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Cassava Variety Tolerance to Spider Mite Attack in Relation to Leaf Cyanide Level

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Abstract
Cassava, *Manihot esculenta* Crantz is grown in the tropics as an important staple root crop. The major herbivore pest is cassava green mite (CGM) of the *Mononychellus* species. The pest mite *Mononychellus progresivus* Doreste collected from the coastal Kenya lowlands was used to evaluate spider mite tolerance of nine varieties grown in three regions of the country; eastern lowlands, western midlands and the humid coastal lowlands. Mite population build-up of 10 individual motile stages of *M. progresivus* reached peak densities on 39th day of the most susceptible varieties and by the 54th day attacked leaves had wilted in 20.0 ± 2 °C and 63 ± 4%, climatic conditions. For the most tolerant varieties it took 47 days to reach peak densities and for one variety (MM97/3567) the mite population did not cause highest damage score even by the 55th day. Equating visual damage score with actual leaf biomass loss (%) enabled indication of the level of photosynthetic leaf area loss due to pest mite attack. Higher leaf cyanide (HCN) content led to higher biomass loss (%) up to HCN 20mg/Kg. Spider mite density increase was similarly positively correlated to the subsequent biomass loss on cassava varieties. This information is beneficial to cassava breeders when developing varieties tolerant to CGM attack and safe for human consumption.
Key words: *Mononychellus progresivus*; damage score; cyanide content; biomass; human consumption

1. 0 Introduction

Cassava (*Manihot esculenta* Crantz) is reported to be tolerant to low moisture and poor soils in most regions of Sub Sahara Africa (SSA) (Hillocks, 2002; Fermont *et al*., 2009). Improved genotypes have led to higher root yield and various utilization options (Jennings and Iglesias, 2002; Westby, 2002; Fermont *et al.,* 2009). With the increased climate change scenario where some regions are no longer considered potential for legume and cereal staples, cassava production is reported as the food insurance crop in most SSA countries (Von Braun, 2007; Ferris *et al.*, 1997). More agro-industries for cassava utilization have been fronted for animal nutrition and industrial raw materials processing (Gomez, 1991; Quenyh and Cecil, 1996; Padmaja *et al.,* 1994). Root cyanide (HCN) level consideration is a pre-requisite to safety for human consumption of raw cassava as reports of acute toxicity in the SSA region increase (Emmanuel *et al*., 2012; Iglesias *et al*., 2002; Balagopalan, 2002). Cassava roots have twice HCN content level to the amount in the leaves (Wheatley and Chuzel, 1993). Invertebrate herbivores like mealybug and phytophagous mites have been reported as being of economic importance and constrain production of the staple roots (Bellotti, 2002; Yaninek, 1994; Yaninek *et al*., 1989; Megevand *et al.,* 1987). Biological control of cassava green mite (CGM) pest has been reported as widely successful in the humid warm regions of SSA with less success in drier regions (Hanna and Yaninek, 2003: Onzo *et al,* 2005). On plant host resistance, cassava mealybugs resistance *Phenacoccus manihot* and *P. herreni* is correlated to leaf pubescence and unexpanded leaves (Hahn, 1984). Similarly spider mite pest resistance or tolerance has been attributed to plant vigor, leaf pubescence and antibiosis mechanism (Bellotti and Byrne, 1979; Kawano and Bellotti, 1980). The cassava cyanogens compounds are mainly the linamarin (85%) with lesser amounts of lotaustralin free HCN glycosides found in both foliage and root content (Iglesias et al, 2002; Alves, 2002). No report has detailed how leaf cyanide levels contribute to repellence of CGM pest. Recent studies have indicated high mortalities of the predatory mite *Phytoseiulus longipes* Evans when fed *Mononychellus progresivus* (Doreste) which feed on cassava leaf tissue (Mutisya *et al.,* 2012). Whether higher cyanogens content on cassava leaf leads to less damage by *M. progresivus* was assumed as an important question in cassava varietal

breeding during formulation of the study.
This study explored how HCN levels of different cassava varieties influence cassava green mite (CGM) *Mononychellus progresivus* Doreste population build up and subsequent leaf tissue damage.

1.1 Materials and Methods

1. 1. 1 Cassava varieties and data score

Nine cassava varieties from coast (3), eastern (3) and western (3) cassava production regions were planted on plastic pots of 18cm-length x 18cm-width and 15cm-height. Sandy loam soil was used in the pots. Each variety had 12 potted cassava plants making a cluster block (4rows x 3plants) in a screen house. The cuttings were watered after every two days to maintain saturated moisture content of the soil. Leaf characteristics of the cultivars were 5-7 lobes in most seedlings. One month after planting when plant height was 32cm, some 10 motile life stages of cassava green mite (CGM) collected from Mtwapa coast area were introduced on each variety. Five days later monitoring of CGM numbers per leaf was carried out by estimating adult stage by aid of magnifying (X 4) lenses. The damage scored was taken from severity level of 1-5 (1=No damage score, 2: ≤25% leaf damage, 3: ≤50% damage, 4: ≤75% damage and 5:=100% wilted leaf) (Yaninek et al., 1989). The estimate number of spider mites per leaf was scored to determine injury level in relation to CGM numbers. The screen house (5m-lenth x 3m-width) conditions were temperature 20.0 ± 2 °C and relative humidity 63 ± 4%, with clear glass cover on the top. The experiment was repeated thrice.

1. 1. 2 Mite peak densities and crash over time

The duration for each variety spider mite density build up to the peak density and drop at estimated damage score level was recorded for varietal comparison. The duration (days) to particular damage score of a variety in comparison with other varieties would indicate susceptibility /tolerance to CGM attack. The period for experimental monitoring was 55 days since introduction of 10 individuals of CGM adult motiles on each variety. On day five, after mite introduction, the number of CGM /leaf on each cassava variety was scored as the start point. Follow up of mite densities on the whole leaf lobes were estimated after every three days by randomly picking (without detaching) the third mature leaf of the apex and estimating the number of the mites/leaf and visual damage score (Yaninek et al., 1989). This was continued up to 55^{th} day of the experiment.

1. 1. 3 Leaf biomass losses

During the determination of leaf biomass loss on cassava varieties, five leaves were picked from each variety representing damage (D) scores of D.1, D.2, D.3, D.4 and D.5. The choice of the sample leaf position was important because the highest numbers of CGM are found at the apex of cassava plant. The five number of leaves for D:1-5 were collected in Khaki paper(No 25) bags and taken to the laboratory for weighing. An electronic weighing balance (Santorius Basic-BA 310s, made in Germany) of three decimal units (000.000 g) was used to weigh the leaves after brittle drying them in oven at 60 °C. To get single leaf mean weight (mg) of the sample leaves the total leaf weight was divided by five. To arrive at the dry weight (DW) loss or biomass loss at each damage score, D.1 was taken as the leaf DW with no CGM damage score. Subsequent weights of D.2, D.3, D.4 and D.5 were taken and weight loss calculated as:

$$\text{DW loss (\%)} = \frac{D.1-(D.x_{1-5})}{D.1} 100$$

Where, D.1 is the weight of non damaged leaf, x representing values 1-5 as damage scores of a particular cassava variety.

1. 1. 4 Cyanide content in relation to biomass losses

Cassava leaf variety cyanide (HCN) content was determined by Picric Acid analysis. The procedure included cutting fresh cassava leaf to disks of specific diameter to the size of test-tube to be used for the test. The cassava leaf disks (of 1.0cm-diameters) were inserted to the bottom of 1.5-cm diameter tube and the prepared Picric acid solution poured into standard size Petri-dish. Five drops of Tuluone volatile liquid was added to leaf disk in the test-tube to vaporize the gaseous cyanide (HCN). Whatman No 1 (6cm x 1cm) strip paper was soaked with the Picric acid solution and inserted into opening of each test-tube to avoid touching the Tuluone-wet leaf and left for 24 hr period for colour change. A control test-tube treatment (with leaf disc) was set along all the variety tubes replicated three times. The colour chart was from the range of 1-9 starting with yellow (no cyanide) to dark brown of highest cyanide level according to Natural Research Institute (1996) described procedure. The reading ranges where calculated for mean values. Later comparison of cassava variety biomass loss (DW loss %) and cyanide content was compared to find out if there was relationship between spider mite damage to cyanide (HCN) level. The procedure was carried out on day 10, 20, 33 and 54 day for each experimental repeat assessment.

1. 1. 5 Statistical analyses

Kruskal-Wallis non-parametric one-way analysis of variance was carried out considering the precarious nature of mite counts. Software GenStat Discovery (2010) edition 3 VSN International was used. Analysis of variance was carried out to determine significance difference of leaf biomass loss (%) and cyanide content of the different cassava varieties, using SPSS Version 12.0 where means were separated with Student Newman Keuls (S.N.K) Post Hog Test. Pearson correlation was used to determine leaf cyanide content correlation (r) level and slope (b) to CGM density growth and the subsequent biomass loss on the cassava varieties.

1. 2 Results

1. 2. 1 Estimation of CGM densities and visual damage score

The nine potted cassava varieties sampled for 55 days were scored for number of cassava green mite (CGM) per leaf and the subsequent leaf damage score severity in specific duration in days. The damage score of D.3 and D.4 were critical since they represented visual leaf damage of 50 and 75% respectively. There was significance difference ($P<0.001$) among spider mite densities as well as number of days among the cassava varieties (Table 1). Susceptible varieties had peak densities by the 39th day from mite introduction time.

Variety Kalezo of the coastal lowlands region had the shortest period of attaining D.3 and D.4 damage levels in 12.7 and 30.3 days of spider mite populations of 320 and 870 CGM/leaf, respectively. Variety MM990183 of the eastern lowlands was the second in attaining D.3 and D.4 in 14.3 and 33 days of pest populations of 560 and 980 mites per leaf. The X-Mariakani variety (eastern lowlands) was the third with the shortest period of 18.0 and 30.1 days of attaining damage scores of D.3 and D.4 with pest populations of 310 and 950 mites /leaf. The most CGM tolerant-varieties were the two from the western midland region of the country; varieties MM97/3567 and MM96/9308 which had the least number of mites through out the experimental period. It was also observed that variety MM97/3567 damage score D.5 was not attainable due to the low mite numbers leading to a maximum of D.4 visual score on the leaves. The susceptible varieties had leaf wilt by 54th day.

1. 2. 2 Leaf biomass losses

The highest biomass losses (%) due to spider mite damage was observed on variety Kalezo from the coastal lowlands of 15, 22, 54 and 67%, of damage scores D.2, D.3, D.4 and D.5, respectively as indicated in Table 2. The second variety with highest leaf biomass loss was Karibuni of the same coastal lowlands with 12, 18, 51 and 64% representing D.2, D.3, D.4 and D.5 damage scores. Variety X-Marikani and MM99005 of eastern lowlands had fairly similar biomass loss across the damage levels from D.2 to D.5.

The variety with the least loss of leaf biomass which could be considered as being relatively tolerant to CGM was MM97/3567 of western midlands region with 4, 15 and 34 %, for D.2, D.3, and D.4 for CGM damage levels. Leaf visual damage score D.5 was not observed on Variety MM97/3567. For all the varieties visual damage score 2 (D.2) was indicative of some early mite damage and was found to be between 9-15% of the evaluated nine varieties. It was determined that second level damage (D.2) had biomass loss >10% for Kalezo, Karibuni, MM990183, X-Mariakani and MM96/2480 varieties.

1. 2. 3 Effect of leaf cyanide on mite infestations

The varieties with cyanide (HCN) content levels >10<30 mg/kg had the highest spider mite population peaks than those of <10 cyanide levels as Table 3 shows. Variety Kalezo from coastal lowlands region had cyanide content of 20 mg/kg with CGM peak score of 1370 CGM/leaf. Karibuni variety from the same coastal region was third with mite density peak of 1170 mites /leaf and similarly with HCN of 20 mg/kg. The X-Mariakani variety from eastern lowlands led in third position with the highest peak mite infestations of 1190 CGM/leaf with same HCN content of 20.0mg/kg. Varieties MM99005 and MM990183 of eastern lowlands had HCN level of 12.5 mg/kg and exhibited mid infestation levels of 1020 and 1050 mites/leaf with biomass loss of 55 and 54%. The three varieties from western midlands had low CGM densities of 585, 580 and 760 for MM97/3567, MM96/9308 and MM96/2480 respectively. These low CGM densities similarly caused low biomass losses.

1. 2. 4 Relationship of cyanide content and biomass losses

Eight of the nine varieties showed a positive correlation of increase of cyanide content (mg/kg) leading to higher biomass loss due to higher number of CGM feeding on the leaf tissue (Table 4 and Fig. 1). Correlation values indicated in Table 4 show increasing slope (b) values with increasing days on CGM densities on the cassava varieties. Similar trend was observed for biomass loss slope. Correlation relationship (r) of HCN leaf content (mg/kg) increased with mite density increase in time and space on the varieties. For the biomass loss the trend was less linear with increase of days and CGM density increase.

1. 3 Discussion

Cassava green mite (CGM) population growth has been reported to be temperature dependent as with other tetranychid mite herbivores (Yaninek *et al*., 1989; Byrne *et al.*, 1982). CGM feeds on the under side of cassava leaf tissue sucking cell-sap and reducing photosynthetic leaf area (Yaninek et al., 1989). Yaninek and Gnanvossou (1993) compared dry matter increase of the spider mite life history and concluded that cumulative dry matter increased as CGM cohorts development progressed upward from juvenile to adult life stages of the mite. Environmental conditions dictate the subsequent damage level of the mite on each cassava variety depending on susceptibility to CGM (Yaninek *et al.,* 1989; Ayanru and Sharma, 1984; Byrne *et al.,* 1983). In this study, the visual damage scoring has been equated to level of leaf biomass losses (%) which allowed indicative loss of the leaf photosynthetic area on the plant. Thus, some varieties always lose higher leaf photosynthetic areas in comparison with others due to their higher susceptibility to mite attack which would subsequently lead to higher root yield loss (Yaninek *et al.,* 1989; Ayanru and Sharma, 1984).

From the duration (days) and level of leaf damage score it was found that the minimum injury level of economic importance on cassava leaf was D.2 caused by ≤100 mites per leaf. It was at this damage level (D.2) reached in about 8.4-9.3 days for susceptible varieties in the indicated climatic conditions that reflected the threshold numbers of CGM which justified control of the pest on the different cassava varieties. All the varieties evaluated did not indicate any of them being resistant but demonstrated some level of tolerance to CGM attack of the species *M. progresivus* Doreste. Food and Agriculture Organization and World Health Organization (FAO/WHO) recommended cyanide level of cassava roots for human consumption to be 10mg ppm (FAO/WHO, 1991). For other industrial use, where cassava flour is the end product little consideration for cyanide level is required since drying and dehydration processes would cater for lowering of cyanogens content in the roots (Balagopalan, 2002; Ferris *et al.,* 1997). Fast population growth of CGM was found positively correlated to cyanide levels on cassava leaves within the medium range (>10<30). However, the evaluation results on cassava leaf cyanogens content (mg/kg) could not be used to conclusively determine or predict raw root cyanogenic potential indicative of safety for human consumption as soil, genotype and environmental interactions play important role to the final cyanogens root content (Iglesias *et al.*, 2002; Attalia *et al.*, 2001). Of interest from the results was the indication that CGM preferred higher cyanide bearing varieties to low cyanogens content ones. Breeders would benefit from the information as they breed against CGM. From the results leaf cyanide level content >10<30 was found to attract high CGM densities and led to increased biomass loss reducing photosynthetic leaf area. Cassava varieties with low levels of cyanide content (<10), have safer roots for human consumption and that would translate to tolerance to CGM of cassava varieties (Emanuel *et al.,* 2012; Iglesias *et al.,* 2002).

1. 3. 1 Acknowledgement

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Table 1: Mean (±SE) cassava green mite motiles density score in relation to visual damage score (D) and duration (days) in greenhouse (days) in 20.0 ± 2 °C, RH 63 ± 4%

	No CGM / duration		No. CGM / duration		No. CGM / duration		No. CGM / duration		No. CGM / duration	
Variety	**(D.1)**	**(Days)**	**(D.2)**	**(Days)**	**(D.3)**	**(Days)**	**(D.4)**	**(Days)**	**(D.5)**	**(Days)**
Kalezo	9	5	75 ±1.4	8.4 ± 0.5	320 ± 4.8	12.7 ± 0.7	870 ± 12.6	30.3 ± 0.4	1370 ± 7.1	33.0 ± 0.5
Karibuni	8	5	65 ± 1.2	9.0 ± 0.6	300 ± 4.7	21.0 ± 0.7	820 ± 11.2	33.0 ± 0.5	1170 ± 7.0	43.0 ± 0.2
Tajirika	11	5	55 ± 2.2	13.0±0.5	280 ± 4.8	28.0 ± 0.2	740 ± 14.5	33.0 ± 0.6	860 ± 4.4	35.7 ± 0.6
MM990183	8	5	75 ± 1.1	8.5 ± 0.5	560 ± 7.1	14.3 ± 0.5	980 ± 11.9	33.0 ± 1.3	1050 ± 3.6	36.7 ± 0.6
MM99005	10	5	90 ± 4.0	8.6 ± 0.5	325 ± 1.2	20.6 ± 0.4	930 ± 11.8	33.0 ± 0.9	1020 ± 5.9	36.0 ± 0.7
X-Mariakani	9	5	77 ± 2.9	9.3 ± 0.7	310 ± 2.4	18.0 ± 0.2	950 ± 11.2	30.1 ± 0.7	1190 ± 4.8	36.7 ± 0.4
MM97/3567	5	5	42 ± 0.7	21.3±1.2	250 ± 3.3	29.3 ± 0.5	770 ± 6.7	55.3 ± 1.0	-	-
MM96/2480	6	5	59 ± 1.2	9.3 ± 0.9	625 ± 8.4	22.3 ± 1.2	890 ± 5.1	30.6 ± 0.5	1100 ± 2.9	33.6 ± 1.3
MM96/9308	8	5	71± 5.7	17.0±0.3	290 ± 6.7	21.7 ± 1.2	580 ± 4.5	28.0 ± 0.8	770 ± 6.7	36 ± 1.3
H-value	-	-	*19.81*	*33.07*	*30.1*	*30.49*	*43.30*	*29.19*	*47.06*	*35.37*
P-value	-	-	*0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*

Kruskal-Wallis one-way analysis of variance at P=0.05

Table 2: Mean (±SE) leaf biomass loss due to CGM damage in 20.0 ± 2 °C, RH 63 ± 4%

	Leaf biomass loss (%) on the cassava varieties				
Variety	**D.1**	**D.2**	**D.3**	**D.4**	**D.5**
Kalezo	0a	15 ± 0.2a	22 ± 0.6a	54 ± 1.1a	67.2 ± 5.7a
Karibuni	0a	12 ± 0.2b	18 ± 0.4b	51 ± 0.5a	64.1 ± 5.1b
Tajirika	0a	9 ± 0.4c	14 ± 0.7c	23 ± 0.9b	29.4 ± 4.9c
MM990183	0a	12 ± 0.5b	18 ± 0.2b	51 ± 0.9a	54.5 ± 3.3d
MM99005	0a	9 ± 0.2c	13 ± 0.7c	44 ± 0.4c	55.6 ± 4.1d
X-Mariakani	0a	12 ± 0.5b	18 ± 1.1b	43 ± 0.2c	54.4 ± 6.5d
MM97/3567	0a	4 ± 1.2d	15 ± 1.4c	34 ± 1.2d	-
MM96/2480	0a	15 ± 0.8a	17 ± 0.7c	42 ± 0.3c	66.3 ± 2.4a
MM96/9308	0a	8 ± 0.9c	16 ± 0.6c	36 ± 0.8d	46.2 ± 3.0e

Similar letters across columns denote no significant difference (P >0.05), S.N.K Test.

Table 3: Mean (± S.D) highest number of CGM /leaf and biomass losses (%) in relation to cyanide (HCN) level on cassava

Cassava variety	HCN (mg/kg)	Peak No. CGM	Net biomass (%) loss
Kalezo	20.0 ± 7.1a	1370 ± 20a	67.2 ± 5.7a
Karibuni	20.0 ± 7.1a	1170 ± 10b	64.1 ± 5.1b
Tajirika	12.5 ± 3.5b	860 ± 60c	29.4 ± 4.9c
MM990183	12.5 ± 3.5b	1050 ± 100d	54.5 ± 3.3d
MM99005	12.5 ± 3.5b	1020 ± 10d	55.6 ± 4.1d
X-Mariakani	20.0 ± 7.1a	1190 ± 20d	54.4 ± 6.5d
MM97/3567	8.5 ± 4.9 c	585 ± 20e	-
MM96/2480	30.0 ± 7.1d	760 ± 40b	66.3 ± 2.4a
MM96/9308	14.0 ± 5.7b	580 ± 20e	46.2 ± 3.0e

Means with different letters across columns are significantly different (P<0.001), S.N.K Test

Table 4: Regression of cassava cyanogens (HCN) content to cassava green mite densities and the subsequent leaf biomass loss in 54 days

Factor		Time period (days) of mite population growth: 10	20	33	54
CGM density:	Slope (b)	57.3	259.4	829.4	900.4
	t-value	6.621	3.869	13.823	6.182
	r	0.2	0.1	0.4	0.5
	F-value	1.734	2.710	0.771	0.114
Biomass loss:	Slope (b)	6.2	9.4	9.6	10.4
	t-value	3.812	7.051	5.322	4.402
	r	0.6	0.7	0.9	0.8
	F-value	0.026	0.028	0.022	0.026

Linear regression effect of leaf cyanide content of cassava varieties, with Pearson correlation (r) values at 5% significant level.

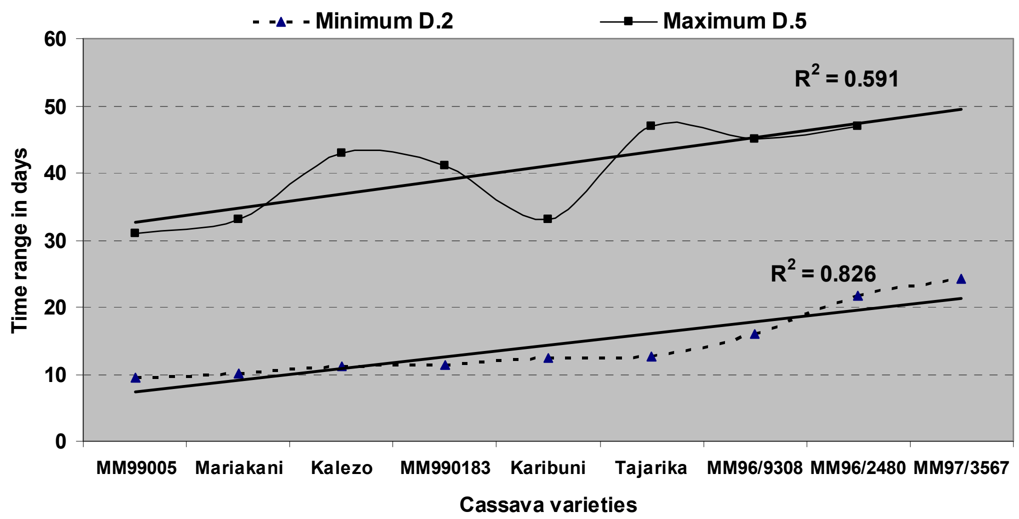

Fig.1: Range in days for minimum (D.2) and maximum (D.5) leaf damage score on cassava varieties under cassava green mite infestation