Correlation of Chemical Compositions of Cassava Varieties to Their Resistance to Prostephanus truncatus Horn (Coleoptera: Bostrichidae)

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RESEARCH

Correlation of Chemical Compositions of Cassava Varieties to Their Resistance to *Prostephanus truncatus* Horn (Coleoptera: Bostrichidae)

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ABSTRACT. The preference of cassava as a major host by *Prostephanus truncatus* Horn is a major constraint to ample production of cassava, *Manihot esculenta* Crantz and storage. This study analyzed the nutritional and secondary metabolite compositions in 15 cassava varieties, evaluated levels of damage and reproduction by *P. truncatus,* and assessed their resistance to attack. One hundred grams of dried cassava chips in 250-ml Kilner jars were infested with 10 adult larger grain borerof 0–10 days old and held for 3 months. The nutritional and secondary metabolites compositions of the dry cassava chips were determined using the method of Association of Analytical Chemists . Chip perforation rates in the cassava varieties ranged from 17.7 to 71.6%. The weight of cassava powder varied by about threefold. The final number of larger grain borer in the cassava varieties varied by about sixfold with 63 in 01/0040 and 379 in 01/1368. Hydrocyanic acid content content varied by over 10-fold and correlated negatively with number of larger grain borer. Flavonoid content varied by ∼10%. Tannins and saponin content of the cassava negatively correlated with number of adult *P. truncatus*. The cassava varieties 95/0166, 92/0326, 01/0040, 05/0024, and 34 91934 had selection index <0.8 and were classified as resistant to larger grain borer damage, while others with selection index >0.8 were classified as susceptible. The resistance to high damage in the resistant varieties was conferred by secondary metabolites such as tannins, saponins, alkaloids, and hydrocyanic acid content. The genetic variation in cassava varieties could be explored to breed resistant cassava varieties for use in larger grain borer-endemic areas.

Key Words: Prostephanus truncatus, Manihot esculentus, secondary metabolites, selection index, resistance

Cassava (*Manihot esculenta* Crantz) is an extensively cultivated annual crop in tropical and subtropical regions for its edible starchy tuberous root that is a major source of carbohydrates (Phillips 1984). The crop is useful in many types of products, and the roots can be processed into chips and pellets used in compounding animal feed for cattle, sheep, goats, pigs, poultry, and farmed fish. Cassava flour is mainly used in bakery products and cassava starch can be used as a generally thickening, binding, texturing, and stabilizing a range of food products such as canned foods, frozen foods, salad dressings, sauces, and infant foods. Various varieties are usually differentiated from one another by their morphological characteristics such as color of stems, petioles, leaves, and tubers (Phillips 1984). The crop is however infested by a number of beetles that feed on dry cassava causing postharvest losses (McFarlane 1982).

The most common insect pests of cassava are *Dinoderus* sp., *Carpophilus* sp., the coffee bean weevil (*Araecerus fasculatus*), the lesser grain borer (*Rhizopertha dominica*), and more recently, the *Prostephanus truncatus* Horn. *P. truncatus* is a serious major pest of farm-stored maize cobs and dried cassava in Mexico, Central America, and recently in Africa (Hodges 1986, Rees 1994). Adults bore into cassava or maize husks, cobs, or grain, making neat holes and tunnel by producing large quantities of dust (Nang'ayo et al. 1993, 2002; Nansen et al. 2004). Since the report of its first outbreak in Africa in 1981, there has been rapid and sustained spread of *P. truncatus* across the African continent (Kega and Warui 1983, Harnisch and Krall 1984, Hodges 1986). The insect has now spread to Benin, Burkina Faso, Burundi, Ghana, Guinea Conakry, Kenya, Malawi, Mozambique, Namibia, Niger, Nigeria, Rwanda, South Africa, Tanzania, Togo, Uganda, and Zambia becoming the most destructive pest of dried cassava and stored maize (Harnisch and Krall 1984, Hodges 1986, Boxall 2002, Nang'ayo et al. 2002, Nansen et al. 2004). *P. truncatus* was reported to have the potential to spread to the entire major maize and cassava-producing regions in Africa (Nang'ayo et al. 1993, 2002; Nansen et al. 2004). *P. truncatus* is not restricted to stores but occurs widely in the natural environment. Although no preferred breeding sites have yet been discovered, laboratory tests show that *P. truncatus* is able to breed successfully on dried wood from a range of trees, as well as the dried stems of cassava and maize plants (Nansen et al. 2004, Osipitan et al. 2011). The rapid spread of *P. truncatus* in Africa was attributed to its ability to fly long distances ranging from 2 to 24 km (Wright 1984, Hodges 1986). *P. truncatus* is highly voracious and destructive, posing a threat of food security to rural populations (Nang'ayo et al. 2002).

The dried roots of cassava may be readily reduced to dust by boring adults of *P. truncatus*. Average losses of 19% have been recorded after 6 months of storage and as high as 30% in some cases (Markham et al. 1991, Espinal et al. 1996). The numerous varieties of cassava are usually grouped in two main categories: *Manihot palmate* and *Manihot aipi*, or bitter and sweet cassava, respectively; the distinction between them rests upon the content of hydrocyanic acid, which causes toxicity in the roots, branches, and leaves of the plant in both free and chemically bound forms. One alternative strategy for the control of insect that is cost effective and environmentally friendly is host resistance (Throne and Eubanks 2001). However, cassava is mostly selected for agronomy traits such as yield and duration of maturity and not for resistance to *P. truncatus* while in dried form in storage.

The preference of cassava as a major host by *P. truncatus* is a major constraint to ample production of cassava and storage. This study therefore analyzed the nutritional and secondary metabolite compositions in 15 cassava varieties and evaluated levels of damage and reproduction by *P. truncatus* in order to assess their resistance to attack.

Materials and Methods

Study Site and Sources of Cassava Varieties. The study was carried out in the Laboratory of the Department of Crop Protection, Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State, Nigeria.

Fifteen varieties of *M. esculenta* screened for infestation and damage by *P. truncatus* were sourced from International Institute of Tropical Agriculture (IITA). They are among elites varieties released by IITA to farmers and some have shown resistance to infestation by cassava mealy bug on the field. The cassava varieties are presented on Table 1. The cassava roots were peeled and cut into cubes of 10–20 cm^3 sizes. They were oven dried to moisture content of ∼13%.

Insect Culture. The adult larger grain borer used for the study was obtained from culture maintained in the Department of Crop Protection, College of Plant Science and Crop Production, FUNAAB. Several adult *P. truncatus* from the culture were introduced into maize kernels ("Solo") kept in two glass jars under ambient condition in the laboratory for 5 days to lay eggs. The jars were observed for larger grain borer emergence as from 20th day after the removal of the introduced larger grain borer adults. Larger grain borer that emerged within the first 10 days were used for the study.

Evaluation of the Dried Cassava Chips to Damage by P. truncatus. One hundred grams of dried cassava chips, each of the 15 varieties were weighed and placed in 250-ml Kilner jars. Ten adult *P. truncatus* of 0–10 days old were introduced into each of the jars containing the dried cassava chips. The treatments were replicated four times and arranged on work table in the laboratory in a completely randomized design (CRD). At 3 months postinfestation of the cassava varieties, the insects and the dust generated by the feeding activities of the insects was sieved out. The following data were collected: 1) weight of cassava chips, 2) weight of cassava powder, 3) total number of chips, 4) number of chips perforated, 5) total number of adult *P. truncatus*, and 6). The following were calculated: 1) % cassava chips perforated and 2) % cassava chips weight loss.

Table 1. Names and descriptions of cassava varieties used for the study

S/N	Cassava varieties	Color
1.	91/02324	White
2.	95/0166	White
3.	98/0581	White
4.	95/0289	White
5.	92/0326	White
6.	01/0040	White
7.	92B/00068	White
8.	91934	White
9.	30572	White
10.	98/2132	Yellow
11.	05/0303	Yellow
12.	96/1672	White
13.	05/0024	Yellow
14.	01/1368	Yellow
15.	07/0824	Yellow

Determination of Resistance Using Selection Index. Resistance was determined using selection index based on number of adult larger grain borer, weight of cassava powder, % cassava chips weight loss, and % cassava chips perforated. Data collected on the four parameters were integrated to define the resistance reaction of the maize varieties by summing the ratios between values and overall mean and dividing by four (number of parameters). Varieties with selection index values less than 0.8 are regarded as resistant and those with a selection index greater than 0.8 as susceptible (Tefera et al. 2011, Mwokolo et al. 2012).

Determination of Nutritional Composition and Secondary Metabolites of the Cassava Varieties. One hundred grams dried cassava chips of each variety were taken and ground to powder. The nutritional composition and secondary metabolites of the cassava varieties was determined using the method of official methods of analysis of the Association of Analytical Chemists (1990).

Experimental Design and Data Analysis. The experiment was conducted using CRD. Data collected were subjected to analysis of variance and significant means were separated using Students's–Newsmankeul Test. Cassava damage indices such as final population of adult *P. truncatus*, weight of cassava powder, % chips perforated were correlated with nutritional composition, and secondary metabolites of the cassava.

Results

Damaged Cassava Chips and Weight of Cassava Powder. The cassava variety 01/0040 had a significantly higher percentage of the chips perforated ($F = 497151$; df = 14, 45; $P < 0.0001$). It was however not significantly higher than the percentage of the chips perforated in 91934, 95/0166, 05/0303, and 05/0024. Significantly lower percentage (17.72) of the cassava chips was perforated in 92B/00068. It was however not significantly higher than the percentage of the chips perforated in 95/0289 (26.22%) and 01/1368 (23.32%). The percentage of the cassava chips perforated in 91/02324 (56.45%), 92/0326 (58.62%), 98/2132 (52.51%), and 30572 (49.72%) were not significantly different from each other (Fig. 1).

The result depicted in Fig. 2 shows that the cassava variety 92B/00068 had a significantly ($F = 661835$; df = 14, 45; $P < 0.0001$) higher weight of cassava powder (82.28 g). It was however not significantly higher than the weight of cassava powder in 95/0289 (73.78 g) and 01/1368 (76.68). The lowest weight (31.11 g) of cassava powder was from 05/0303. It was however not significantly lower than the weight of cassava powder in 05/0024 (33.82 g), 95/0166 (33.42 g), 91934 (32.33 g), 01/0040 (28.43 g), and 92/0326 (41.39 g).

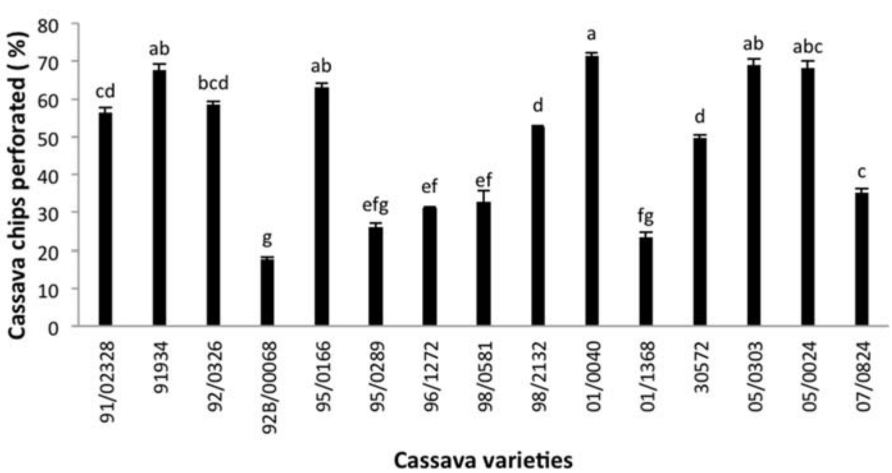

Fig. 1. Perforation of cassava chips by larger grain borer.

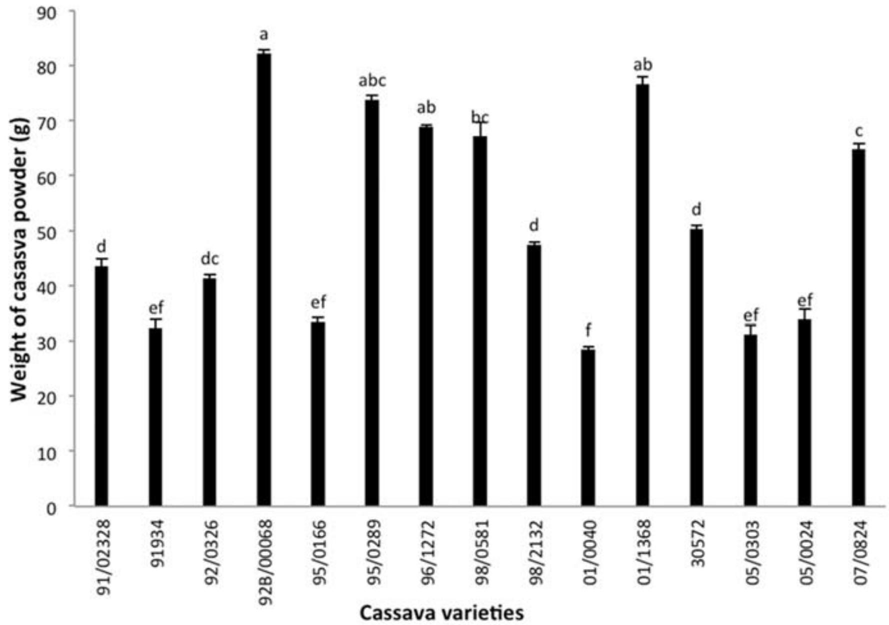

Fig. 2. Weight of cassava chips powder in the cassava varieties.

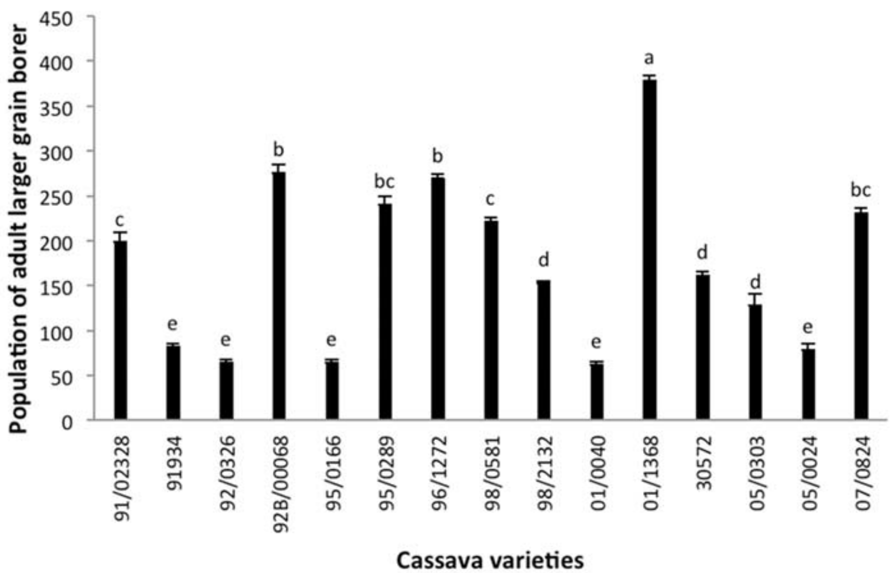

Fig. 3. Population of larger grain borer in infested cassava.

Reproductive Success of Larger Grain Borer in Infested Cassava Chips. The final population of adult *P. truncatus*. The variety 92B/00068 had a significantly higher number (277.0) of adult *P. truncatus* ($F = 3502.71$; df = 14, 45; $P < 0.0001$). The number of adult *P. truncatus* in 95/0289 (241.0), 96/1672 (270.0), and 07/0824 (232.0) was not significantly different from each other (Fig. 3).

Resistance Rating of the Cassava Varieties. The selection index value based on integration of number of adult larger grain borer, weight of cassava powder, % cassava chips weight loss, and % cassava chips perforated shows that the cassava varieties 95/0166, 92/0326, 01/0040, 05/0024, and 91934 had selection index value <0.8 while the selection index of others were >0.8 (Fig. 4).

Secondary Metabolites of the Cassava Varieties. The results presented in Fig. 5 shows the secondary metabolites of the cassava varieties. The alkaloids (2.87) in 92B/00068 was significantly ($P < 0.05$) higher than what was obtained in other cassava varieties. The alkaloid composition (2.08) was however significantly ($P < 0.05$) lower in 98/0581. The oxalate composition (7.42) was higher in 05/0024. It was however not significantly different from the composition in other cassava varieties. Flavonoid content (1.85) was significantly ($P < 0.05$) higher in 91/02324 relative to what was obtained from other varieties, except the varieties 92B/00068 and 05/303 that has 1.83 oxalate content at a piece. The tannins and saponins content (3.67 and 3.03, respectively) in the variety 98/2132 was significantly ($P < 0.05$) higher than the content in other cassava varieties. Conversely, tannins and saponins content (2.43 and 3.01, respectively) in the variety 07/0824 was significantly ($P < 0.05$) lower. However, the saponin content of 3.03 and 3.01 in 95/0289 and 07/0824, respectively. Tannins content of 2.41 and 2.43 in 95/0289 and 05/0303, respectively, were not significantly ($P > 0.05$) different from each other.

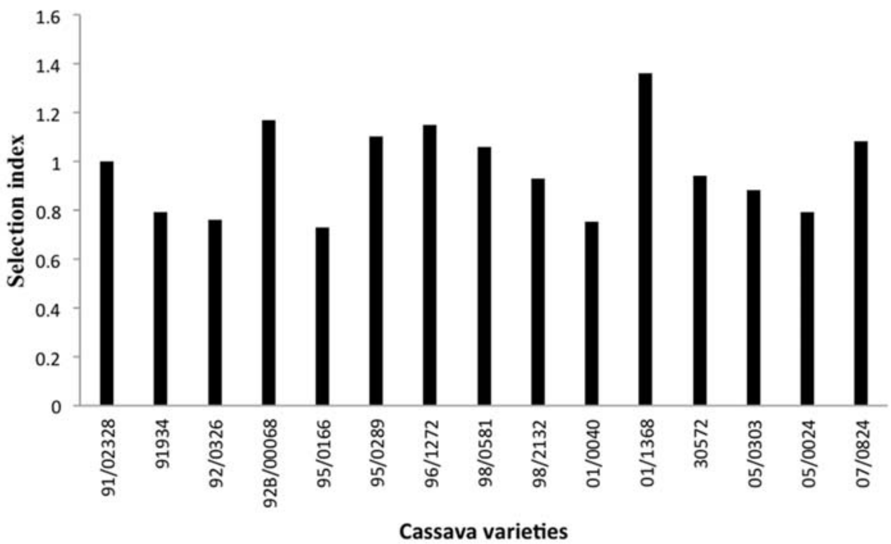

Fig. 4. Selection index for resistance in the cassava varieties.

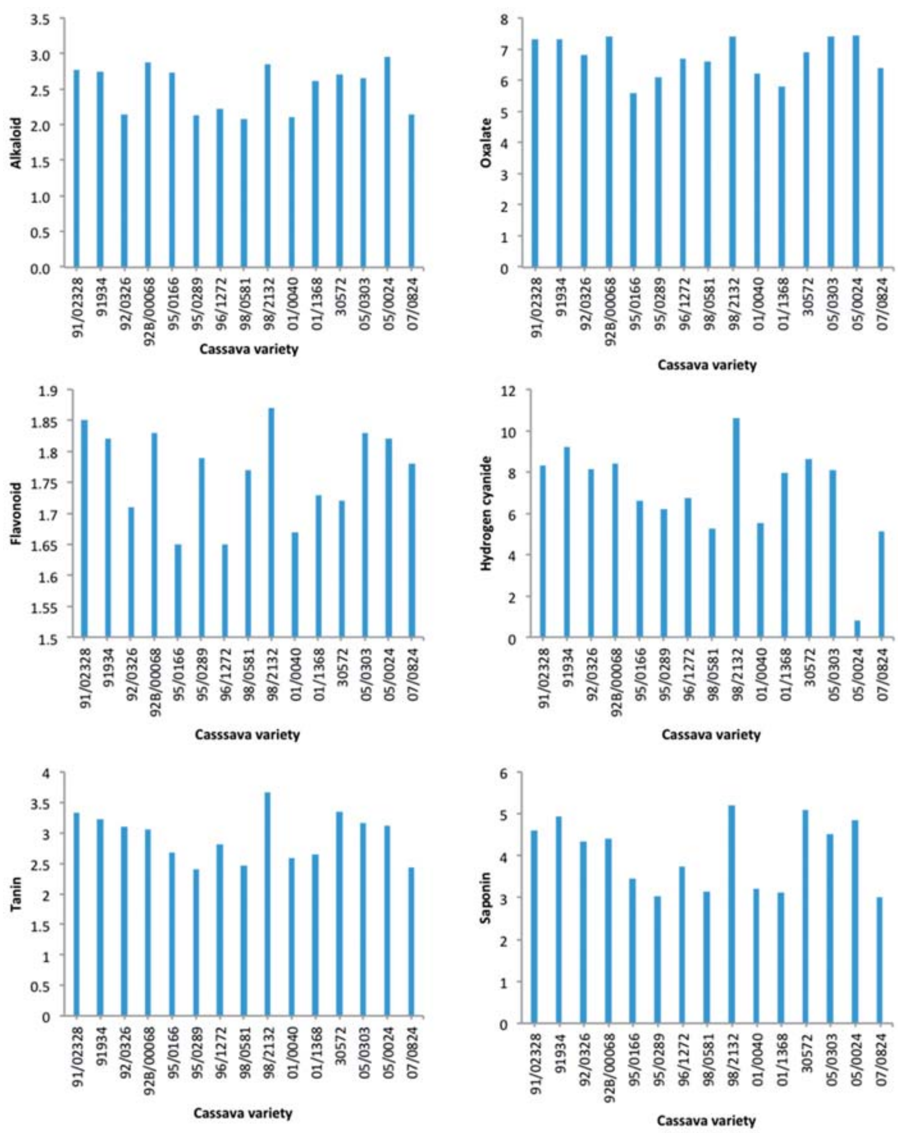

Fig. 5. Secondary metabolites of the cassava varieties.

Table 2. Correlation of *P. truncatus* damage indices and nutritional composition

	NA	NCCP	WCPD	MC	DM	FC	AC	CF	CP	CHO
NA	1									
NCCP	0.879**	1								
WCPD	0.885**	−0.999**	1							
MC	0.111	−0.062	0.065	1						
DM	0.011	−0.046	0.043	−0.897**	1					
FC	−0.103	−0.006	0.002	−0.274	0.478	1				
AC	0.088	0.058	−0.057	0.495**	−0.545	−0.054	1			
CF	0.217	−0.098	0.095	0.583**	−0.617	−0.099	0.943**	1		
CP	−0.011	−0.064	0.053	−0.635**	0.520	−0.005	−0.257	−0.234	1	
CHO	0.102	−0.198	0.199	−0.668**	0.634	−0.137	−0.765**	−0.776**	0.602**	1

NA, number of adult LGB; NCCP, number of cassava chips perforated; WCPD, weight of cassava powder; MC, moisture content; DM, dry matter content; FC, fat content; AC, ash content; CF, crude fiber content; CP, crude protein content; CHO, carbohydrate content.

*significant at $p \leq 0.05$.

**significant at $p \leq 0.01$.

Table 3. Correlation of *P. truncatus* damage indices and secondary metabolites

	NA	NCCP	WCPD	HCN	TAN	SAP	ALKAL	OXAL	FLAV
NA	1								
NCCP	0.879**	1							
WCPD	0.885**	−0.999**	1						
HCN	−0.217*	−0.289	−0.278	1					
TAN	−0.321*	−0.419**	−0.405	0.857**	1				
SAP	−0.391**	−0.437**	−0.423**	0.874**	0.964**	1			
ALKAL	−0.123	−0.225	−0.231	0.822**	0.703**	0.722**	1		
OXAL	−0.225	0.220	−0.215	0.369*	0.097	0.208	0.284	1	
FLAV	0.072	−0.000	0.021	0.534**	0.498**	0.505**	0.486**	0.136	1

NA, number of adult LGB; NCCP, number of cassava chips perforated; WCPD, weight of cassava powder; HCN, hydrocyanic acid content; TAN, tannin content; SAP, saponin content; ALKAL, alkaloid content; OXAL, oxalate content; FLAV, flavonoid content.

*significant at $p \leq 0.05$.

**significant at $p \leq 0.01$.

Correlation of *P. truncatus* Damage Indices with Nutritional Composition. The correlation of damage indices of the cassava with nutritional composition of the cassava is shown in Table 2. The number of *P. truncatus* adult correlated positively with the weight of adult *P. truncatus* ($r = 0.554$; $n = 58$). Similarly, the number of cassava chips perforated and weight of cassava powder correlated positively with number of adult *P. truncatus*. This indicates that the higher the number of adult *P. truncatus*, the more the number of cassava chips perforated and the more the cassava powder.

Correlation of *P. truncatus* Damage Indices to Secondary Metabolites. The correlation of damage indices of the cassava with secondary metabolites of the cassava is shown in Table 3. Tannin content of the cassava negatively correlated with the number of adult *P. truncatus*. ($r = -0.321$; $n = 58$). Similarly, the saponin content negatively correlated with the number of adult *P. truncatus* and weight of cassava chips powder (−0.391 and −0.423), indicating that high tannin and saponins content has a restrictive effect on the number of adult *P. truncatus* and weight of cassava powder. The correlations of oxalate contents of the cassava with damage indices of the cassava by *P. truncatus* and flavonoids content with weight of cassava chips damaged ($r = 0.021$; $n = 58$) were insignificant. The correlation of the hydrocyanic acid content of the cassava with number of *P. truncatus* was negative and significant ($r = -0.217$; $n = 58$)

Discussion

The results of the study indicated variations in the nutritional composition of the cassava varieties and ability of *P. truncatus* to damage dried cassava irrespective of their varieties and nutritional composition. Chijindu and Boateng (2008) reported that processed cassava chips differed in their overall chemical contents and their responses to *P. truncatus* attack. Akunne (2008) attributed differences in the development periods of *P. truncatus* in some cassava cultivars to differences in the nutritional values of the cassava cultivars as earlier reported by Okeke et al. (1989). Giles and Leon (1975) and Hodges et al. (1985) reported *P. truncatus* as a highly voracious insect pest that is capable of causing up to 40% yield loss in stored maize grains within 6 months and 75% in fermented cassava roots within 4 months. Espinal et al. (1996) report the ability of the adult beetle of *P. truncatus* and its larval stages to damage wide range of commodities including some roots and tubers, cereals, pulses, cocoa, coffee, groundnut, and wooden structures. Similarly, Nyakunga (1982) and Osipitan et al. (2011) also reported the ability of *P. truncatus* to damage and breed on dried root and tuber crops. In this study, high percent weight loss of 82% was recorded in 92B/00068 within 3 months of infestation, indicating the ability of *P. truncatus* to cause huge damage in infested cassava. This result is similar to that of Hodges et al. (1985) who reported 74% weight loss in fermented cassava roots and 52% in unfermented ones after 4 months of infestation. Giles and Leon (1975) and Hodges et al. (1983) noted that the most obvious cause of weight loss in *P. truncatus* infested grains is conversion of the grain to maize powder by adults and larvae-boring activity, and this could completely reduce grains to powder.

The rapid multiplication of *P. truncatus* from the initial 10 *P. truncatus* adults to 380.0 in 01/1368 and 277.0 in 92B/00068 suggest that *P. truncatus* has high fecundity and high reproductive ability in preferred host. Li (1988) reported that oviposition by *P. truncatus* spanned through life and peaked at 20 days after mating. Nyakunga (1982) reported daily fecundity of 1.4–3.9 eggs per day for adult *P. truncatus* depending on the quantity and type of food. Li (1988) reported that adult *P. truncatus* commonly live for more than 100 days under laboratory conditions.

In this study, the correlation of the dry matter, fat, ash, crude fiber, crude protein, and carbohydrate content with damage indices were not significant, suggesting that *P. truncatus* damaged the cassava varieties irrespective of their nutritional composition. Hodges et al. (1983) reported the ability of *P. truncatus* to penetrate materials in which there is no evidence of breeding such as wood, perspex, and polythene, suggesting the ability of the insect to bore into solid substrate irrespective of their nutritional quality. The ash and crude content of the cassava varieties correlated positively with number of dead *P. truncatus* adult, suggesting that ash and crude content are likely to play a key role in adult mortality of *P. truncatus*.

The result of the study indicated varietal differences in the response of the cassava varieties to damage by *P. truncatus*. The cassava variety 01/0040 had a significantly higher number (71.55) of the cassava chips perforated and significantly lower % (28.43) converted to powder. This indicates that the variety was perforated by the insect but was not a preferred variety for feeding by the insect. The variety 92B/00068 that had a significantly higher % (82.28) of the cassava chips converted to powder suggests preference of the variety for feeding by *P. truncatus*. In a similar study, Akunne (2008) studied the effect of cassava varieties on oviposition and development of larger grain borer, *P. truncatus* and attributed low oviposition preference for the variety Nwugo to the presence of oviposition deterrents.

In this study, the correlation of the nutritional composition and secondary metabolites of the cassava with damage indices in infested cassava shows that high tannin and saponin has restrictive effect on multiplication of *P. truncatus* and their weight. Of the phenolic metabolites, greatest emphasis has been placed on the group of compound collectively known as tannins (condensed tannins, hydrolysable tannins, polymeric tannins), with overwhelming evidence that in domestic animals, excessive levels of tannins in foodstuffs do have deleterious effect on nitrogen imbalance (Haslam 1989, CIBA Foundation 1992). Tannins are naturally occurring plant polyphenols that bind and precipitate proteins and have large influence on the nutritive value of many foods eaten by humans and feed stuff eaten by animals. Haslam (1989) reported tannin as having major impact on animal nutrition because of their ability to form complexes with numerous types of molecules such as carbohydrate, proteins, polysacaccharide, and enzymes involved in protein and carbohydrate digestion. Reed (1995) reported that tannins negatively affect animal feed intake by affecting its digestibility and palatability. Studies also reported higher feed intake and weight gains when tannin-free diets were compared with tannin-containing ones (Haslam 1989, Reed 1995). Jain and Tripathi (1991) reported that saponins and sapogenins isolates had insect antifeedant activity against the larvae of the noctuid, *Spilosoma oblique*, with the most active compound being diosgenin-3-0-[α-L-rhamnopyranosyl(1→2)]-[β-D-glycopyranosyl (1→3)-β-D-glucopyranosyl(1-4)]-(β-D-glucopyranoside (X) inhibiting 73.63% feeding at 0.022% concentration. Saponins, iridoids, sesquiterpene lactones, and nonprotein amino acids were reported to exhibit insect-deterrent activity. Terpenes and its derivatives such as Azadirachtin and Pyrethriin-1 were reported to be effective as antifeedants with specific toxicity (Jain and Tripathi 1991).

The study shows that high hydrocyanic acid has a restrictive effect on the number of *P. truncatus* adult. The result is similar to the findings of Akunne (2008), which indicated low preference of cassava variety Anti-Ota for damage by larger grain borer as a result of its high cyanide content as earlier reported by Dufour (1987). Tewe et al. (1980) reported that bitter roots of the cassava plant may contain up to 1 g of hydrocyanic acid per kilogram. The cyanogenicglucosides of cassava (Linamarin and Lotaustralin) on hydrolysis releases hydrocyanic acid. The presence of cyanide in cassava has caused a global scare as to the safety of cassava and its products for human and animal consumption. Bellotti and Riis (1994) reported that over 1,000 plant species produce cyanogenic glucosides in variable concentrations, which includes cassava a highly cyanogenic species. Hydrocyanic acid was reported as a feeding deterrent in plants such as sorghum, white clover, and peach. A growing cassava is an unacceptable food for the grasshopper, *Zonocerus variegatus*, because of the relatively large amounts of cyanogens produced in the leaves. Similarly, the burrowing bug, *Cyrtomenus bergi*, prefers feeding on roots with low rather than high cyanogen content. Nymphal mortality was greater on a cassava clone with high cyanogenic potential, while adult insects lived longer and produced more eggs on a clone with low cyanogenic potential. In field trials, considerably more feeding damage was observed on clones with low cyanogenic potential, and in free choice tests, maize was a preferred host.

The study indicated varietal differences in response of cassava to damage by *P. truncatus*. The high perforation index and % weight loss in some of the varieties suggest that cassava is a preferred host for infestation and damage by *P. truncatus*. Damage by *P. truncatus* to infested dried cassava chips correlated negatively with the hydrocyanic acid content and secondary metabolites such as tannins, saponins, and alkaloid, suggesting that the relative resistance to damage by *P. truncatus* in some of the cassava varieties may likely be conferred by hydrocyanic acid and secondary metabolites of the cassava varieties, among other factors. The study also shows that the nutritional and secondary metabolites composition of the cassava varieties varied. The cassava varieties 95/0166, 92/0326, 01/0040, 05/0024, and 91934 were resistant to *P. truncatus* damage, while others were susceptible. The genetic variation in cassava varieties could therefore be explored to breed resistant cassava varieties for use in larger grain borer-endemic areas.

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