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Full Length Research Paper

Quantification and correlation of African cassava mosaic disease parameters on cassava genotypes (*Manihot esculenta,* Crantz) in Buea, Cameroon.

[1]Njock TE and [2]Sama V

[1]Department of Agronomic and Applied Molecular Sciences, University of Buea, P.O. Box 63 Buea, Cameroon
[2]Institute of Management and Financial Accounting (IMFA), Molyko, Buea, Cameroon

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Cassava, *Manihot esculenta* Crantz is an important staple in sub-Saharan Africa, feeding over one billion people but cassava mosaic disease militates against its productivity. We quantified and correlated disease parameters of resistance-graded cassava genotypes to African cassava mosaic geminivirus disease in a completely-randomized block design. Resistant (8061), moderately-resistant (local) and susceptible (AA) cassava was planted 1.0 m, 0.70 m and 0.35 m apart randomized within each planting distance. The data on the disease parameters: incidence, index of severity of symptoms, and whitefly, egg and larval counts were subjected to analysis of variance (ANOVA) and correlations among the parameters determined by Pearson's correlation coefficients. There were positive correlations among disease parameters. Higher whitefly population (4.07±1.74) occurred among plants with lower plant density (1.0 m) and lower populations (1.89±0.914) with those of higher density (0.35 m) for variety AA. Similarly, incidence (92.81±13.36%), severity (3.18±0.87), number of eggs (2.97±1.37) and larvae (1.97±1.11) were higher among plants of lower than those of higher plant density. Thus, cassava may be planted at relatively higher density to provide a favorable microclimatic environment for greater whitefly vector whitefly for its reduced build up hence lower chances of virus transmission and disease.

Keywords: Quantification, African cassava mosaic, cassava genotypes

INTRODUCTION

In Africa cassava, *Manihot esculenta* Crantz, grows at varying altitudes from sea level to 1800 m above sea level and from the sub-sahel semi-arid region to latitude 20^{o} South (Hahn *et al*., 1980), as well as throughout the year and does well on all soil types except in the swamps (Fauquet and Fargette, 1990). It has a potential yield of between 30.5 t/ha to 51.0 t/ha. Limiting this potential is the African cassava mosaic which is the most widespread and economically important disease of cassava in tropical Africa, caused by the African cassava mosaic geminivirus (Thresh *et al*., 1998a). The disease is a major constraint to cassava production in Cameroon. The occurrence of African cassava mosaic virus (ACMV), East African cassava mosaic Cameroon Virus (EACMCV) and East African cassava mosaic virus (EACMV) in cassava mosaic disease etiology has been confirmed (Fondong *et al*., 2000). Akinbade *et al*. (2010) reported in Cameroon, for the first time, the presence of East African cassava mosaic

*Corresponding Author's Email: tnjockeku@yahoo.com

Uganda virus (EACMV-UG), a recombinant virus responsible for the severe CMD epidemic in East and Central Africa, The Democratic Republic of Congo and Gabon (Legg and Fauquet, 2004). A mix infection of up to 23 % of ACMV with EACMV/EACMCV was detected and its incidence in the field ranged from 11 % to 83 %. Its occurrence in areas bordering EACMV-UG countries suggested the spread of the virus by whiteflies or infected cassava cuttings.

This work therefore sought to quantify and correlate the different African cassava mosaic disease (ACMD) parameters on resistant, susceptible and tolerant cassava genotypes. Specifically to, quantify the incidence, severity and whitefly vector abundance; assess the correlation of the different disease parameters and determine the effect of micro-climatic conditions on vector abundance, disease incidence and severity.

MATERIALS AND METHODS

Stem cuttings of three cassava genotypes were planted in three blocks of 5 m by 7 m at 1 m, 0.7 m and 0.35 m apart along rows. A block had three segments each having plants of a particular planting distance randomized. Thus in Block I, the upper segment had plants at 1 m, the middle segment at 0.7 m apart and the lower segment at 0.35 m apart. Each of the segments had three rows of plants with each row consisting of a particular clone. In block II, unlike in Block I, the upper segment consisted of plants at 0.7 m, the middle segment at 1.0 m apart then the lower segment at 0.35 m apart while the same cassava varieties were maintained across the rows. In block III, the upper segment had plants at 1.0 m, the middle segment at 0.7 m and the lower segment at 0.35 m apart. The variation in the planting distances sought to create different micro-climatic conditions in the plantings and changes in position of particular planting distances across the blocks ensured complete randomization. No fertilizer, nor manure, was applied and the field was maintained weed-free to enhance the effect of the microclimatic conditions due to the variations in planting distance and free movement of the whitefly vector.

The approximately 20-cm long (5-6 nodes) stem cuttings of the cassava clones 8061, AA and 'Local' were donated by the Institute of Agricultural Research for Development (IRAD), Ekona and reported to be resistant, moderately-resistant and susceptible to ACMD, respectively. Data collection started four weeks after planting (4 WAP) and assessed every fortnight for three months on the disease parameters incidence and severity of mosaic symptoms, adult whiteflies, larvae and eggs on leaves thus:

Determination of disease severity

Leaves infected by African cassava mosaic virus were rated by an index of severity of symptoms (ISS) according to severity scores on single leaves of five randomly selected plants per genotype on a scale 1-5 (Hahn et al., 1989) where class 1 = no symptoms, class 2 = a mild chlorotic pattern over the entire leaf while the latter remained green and healthy. Class 3 = a moderate mosaic pattern throughout the leaf, narrowing and distortion in the lower one third of the leaflets, class 4 = severe mosaic, distortion in two-thirds of the leaflets and general reduction in the leaf sizes and class 5 = severe mosaic and distortion and reduction in the entire leaf. The mean index of severity of symptoms on diseased plants only (ISS_{DP}) was computed thus:

$$ISS_{DP} = \left(\left(\sum_{S=2}^{5}(SXs)\right)/\left(\sum_{S=2}^{5}(Xs)\right)\right)$$

Where S is the severity scale 1-5, Xs the number of plants given the score s and DP refers to diseased plants.

Determination of the disease incidence

Plants with mosaic symptoms of the ACMD were visually scored per genotype and planting distance and disease incidence was computed as the ratio of diseased plants to the total number of plants scored expressed as a percentage thus:

$$\%\ \text{disease incidence} = \left(\left(\sum_{S=2}^{5}(SXs)\right)/\left(\sum_{S=1}^{5}(Xs)\right)\right) \times 100$$

Determination of eggs and larvae

Whitefly eggs and larvae on the underside of cassava leaves were counted by destructive sampling. The first fully-developed leaf from the top of the shoot of five randomly sampled plants each of three cassava genotypes per segment per block were carefully removed at the base of the petiole and labeled. This therefore gave a total of 135 leaves each sampling date. In vitro, under a stereo microscope, the fresh leaves were observed at a magnification of the 20x objective for presence of eggs and larvae of the whitefly *Bemisia tabaci* Genn (Hemiptera, Aleyrodidae) on full leaves (complete lobes), every fortnight. Whitefly eggs were rounded while larvae were ovoid with tapered ends.

Table 1. Mean incidence and severity of African cassava mosaic disease on cassava genotypes relative to planting distance.

Cassava genotype	Resistance Status	Planting distance (m)	% disease incidence (Mean±STD)	disease severity (Mean±STD)
AA	Moderately-resistant	0.35	91.13±21.21	2.84±0.50
		0.70	91.98±15.03	2.99±0.64
		1.00	92.81±13.36	3.18±0.87
8061	Resistant	0.35	34.92±11.26	2.31±0.23
		0.70	43.15±16.40	2.29±0.59
		1.00	51.33±18.63	2.39±0.66
Local	Susceptible	0.35	40.67±13.72	2.39±0.29
		0.70	55.58±17.57	2.55±0.37
		1.00	54.97±20.07	2.66±0.79

STD = Standard deviation

Determination of whitefly population

Direct visual counts were taken in situ on intact leaves early in the mornings by carefully turning the underside of the first three fully-developed leaves from top of three randomly sampled plants per genotype. This was repeated every fortnight.

RESULTS

Incidence of ACMD on cassava genotypes relative to planting distance

The highest disease incidence was recorded on plants of genotype AA at 1.0 m while it was relatively lower on those at 0.7 m and least on those at 0.35 m. For genotype 8061, again the highest incidence was on the plants at 1.0 m, relatively lower on those at 0.7 m and least on those at 0.35 m apart and this was similar on plants of the local genotype (Table 1).

Severity of ACMD on cassava genotypes to planting distance

In the cassava genotype 8061, the most resistant, the highest severity was recorded on plants at 1.0 m, relatively low on those at 0.7 m and lowest on those at 0.35 m apart (Table 1).

Of all the genotypes, variety AA was the most susceptible, and recorded the highest mean severity index among the plants which were planted 1.0 m apart (3.18±0.87). For plants at 0.7 m apart, the severity was relatively lower but the lowest severity was recorded among the plants at 0.35 m apart.

For the variety Local, the least incidence was recorded on plants that were 0.35 M apart and a relatively higher severity was the case in the plants at 0.7 m apart and the highest mean severity index was recorded on plants that were 1.0 m apart.

Whitefly, larval and egg abundance on cassava genotypes relative to planting distance

Generally, there was an increase in adult whiteflies, larvae and eggs with increase in planting distance irrespective of the cassava genotype. For instance, the highest number of adult whiteflies (4.07±1.74) occurred on plants of 8091 and AA, larvae (1.97±1.11) on AA and eggs (3.38±1.65) on 8061 genotypes at 1.0 m apart, while the lowest number of all three whitefly developmental stages occurred at 0.35 m apart in all cassava genotypes (Table 2).

Correlations of adult whitefly abundance with ACMD parameters and its developmental stages

Correlation of adult whitefly abundance with disease incidence, severity, planting distance, and eggs and larvae.

A correlation analysis of the whitefly number with the percentage incidence was carried out using the Pearson correlation coefficient and a 2-tailed test at 95 % and 99 % confidence intervals. A coefficient of 0.322 showed a weak positive correlation and this was significant at both 95 % and 99 % confidence intervals (Figure 1a). For whitefly number versus the index of severity of ACMD, a coefficient of 0.393 indicated a weak positive correlation significant at 95 % but not at 99 % confidence interval (Figure 1b). Whitefly number versus the planting distance showed a strong positive correlation (0.506) which was significant both at 95 % and 99 % confidence intervals (Figure 1c), while coefficients of 0.731 and 0.722, respectively for egg and larval counts showed very strong positive correlations between these parameters and were significant at both 95 % and 99 % confidence intervals (Figure 1 d, e).

Table 2. Whitefly, larval and egg abundance on cassava genotypes relative to planting distance.

Cassava genotype	Planting distance (m)	Whitefly number (Mean ± STD)		
		Eggs	Larvae	Adults
AA	0.35	2.02±1.19	0.83±0.90	1.89±0.91
	0.70	2.42 ±1.20	1.48±0.87	3.22±1.49
	1.00	2.97±1.37	1.97±1.11	4.07±1.74
8061	0.35	2.55±1.14	1.25±0.89	2.18±1.08
	0.70	2.22±1.09	1.18±0.76	3.13±1.51
	1.00	3.38±1.65	1.90±1.07	4.07±1.74
Local	0.35	1.32±0.84	0.82±0.68	1.86±0.97
	0.70	2.35±1.09	1.32±0.86	2.82±1.27
	1.00	2.56±1.28	1.59±0.93	3.80±1.71

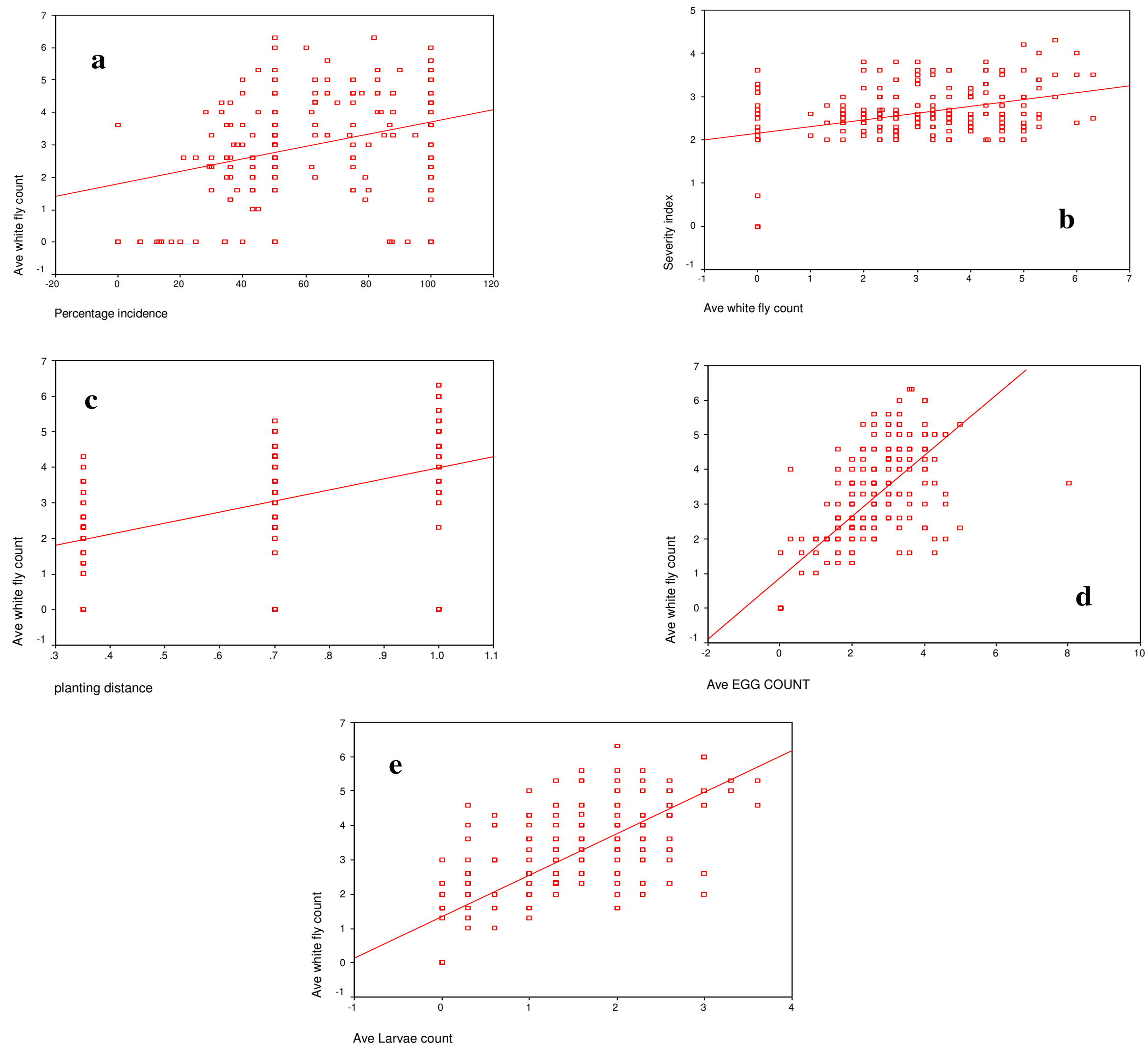

Figure 1. Correlation of whitefly abundance with disease incidence (a) severity (b) planting distance (c) eggs (d) and larvae (e) on

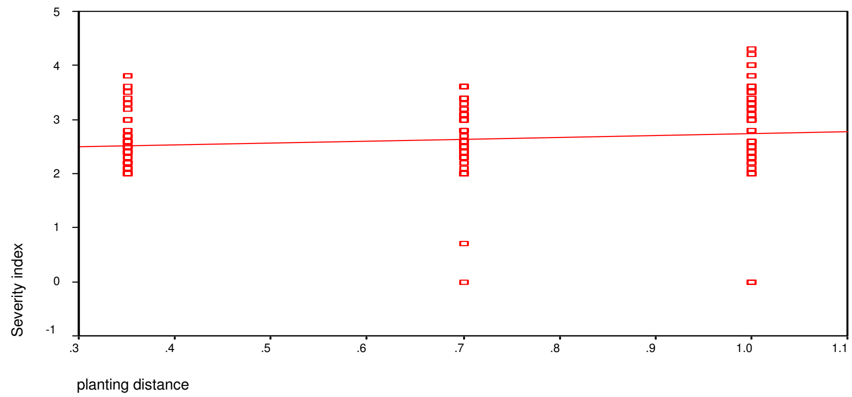

Figure 2. Correlation of planting distance with the index of severity of symptoms on all cassava genotypes.

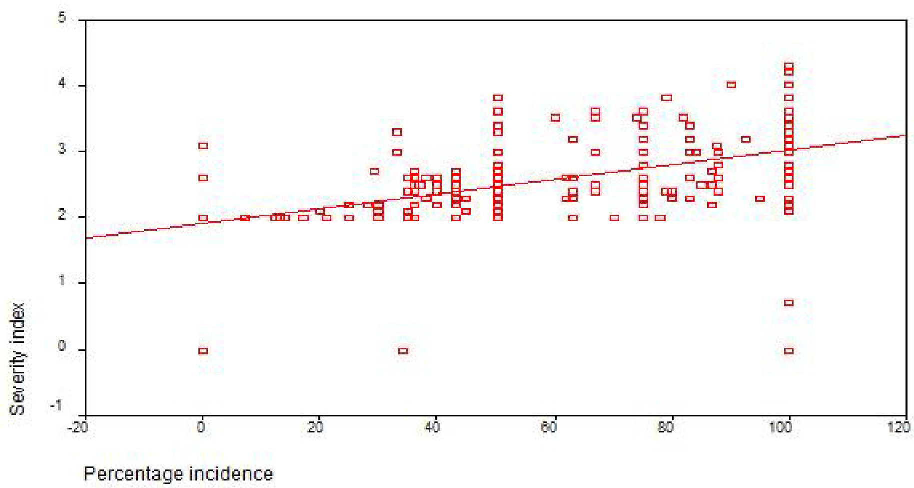

Figure 3. Correlation of ACMD incidence with index of severity of symptoms on all cassava genotypes.

Correlation of planting distance with ACMD incidence and severity

A correlation of planting distance with ACMD incidence showed a weak positive correlation coefficient (0.229) significant at both 95 % and 99 % confidence intervals. However, a correlation of planting distance with index of severity of symptoms showed a weak positive correlation (0.033) which was significant at 95 % but not at 99 % confidence interval (Figure. 2).

Correlation of ACMD incidence with index of severity of symptoms on all cassava genotypes.

Similarly, a correlation of ACMD incidence with index of symptom severity showed a positive correlation coefficient (0.466) which was significant at both 95 % and 99 % confidence intervals (Figure. 3).

DISCUSSION

Cassava plants at 1.0 m apart of the genotype AA, recorded the highest incidence and severity. This was probably because genotype AA was more susceptible to ACMD than genotypes 8061 and local'. This susceptibility to ACMD which led to the development of symptoms faster and greater damage within a shorter period could be attributed to the low inherent resistance to ACMD. In contrast genotype 8061 which was a resistant genotype had the highest mean percentage incidence (51.3±18.6%) compared to variety AA (98.8±13.3%). Also the severity index in 8061 (2.4±0.67) was much lower compared to that of the variety AA (3.2±0.9). Genotype 8061 which recorded the lowest incidence and severity index probably owed its inherent resistance to the virus and not to the whitefly vector. Similar trials with a wide range of cassava genotypes in the Ivory Coast found a weak relationship between field resistance (proportion of infected plants) and vector resistance (number of whitefly vectors effectively feeding on plant) (Fauquet and Fargette, 1990).

Within cassava genotypes, the incidence and severity index were highest for plants spaced 1.0 m, high in those at 0.7 m and least in those at 0.35 m apart. This was probably due to relatively higher temperatures with the wider planting distances compared to the cooler and more favorable micro-climatic conditions found among plants with higher plant densities. The more conducive microclimatic conditions probably enabled higher whitefly mobility in the cooler and more favorable environment of high plant density thus resulting in lower counts on the leaves compared to the higher radiation, higher temperatures and less favorable environmental conditions that prevailed in the low density plots resulting in the whitefly resting on the undersurface of the leaves for longer periods thus feeding and increasing the chances of viral transmission. Conversely, reduced vector populations resulting from higher incidence of virus-infected cassava plants were proposed as a possible cause for super abundance. Sseruwagi *et al.* (2004), showed that mean whitefly abundance was significantly correlated with current-season infection.

Our findings indicated a strong positive correlation between the number of whiteflies and planting distance. This was in conformity with Dengel (1981) that solar radiation has been associated positively with fluctuation of *B. tabaci* populations. In Kenya, Seif (1981) indicated that the strongest association with numbers was provided by the interaction between relative humidity and temperature. This relationship is generally considered, however, to be rather less clear as a determinant of *B. tabaci* numbers than temperature (Fauquet *et al*., 1985). We also found that incidence of ACMD increased relative to the number of whiteflies at a given planting distance. Similar findings by Dengel (1981) associated large numbers of whiteflies with rapid ACMV spread. However, Robertson (1986) found no such association in coastal Kenya probably because he monitored adult whiteflies using yellow attractive sticky traps 1.5 m above ground rather than direct counts on the leaves. Similarly, in the Ivory Coast, Fargette *et al*. (1985, 1990) demonstrated the relationship between the spatial patterns of spread of ACMV and infestation by *B. tabaci* in the field and in Uganda, Otim-Nape (1993) showed a positive correlation between number of adult whiteflies and virus disease incidence.

Paradoxically, high populations of *B. tabacci* have also been recorded in cooler and higher altitudes. Rapidly growing cassava supported high whitefly populations (Dengel, 1981) and more likely to be more susceptible to virus infection and the virus multiplied more efficiently.

The strong positive correlation between whitefly population and egg and larval counts could be due to the fact that, there will be greater oviposition on the leaves on which the adult whiteflies rest for a longer period compared to the low whitefly counts recorded on plants of high density due to the favorable micro-climatic conditions which probably led to more frequent whitefly mobility hence less oviposition.

In conclusion, there were positive correlations among various disease parameters with the implication that increases in whitefly populations resulted in a concomitant increase in the larval and egg counts. The highest whitefly populations corresponded to the highest incidence and severity index. Conversely, the lowest severity index corresponded with the lowest incidence, the least count of the whitefly and, consequently, the least eggs and larvae. The microclimatic conditions conferred on the plants by the differences in planting distance hence planting densities had effects on the disease parameters (severity index and incidence) as well as a bearing on the number of adult whiteflies, eggs and larvae. The least number of whiteflies occurred on plants with highest plant density (0.35 m apart) while the highest numbers among those with the least plant density (1.0 m apart).

Based on the findings of this study, it is therefore recommended that:

1) Cassava may be planted at relatively higher density to provide favorable microclimatic environment for greater mobility of the viruliferous whitefly vector to reduce its infestation and the chances of transmission, thus disease incidence and severity.

2) There is need to address the recombinant East African cassava mosaic Cameroon virus reported in Cameroon. This might be the source of future severe cassava mosaic disease infections.

3) Encourage the enhanced multiplication and distribution of improved ACMD-resistant propagative “seed” planting cassava material to the local farmers to mitigate disease incidence.

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