, it was centrifuged at 2000 rpm for 5 min at 20° C and the supernatant was removed. A volume of 1 ml of ninhydrin reagent was added to 1 ml of extract and boiled in a specimen tube over water bath for 20 min. The specimen tubes were cooled under running water and the volume was made up to 10 ml with diluents solution till it developed a purple color and absorbance was read at 570 nm. A standard curve Y=0.0482X - 0.048; R^2=0.9684 was used with glutamate to calculate the concentration (µg/100 g glutamine equivalent) of total soluble amino acids (Kandakoor *et al*., 2014).

Total carbon (Sugar): Total sugar and non-reducing sugars were hydrolyzed in 1 ml of 1.0N H_2SO_4 to 0.5 ml of aliquot and heated over boiling water bath for 30 min. After cooling under running water, one to two drops of phenolphthalein indicator were added. Later, 1.0N NaOH was added drop by drop to neutralize the acid in the hydrolysate till it developed pink colour. Further, 1.0N H_2SO_4 was added to make it colourless. Finally the volume was made up to 10.0 ml with distilled water and absorbance was read at 510 nm. The total carbohydrates content (%) was calculated from the calibration curve Y=0.0151X - 0.0151; R^2= 0.9935.

Reducing sugars: Reducing sugars were estimated in 0.4 ml of aliquot by adding 1 ml of Nelson's reagent A [25 g of carbonate (anhydrous), 25 g of Rochelle's salt (Sodium potassium tartrate), 20 g of sodium bicarbonate and 200 g of sodium sulfate were dissolved in 800 ml distilled water and diluted to one liter] + Nelson's reagent B [15 g of copper sulfate were dissolved in a small quantity of distilled water and made up to 100 ml and a few drops of concentrated H_2SO_4 were added]. The mixture was heated for 20 min. After cooling in running water, 1 ml of arsenomolybdate solution was added and finally the volume was made up to 10 ml with distilled water. The absorbance was read at 510 nm. A standard graph was constructed using glucose solution as a standard (Kandakoor *et al.,* 2014). The concentration of reducing sugars (%) was calculated using the calibration curve Y=0.0151X - 0.0151; R^2= 0.9935.

Total phenol content: One hundred milliliter (100 ml) of oven-dried powdered was extracted in 10 ml of warm 80% ethanol for 1hr at room temperature. The extract was centrifuged at 6000 rpm for 15 min. The supernatant was evaporated to dryness on a water bath and the residue was dissolved in 5 ml water. The total phenol content was measured using the Folin-Ciocalteu method (Singleton *et al*., 1999). Aliquot (0.5 ml) of each extract was mixed with 10% (volume/volume) Folin reagent (2.5 ml) and 7.5% (weight/volume) sodium carbonate (2.0 ml). The mixture was incubated in a water bath at 40° C for 30 min. Absorbance was measured at 765 nm using a Bio-Wave UV-VIS spectrophotometer (Guangzhou Co., Ltd). Gallic acid was used as standard. Total phenolic compound was expressed as mg per Gallic Acid Equivalent (GAE) per gram using the curve (Y=0.0765X-0.1477)*100; R^2=0.941(Shad *et al.*, 2013).

Total tannin content: One hundred milliliter (100 ml) of oven-dried powdered sample was extracted with 5 ml of methanol for 24 hours at room temperature with occasional stirring. The extract was centrifuged at 5000 rpm for 10 min. The supernatant was used to estimate the total tannins (Burns, 1971). A standard graph was constructed using catechin as a standard Y= 0.0076X-0.0088; R^2=0.9884. The total tannin content was expressed as $mg.g^{-1}$d.wt (Kandakoor *et al.,* 2014).

Total flavonoids content: The total flavonoids were extracted by soaking 1 g of plant sample in aqueous methanol (70% volume/volume) for 24 hours at room temperature. The total flavonoids content in methanolic extract obtained after filtration was estimated by Aluminium chloride (AlCl3) method described by Michalaska *et al.* (2007). An aliquot (1 mL) of methanolic extract was treated with 5% Sodium nitrate (NaNO3) (1 ml). After 6 min, 10% AlCl3 (1 ml) was added and volume was made up to 25 ml with 50% ethanol. The solution was allowed to stand at room temperature for 15 min and absorbance was recorded at 510 nm using UV-visible Spectrophotometer. The flavonoids content was calculated as catechin equivalent (gram/100 gram of dry weight) from the calibration curve Y= 0.2515X - 0.048; R^2 =0.9991 (Shad *et al.*, 2013).

Total antioxidant content: The total antioxidants content in methanolic extracts of plant parts were determined by the method described by Shad *et al.* (2013). Methanolic extract (1 ml) was mixed with 40 µM methanolic solution (3 ml) of stable 2, 2-dipheny- 1-picrylyhydrazyl (DPPH) radical. The solution was made

stable for 30 min at room temperature and absorbance was recorded at 517 nm. The amount of the total antioxidant activity was calculated as mg Trolox and ascorbic acid equivalent/100 g of extract by applying the linear regression equation obtained from a calibration curve Y=0.0218X -0.0318; R^2=0.9831.

Data analysis: Thrips damage scores, thrips counts in flowers and biochemical constituent concentrations were subjected to analysis of variance using Genstat 12th edition computer software (Payne *et al*., 2009). The means were separated using Student Newman-Keuls Test (SNK) at 5% of significance.

Thrips damage scores, thrips counts in flowers and biochemical constituent concentrations of the six cowpea cultivars were also subjected to Pearson correlation analysis to assess the association among the parameters. A multiple linear regression analysis was performed between biochemical constituents and thrips damage scores. A similar analysis was performed between thrips counts in flowers and the biochemical constituents as well.

RESULTS

Thrips damage scores and thrips counts in flowers as influenced by cowpea genotypes and seasons: The results from the analysis of variance showed that there was a significant (P<0.001) difference among cowpea genotypes for thrips damage scores and thrips counts in flowers. Also, season significantly (P<0.001) influenced thrips damage and thrips counts in flowers on cowpea. The results also showed that there was a significant (P<0.01) genotype by season interaction for thrips damage scores but not significant for thrips counts in flower (Table 2).

In the two seasons, the cultivar WC36 had the highest thrips counts in flowers (5.06) and was significantly (P<0.001) different from others while Sanzi had the lowest value (1.00) (Table 3). With regards to thrips damage scores, the cultivar WC36 was severely damaged by thrips (6.55) as compared to all the other cultivars. TVU-1509 suffered the least damage (1.05) in the late season but it was not significantly different from damage observed on Sanzi (1.05) and the resistant cultivar IT 2841*Brown (1.15). The thrips damage observed on WC36, WC52 and NE4 was significantly (P< 0.05) higher than those observed on Sanzi, IT284*Brown and TVU-1509 in the late season. However, WC36 was significantly (P < 0.01) more damaged compared with the other susceptible cultivars WC52 and NE4 that harbored similar number of thrips in the late season (Table 3).

Variation of biochemical constituent concentrations in cowpea genotypes: The analysis of variance on the biochemical constituents showed that there were significant (P<0.01) differences among the genotypes for the total reducing sugar, flavonoids, soluble amino acid, antioxidant, total carbon and tannins content but no significant differences among genotypes for the protein and phenol (Table 4).

The coefficients of variation of the biochemical constituent concentrations in cowpea cultivars were presented in Table 4. Tannins content recorded the least variation 11.20%, in contrast to the total reducing sugar, flavonoids, total carbon and soluble amino acid which recorded higher coefficients of variation of 25.60, 23.50%, 22.20 and 20.70%, respectively.

The means concentrations of the different biochemical constituents in cowpea genotypes are presented in Table 5. The results showed that total protein concentration (%) ranged from 8.34 in TVU-1509 the resistant control to 11.55 in WC52 a susceptible cultivar. Total reducing sugar concentration (%) ranged from 2.21 in Sanzi and WC36 cultivars to 6.63 in TVU-1509 the resistant control. Flavonoids concentration (mg/100 g) ranged from 4.22 in Sanzi to 6.76 in TVU-1509. Phenol concentration (mg/100 g) ranged from 514.10 in NE4 to 683.10 in WC52. Soluble amino acid concentration (µg/100 g of glutamine equivalent) ranged from 1.87 in sanzi to 4.80 in TVU-1509. Antioxidant concentration (µ mole trolox equivalent) ranged from 2.87 in Sanzi to 7.83 in IT241*Brown. Total carbon concentration (%) ranged from 10.05 in WC36 the susceptible control to 16.01in IT2841*Brown. Tannin concentration (mg/100 g) ranged from 3.48 in NE4 to 5.79 in IT2841 *Brown.

Relationship between thrips damage, thrips counts in flowers and biochemical constituents in cowpea genotypes: The results of the Pearson correlation showed that the thrips counts in flowers was significantly (P<0.05) and positively correlated with the thrips damage scores (r=0.54) (Table 6). The antioxidant concentration was significantly (P<0.05) and positively correlated with tannin (r=0.48) and total carbon (r=0.52). The flavonoids were positively correlated with phenols (r=0.57), proteins (r=0.48) and soluble amino acid (r=0.74). Tannins and phenol contents were significantly (P<0.001) positively correlated (r=0.77). Soluble amino acid was positively correlated with total reducing sugar (r= 0.62) and phenol content (r=0.47). However, thrips damage scores and total carbon concentration were significantly (P<0.05) negatively correlated (r=-0.54) (Table 6). All correlations that were significant when considering all the six cowpea cultivars, were also significant for the three resistant cultivars as presented in table 7, except for the correlations between antioxidant content and thrips counts in flowers (r = 0.61), total reducing sugar and thrips damage scores (r = - 0.51) that became significant (P<0.05).

The multiple linear regression performed between the thrips damage scores and the biochemical

constituents revealed that there was a significant (P<0.05) linear relationship between thrips damage scores and the all biochemical constituents (Table 8). The results from Wald test on the regression coefficients showed that the coefficients of regression of flavonoids content (-1.47), total reducing sugar (-0.61), total carbon (-0.48) and soluble amino acid (2.10) were significant (P<0.05) (Table 9). These biochemical constituents explained 77% of the total variation observed in thrips damage scores on the cowpea genotypes (R^2 = 0.77). The biochemical constituents such as flavonoids, total reducing sugar and total carbon with high concentration in the terminal leaves, flower bud and flowers favored less thrips damage in cowpea while the low concentration of soluble amino acid permitted low thrips damage. However, the regression between thrips counts in flowers and the biochemical constituents was not significant (P > 0.05).

Table 1. Scale for rating flower bud thrips damage on cowpea.

Rating	Appearance
1	no browning/drying (i.e scaling) of stipules, leaf or flower buds; no bud abscission
3	initiation of browning of stipules, leaf or flower buds; no bud abscission
5	distinct browning/drying of stipules and leaf or flower buds; some bud abscission
7	serious bud abscission accompanied by browning/drying of stipules and buds; non-elongation of peduncles
9	very severe bud abscission, heavy browning, drying of stipules and buds; distinct non-elongation of (most or all) peduncles

Source: Jackai and Singh (1988)

Table 2. Means squares for thrips damages scores and thrips counts in the flowers of six cowpea cultivars in Uganda, 2016.

Source of variation	d.f.	Means squares	
		Thrips damage scores	Thrips counts
Season/Blocks	4	0.80^{ns}	4.62^{ns}
Genotypes	5	43.68^{***}	8.44^{**}
Seasons	1	5.38^{***}	14.69^{*}
Genotypes x Seasons	5	1.16^{**}	3.69^{ns}
Residual	22	0.27	1.97
CV (%)		15	55.9

***, **, * significant at P<0.001; 0.01 and 0.05 respectively and ns: non-significant at P> 0.05

Table 3. Means number of thrips count in flowers and thrips damages scores of six cowpea cultivars in Uganda, 2016.

Cultivars	Adults thrips/flower	Thrips damage scores		
	Means	Early season	Late season	Means
IT2841*Brown	1.46^{a}	1.15^{a}	1.15^{a}	1.15^{a}
NE4	2.60^{a}	3.25^{b}	6.05^{b}	4.65^{b}
Sanzi	1.00^{a}	1.14^{a}	1.05^{a}	1.10^{a}
TVU-1509	1.10^{a}	1.00^{a}	1.05^{a}	1.03^{a}
WC36	5.06^{b}	6.08^{c}	7.01^{c}	6.55^{c}
WC52	2.19^{a}	3.71^{b}	6.03^{bc}	4.87^{b}

Columns followed by the same number of letters are not significantly different at P<0.05 using Student-Newman-Keuls test.

Table 4. Means squares for the biochemical constituents from six cowpea cultivars in Uganda, 2016.

Source of variation	d.f.	Means squares							
		Protein	Total Reducing sugar	Flavonoid	Phenol content	Soluble amino acid	Anti-oxidant	Total carbon	Tannins
Genotypes	5	5.00^{ns}	11.46^{**}	3.24*	14575^{ns}	2.87^{**}	9.29^{***}	16.32^{*}	2.28^{**}
Residual	12	3.40	1.44	1.43	13970	0.55	0.26	7.199	0.29
Total	17								
CV (%)		19.7	25.6	23.5	18.9	20.7	11.7	22.2	11.2

***, **, * significant at P<0.001 and 0.01 and 0.05 respectively and ns: non-significant at P> 0.05

Table 5. Means concentrations of biochemical constituents in six cowpea cultivars in Uganda, 2016.

cultivars	Protein (%)	Total reducing Sugar (%)	Flavonoids (g/100g)	Phenol content (mg/g)	Soluble Amino Acid (µg/100g)	Anti oxidant activity (mg/100	Total carbon (%)	Tannin (mg/g)
IT2841*Brown	8.67^{a}	5.73^{b}	4.91^{ab}	678.6^{a}	3.55^{bc}	7.83^{c}	16.01^{b}	5.79^{d}
NE4	10.29^{a}	5.64^{b}	4.99^{ab}	514.1^{a}	3.75^{bc}	3.55^{ab}	10.97^{a}	3.48^{a}
Sanzi	8.41^{a}	2.21^{a}	4.22^{ab}	568.2^{a}	1.87^{a}	2.87^{a}	11.47^{a}	4.38^{ab}
TVU-1509	8.34^{a}	6.63^{b}	6.76^{b}	630^{a}	4.80^{c}	4.06^{b}	13.72^{a}	4.49^{bc}
WC36	8.86^{a}	2.21^{a}	4.89^{ab}	674.8^{a}	3.32^{b}	3.85^{b}	10.05^{a}	5.41^{cd}
WC52	11.55^{a}	5.70^{b}	5.38^{a}	683.1^{a}	4.13^{bc}	3.88^{b}	10.25^{a}	5.48^{d}

Columns followed by the same letters are not significantly different at P<0.05 using Student-Newman-Keuls test.

Table 6. Correlation coefficients between thrips counts in flowers, damages scores and the biochemical constituents in six cowpea cultivars in Uganda, 2016.

	AntiA	Flav	NT	Tan	TDS	TRS	TC	Phe	Prot	SAA
AntiA	-									
Flav.	0.11	-								
NT	0.02	-0.11	-							
Tan.	0.48*	0.17	0.23	-						
TDS	-0.32	-0.05	0.54*	0.11	-					
TRS	0.29	0.37	-0.45	0.09	-0.23	-				
TC	0.52*	-0.13	-0.13	0.04	-0.54*	0.1	-			
Phe	0.26	0.57*	0.13	0.77***	0.12	0.19	-0.1	-		
Prot.	-0.08	0.48*	0.14	0.04	0.34	0.19	-0.21	0.32	-	
SAA	0.18	0.74***	-0.05	0.15	0.09	0.62**	0.11	0.47*	0.36	-

***, **, * significant at P<0.001, 0.01 and 0.05 respectively (two-sided test)

AntiA: Concentration in Antioxidant Activity; Flav: Concentration in flavonoids (mg/100 g of leaves and flowers); NT: Numbers of thrips per flower; Tan: Concentration in tannins (mg/100 g of leaves and flowers); TDS: Thrips damage scores; TRS: Total reducing sugar (%); TC: Concentration in total carbon (%); Phen: Concentration in phenol (mg/100 g of leaves and flowers); Prot: Concentration in protein (%); SAA: Concentration in Soluble Amino Acid (ug/100 g glutamine equivalent).

Table 7. Correlation coefficients between thrips counts in flowers, damages scores and the biochemical constituents in the three resistant cowpea cultivars in Uganda, 2016.

	AntiA	Flav.	NT	Tan.	TDS	TRS	TC	Phe	Prot	SAA
AntiA	-									
Flav.	0.07	-								
NT	0.61*	0.27	-							
Tan.	0.77*	0.16	0.19	-						
TDS	0.27	-0.29	0.43	0.31	-					
TRS	0.4	0.59	0.03	0.47	-0.51*	-				
TC	0.49	-0.11	0.29	0.38	0.17	0.12	-			
Phe	0.31	0.70*	0.16	0.71*	0.19	0.52	0.06	-		
Prot	0.12	0.73*	0.33	0.28	0.19	0.07	-0.04	0.71*	-	
SAA	0.25	0.78*	0.2	0.22	-0.45	0.78*	0.32	0.49	0.3	-

* Significant at P<0.05 (two-sided test)

AntiA: Concentration in Antioxidant Activity; Flav: Concentration in flavonoids (mg/100g of leaves and flowers); NT: Numbers of thrips per flower; Tan: Concentration in tannins (mg/100g of leaves and flowers); TDS: Thrips damage scores; TRS: Total reducing sugar (%); TC: Concentration in total carbon (%); Phen: Concentration in phenol (mg/100g of leaves and flowers); Prot: Concentration in protein (%); SAA: Concentration in soluble Amino Acid (ug/100g glutamine equivalent).

Table 8. Analysis of variance of the linear regression on thrips counts in flowers, thrips damage scores and biochemical constituents in six cowpea cultivars in Uganda, 2016.

Source of variation	Degree of freedom	Mean sum of squares	
		Thrips damage scores	Thrips counts in flower
Regression	8	7.78*	4.07ns
Residual	9	2.07	3.12
Total	17	4.755	

* Significant at P< 0.05; ns=non-significant at P>0.05

Table 9. Estimates of parameters in thrips damage scores and biochemical constituents linear regression.

Parameters	Estimates	Standard error	t(9)	Wald statistic	F pr.
Constant	7.09	3.32	2.14	-	0.06
Antioxidant	0.05	0.31	0.15	0.02	0.88
Flavonoids	-1.47*	0.56	-2.62	6.87	0.03
Phenol	-0.01	0.01	-0.79	0.63	0.45
Protein	0.42	0.21	2.01	4.06	0.08
Soluble amino acid	2.10**	0.62	3.42	11.70	0.01
Tannins	0.83	0.82	1.01	1.03	0.34
Total reducing sugar	-0.61*	0.23	-2.66	7.06	0.03
Total carbon	-0.48*	0.15	-3.25	10.55	0.01

**, * significant at P<0.01 and 0.05

The linear regression equation was: Thrips damage scores = - 0.61*total reducing sugar - 1.47* flavonoids + 2.10*soluble amino acid - 0.48* total carbon.

DISCUSSION

The study showed the cultivars TVU-1509, Sanzi and IT2841*Brown to be resistant to flower bud thrips damage as previously reported on TVU-1509 and Sanzi by Alabi *et al.* (2004), Abudulai *et al.* (2006) and Omo-Ikerodah *et al.* (2008) in West Africa where these cultivars were found resistant to flower bud thrips. The significant genotype by season effect for thrips damage could be explained by the variation in the climatic conditions since the reaction of cowpea to thrips damage is dependent on the environmental factors as reported by Ekesi *et al.* (1999) and Murage *et al*. (2012). The positive correlation coefficient obtained between the thrips count in flowers and the thrips damage scores in these cultivars indicated that the increase in the number of thrips in flowers of these cultivars provoked the increase of the damage caused by thrips.

The variations in the levels of thrips damage among resistant cultivars harboring similar number of thrips as vividly exemplified by TVU-1509, suggests that there are inherent underlying plant factors that are responsible for such differences. Plant defensive secondary compounds such as phenolics, tannins, alkaloids, terpenoids and flavonoids have been reported to increase resistance to thrips in cowpea and groundnut by Alabi *et al.* (2011) and Kandakoor *et al.* (2014). Kandakoor *et al.* (2014) reported that plant phenolics reduced feeding, altered fecundity and the duration of post-embryonic development of thrips in groundnut. The varietal resistance to *M. sjostedti* that was observed in the test cowpea cultivars in this study can partially be explained by the total reducing sugar, total carbon, soluble amino acid, antioxidant, flavonoids and tannin contents. The higher coefficient of variation of these biochemical parameters among cultivars could explain the differences observed in the thrips populations and damage. Concentration of the total reducing sugar, soluble amino acid, antioxidant, total carbon and tannin were found to be higher in the cultivars IT2841*Brown, Sanzi and TVU-1509 (resistant control), suggesting that these compounds were probably the biochemical constituents conferring the resistance to flower thrips in these cultivars. The highly significant and negative correlations observed between thrips damage scores and total carbon content and total reducing sugar when all the cultivars were considered, may suggest that resistance/susceptibility of cowpea cultivars to *M. sjostedti* was more influenced by the total carbon content than the other biochemical constituents. The negative relationship observed between thrips damage scores and total carbon and total reducing sugar may suggest that the higher the total carbon and total reducing sugar content in cowpea, the less thrips damage in these cultivars. However, McNeill and Southwood (1978) reported that primary metabolites such as sugars stimulate feeding of insects. Furthermore, Alabi *et al.* (2005), found a positive and significant relationship between glucose content and damage indices in all the cultivars. This kind of variations

between studies could be attributed to the difference in the cultivars, the environmental factors and pest biotypes.

On the other hand, terminal leaves, floral buds and flowers of the susceptible cultivars WC36, WC52 and NE4 contained higher levels of total proteins than all the resistant cultivars, suggesting that the higher quantity of the total protein in these cowpea could have contributed to their susceptibility to flower thrips damage. In fact, it was reported that cultivars with higher quantity of total proteins enhanced susceptibility to thrips damage (Alabi *et al.*, 2005). Also, according to Ananthakrishnan (1993), plants containing high amino nitrogen induce thrips to lay more eggs. In a similar study, Olatunde and Odebiyi (1991) concluded that cultivars with higher crude protein content were most often preferred for feeding by *C. tomentosicollis* and supported faster developmental period of the nymphs as well as higher egg production by females. In addition, the effects of soluble amino acid on *Frankliniella occidentalis* populations have been reported by Brodbeck *et al.* (2001) where the population of thrips was significantly higher on hosts with higher rates of soluble amino acid in tomato.

The highly significant and positive correlations observed between certain biochemical constituents indicates that these biochemical compounds are probably controlled by the same set of genes and, breeder may target those biochemical parameters to influence the other parameters.

The multiple linear regressions analysis showed that the increase in the flavonoids concentration, total reducing sugar content, total carbon caused the reduction of the thrips damages in the resistant cowpea cultivars. The flavonoids were known to possess antioxidant activities due to the presence of hydroxyl groups in their structures and their contribution to defense system against the oxidative damage due to endogenous free radicals is extremely important (Miranda and Buhler, 2002; Boskou, 2006). The effects of total carbohydrates and reducing sugar on *Thrips tabaci* and *Frankliniella occidentalis* were reported by Pobożniak and Koschier (2014); Žnidarčič *et al.* (2007) where thrips showed weak preference on cabbage heads with high amount of total carbohydrate, fructose and glucose.

The biochemical constituents found in this study, complete the list of the primary and second metabolites influencing the resistance to *M. sjostedti* reported by Alabi *et al*. (2005 and 2011) in cowpea. Alabi *et al.* (2005) found that total protein and glucose contents are responsible for resistance of cowpea cultivars to *M. sjostedti* and are embedded in the floral buds and flowers. In addition, Alabi *et al.* (2011) reported that terpenoids extracts from racemes of resistant cowpea cultivars caused significant mortality to second instar larvae of *M. sjostedti*. This study, revealed that increase in flavonoids, total reducing sugar, total carbon contents, conferred resistance to *M. sjostedti* in cowpea whereas the increase in soluble amino acid brought susceptibility.

Conclusion: The cowpea cultivars (TVU-1509, Sanzi and IT2841*Brown) were found to be resistant to flower thrips. These genotypes may prove promising in breeding programme concerning *Megalurothrips sjostedti* resistance. Cowpea plant metabolites investigated in this study, revealed that flavonoids, total reducing sugar and total carbon conferred resistance to *M. sjostedti* and when present in large amounts in the stipules, floral buds and flowers of the resistant cultivars, whereas soluble amino acid brought susceptibility. Flavonoids, total reducing sugar and total carbon could be promising candidates for enhancement to bolster cowpea cultivars' resistance.

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REFERENCES

Abudulai, M., A. B. Salifu and M. Haruna (2006). Screening of cowpea for resistance to the flower bud thrips, *Megalurothrips sjostedti* Trybom (Thysanoptera: Thripidae). J. Appl. Sci. 6: 1621–1624.

Agbahoungba, S., J. Karungi, T.L. Odong, A. Badji, K. Sadik and P.R. Rubaihayo (2017). Stability and extent of resistance of cowpea lines to *Megalurothrips sjostedti* Trybom (Thysanoptera: Thripidae) in Uganda. Afr. Crop Sci. J. 25(1): 1 – 24.

Alabi, O.Y., J. A. Odebiyi and M. Tamo (2004). Effect of host plant resistance in some cowpea (*Vigna unguiculata* L.Walp) cultivars on growth and developmental parameters of the flower bud thrips, *Megalurothrip sjostedti* (Trybom). J. Crop Prot. 23: 83–88.

Alabi, O. Y., J. A. Odebiyi and M. Tamo (2005). The relationship between primary metabolites in reproductive structures of cowpea *Vigna unguiculata* (Fabaceae: Papilionidae) cultivars and field resistance to the flower bud thrips *Megalurothrips sjostedti* (Thysanoptera: Thripidae). Int. J. Trop. Insect Sci. 26 (1): 8–15.

Alabi, O. Y., J. A. Odebiyi, M. Tamò and A. Omoloye (2011). The roles of plant secondary metabolites from cowpea floral structures in resistance to the flower bud thrips. J. Agr. Sci.1: 262–269.

Ananthakrishnan, T. N. (1993). Bionomics of thrips. Annu. Rev. Entomol. 38: 71–92.

Bradford, M.M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 722: 248-254.

Boskou, D. (2006). Sources of natural phenolic antioxidants. Trends Food Sci. Technol. 17:505–512.

Boukar, O., F. Massawe, S. Muranaka, J. Franco, B. Maziya-Dixon and B. Singh (2011). Evaluation of cowpea germplasm lines for protein and mineral concentrations in grains. Plant Genet. Resour. 9: 515–522.

Brodbeck, B.V., J. Stavisky, J.E. Funderburk, P.C. Andersen and S.M. Olson (2001). Flower nitrogen status and populations of *Frankliniella occidentalis* feeding on *Lycopersicon esculentum*. Entomol. Exp. Appl. 99(2): 165–172.

Burns, R.E. (1971). Method for estimation of tannin in sorghum grain. Agron. J. 63: 511-512.

Ekesi, S., N. K. Maniania and I. Onu (1999). Effects of temperature and photoperiod on development and oviposition of the legume flower thrips, *Megalurothrips sjostedti*. Entomol. Exp. Appl. 93: 145–155.

Fungo, B., S. Grunwald, M.M. Tenywa, B. Vanlauwe and P. Nkedi-Kizza (2011). Lunnyu soils in the Lake Victoria basin of Uganda: Link to toposequence and soil type. Afr. J. Environ. Sci. Technol. 5(1): 15–24.

Jackai, L.E.N. and S.R. Singh (1988). Screening techniques for host plant resistance to insect pests of cowpea. Trop. Gr. Leg. Bull. 35: 2–18.

Kabas, O., E. Yilmaz, A. Ozmerzi and I. Akinci (2007). Some physical and nutritional properties of cowpea seed (*Vigna sinensis* L.). J. Food Eng. 79 (4): 1405–1409.

Kandakoor, S.B., H. Khader Khan, A.K. Chakravarthy, C.T. Ashok Kumar and P. Venkataravana (2014). Biochemical constituents influencing thrips resistance in groundnut germplasm. J. Environ. Biol. 35: 675–681.

Karungi, J., E. Adipala, P. Nampala, M.W. Ogenga-Latigo and S. Kyamanywa (2000c). Pest management in cowpea. Part 3. Quantifying the effect of cowpea field pests on grain yields in eastern Uganda. J. Crop Prot. 19: 343–347.

Machuka, J. and G.O. Okeola (2000). One and two dimensional gel electrophoresic identification of African yam bean seed proteins. J. Agric. Food Chem. 48: 2296–2299.

McNeill, S. and T.R.E. Southwood (1978). The role of nitrogen in the development of insect/plant relationships. Biochemical Aspects of Plant and Animal Coevolution. Proceedings of the Phytochemical Society. Academic Press, San Francisco, (California). 98p.

Michalaska, A., A. Ceglinska and H. Zielinski (2007). Bioactive compounds in rye flours with different extraction rate. Eur. Food Res. Technol. 225: 545–551.

Miranda, A., and R.D. Buhler (2002). Antioxidant activities of flavonoids. Department of Environmental and Molecular Toxicology, Linus Pauling Institute, (Oregon State University).

Murage, A.W., G. Obare, J. Chianu, D.M. Amudavi and C.A.O. Midega (2012). The effectiveness of dissemination pathways on adoption of "push-pull" technology in Western Kenya. Int. J. Agr. Sci. 51: 51–71.

Ngakou, A., M. Tamò, I.A. Parh, D. Nwaga, N.N. Ntonifor, S. Korie and C.L.N. Nebane (2008). Management of cowpea flower thrips, *Megalurothrips sjostedti* (Thysanoptera: Thripidae), in Cameroon. J. Crop Prot. 27: 481–488. doi: 10.1016/j.cropro.2007.08.002.

Olatunde, G.O. and J.A. Odebiyi (1991). The relationship between total sugar, crude protein and tannic acid contents of cowpea, *Vigna unguiculata* L. Walp. and varietal resistance to *Clavigralla tomentosicollis* Stal. (Hemiptera: Coreidae). Int. J. Pest Manage. 37: 393–396.

Olawale, M.A. and O.M. Bukola (2016). Phenotypic analysis of seed yield and yield components in cowpea (*Vigna unguiculata* L., Walp). Plant Breed. Biotechnol. 4(2): 252–261.

Omitogun, O.G., L.E.N. Jackai and G. Thottappilly (1999). Isolation of insecticidal lectin-enriched extracts from African yam bean. (*Sphenostylis stenocarpa*) and other legume species. Entomol. Exp. Appl. 90: 301–311.

Omo-Ikerodah, E.E., C.A. Fatokun and I. Fawole (2009). Genetic analysis of resistance to flower bud thrips (*Megalurothrips sjostedti*) in cowpea (*Vigna unguiculata* [L.] Walp). Euphytica. 165: 145–154.

Omo-Ikerodah, E.E., I. Fawole and C.A. Fatokun (2008). Genetic mapping of quantitative trait loci (QTLs) with effects on resistance to flower bud thrips (*Megalurothrips sjostedti*) identified in recombinant inbred lines of cowpea (*Vigna unguiculata* (L.) Walp). Afr. J. Biotechnol. 7(3): 263–270.

Payne, R.W. (2009). The Guide to Genstat: Release 12, Part 2, Statistics. Lawes Agricultural Trust, Rothamsted, Experimental Station, Harpenden, Herts, (UK). 792p.

Pobożniak, M. and E.H. Koschier (2014). Effects of pea (*Pisum sativum* L.) cultivars on *Thrips tabaci* Lindeman preference and performance. J. Agric. Sci. 152 (6): 885–893.

Rivas-Vega, M.E., E. Goytortúa-Bores, J.M. Ezquerra-Brauer, M.G. Salazar-García, L.E. Cruz-Suárez, H. Nolasco and R. Civera-Cerecedo (2006). Nutritional value of cowpea (*Vigna unguiculata* L. Walp) meals as ingredients in diets for Pacific white shrimp (*Litopenaeus vannamei* Boone). Food Chem. 97(1): 41–49.

Salifu, A.B. (2001). Bases of resistance among cowpea genotypes to the bean flower thrips, *Megalurothrips sjostedti* (Trybom) (Thysanoptera: Thripidae). Nig. J. Entomol. 18: 68–79.

Santos, C. A. F., D. C. Campos da Costa, W. Roberto da Silva and L.S. Boiteux (2012). Genetic analysis of total seed protein content in two cowpea crosses. J. Crop Sci. 52: 2501–2506.

Saxena, K. N. (1985). Behavioural basis of plant susceptibility to insects. Insect Sci. Appl. 6: 303–313.

Shad, M. A., H. Nawaz, T. Rehman and N. Ikram (2013). Determination of some biochemicals, phytochemicals and antioxidant properties of different parts of *cichorium intybus* L.: a comparative study. The J. Anim. Plant Sci. 23 (4): 1060–1066.

Singleton, V.L., R. Orthofer and R.M. Lamuela-Raventos (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. Methods Enzymol. 299: 152–178.

Smith, C.M., Z.R. Khan and M.D. Pathak (1994). Techniques for evaluating insect resistance in crop plants. CRC/Lewis Press, Boca Raton, (Florida). 321p.

Snook, M.E., B.R. Wiseman, N.W. Widstrom and R.L. Wilson (1997). Chemicals associated with maize resistance to corn earworm and fall armyworm. Proceedings of International Symposium, Insect Resistance Maize: Recent Advances and Utilization. CIMMYT, (Mexico). 313p.

Sserumaga, J.P., S.O. Oikeh, S. Mugo, G.A.M. Otim, Y. Beyene, G. Abalo, and J. Kikafunda (2015). Genotype by environment interactions and agronomic performance of doubled haploids testcross maize (*Zea mays* L.) hybrids. *Euphytica*. doi: 10.1007/s10681-015-1549-2.

Tamo, M., S. Ekesi, N.K. Maniania and A. Cherry (2003). Biological control, a non-obvious component of integrated pest management for cowpea. Biological control in integrated pest management systems in Africa. Wallingford, CABI Publishing, Oxon. 309p.

Žnidarčič, D., R. Vidrih, M. Germ, D. Ban, and S. Trdan (2007). Relationship between water-soluble carbohydrate composition of cabbage (*Brassica oleracea* L. var. capitata) and damage levels of onion thrips. Acta. Agric. Slov. 89 (1): 25–33.