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Original Research Article

Effects of phytosanitation and cassava (*Manihot esculenta*) variety on the incidence of cassava mosaic disease and whitefly abundance in a forest zone of Cameroon

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Cassava Mosaic disease (CMD) is transmitted either through infected cuttings or by whiteflies. We investigated how phytosanitation methods could affect cassava varieties responses to CMD infection and its vector and the relationship between vector abundance and CMD incidence. The experiment was set in a completely randomized design with three phytosanitation methods applied on four cassava varieties. CMD severity and whitefly population were assessed at 1, 3, 6 and 9 months after planting. Yield data was recorded at harvest, 12 months after planting. Phytosanitation had effect on CMD incidence on all varieties except the local check which had more than 90% of its plants infected. There was no difference in whitefly population abundance between treatments. We found a negative correlation between vector abundance and CMD incidence with variety 92/0326 having lowest CMD incidence and more whitefly counts. At harvest, main difference in the fresh root yield was observed between the removal treatment and the others. Large difference in yield were observed between varieties, with the local variety producing less than the other varieties. The combination of phytosanitation and cassava varieties with initial level of CMD resistance can reduce the transmission of CMD in cassava fields in the humid tropical forest.

Key words: Cassava varieties, disease severity, initial resistance, *manihot esculenta*, phytosanitation and whitefly

INTRODUCTION

Cassava remains the mains sources of carbohydrates and microelements for population in sub-Saharan Africa, with Nigeria being the number one producer in the world. It can be grown on marginal lands (Fu et al., 2014) and some varieties are drought tolerant (Okogbenin et al., 2013; Carvalho et al., 2016; Brown et al., 2016). However, cassava in farmer's field does not produce at its potential, partly due to the lack of improved varieties and the susceptibility of landrace to pests and diseases among which the cassava mosaic disease (CMD) (Thresh and Cooter 2005).

CMD is one of the main threat cause of decrease in cassava yield in many parts of Africa, Sri Lanka and India (Geddes, 1990; Calvert and Thresh, 2002; Sseruwagi et al., 2004a, b). Among several viruses infecting cassava, the

African cassava mosaic virus (Genus: Begomovirus; Family: Geminiviridae) is the most common in Africa and India (Alabi et al., 2011). It is initially transmitted through whiteflies and subsequent transmission in done through the exchange of infected plant material between producers (Nweke, 2009). The latter remain the main mode of disease dissemination of the CMD between fields.

CMD can be controlled either by using improved varieties which are resistant or tolerant to the disease, or through cultural practices such as phytosanitation (Mallowa et al., 2011). Clean cassava cuttings can be produced in the laboratory via tissue culture technique or thermotherapy to eliminate the virus from infected plants (Kaiser and Teemba, 1979), but such techniques are relatively difficult to use. Simple field techniques are generally adequate for small scale producers (Guthrie, 2003). Control of cassava mosaic by phytosanitation has been tested by some authors under different conditions and is very simple in principle (Guthrie, 2003; Thresh and Cooter, 2005). According to Guthrie (2003), this technique relies on visual check of the status of the plant to select only those with no visible disease symptoms. This is made possible because even in highly infected cassava fields, there are always a few mosaic-free plants from which planting material can be selected. The planting material that has been selected and planted can later show symptoms either on the leaves or the stems. Therefore, those plants with severe mosaic symptoms in the field after planting are uprooted (removal) to reduce the source of infestation and then limit the transmission to other plants by the whiteflies. However, the limitation of the sanitation method relies on the fact that if the variety cannot display initial resistance to CMD infection, it will get infected immediately when planted, especially when the disease pressure is high, giving no practical value to the sanitation method applied (Mallowa et al., 2011). This has been demonstrated in lowland areas with high rainfall and sanitation is not generally recommended for such areas (Guthrie, 2003). In this study, we investigated the effectiveness of phytosanitation on CMD incidence and whitefly population using three improved cassava varieties (TME 419, TMS 92/0326, 8034) and a common local variety (Zaebomadje) in a highland of a humid forest zone with high rainfall in Cameroon.

MATERIAL AND METHODS

The experiment was carried out in Ongot (N 3.84364°; E11.37752°; 740 m; Annual rainfall: 1720 mm), a village near Yaoundé in the center region of Cameroon. The plot was selected in a four-year fallow dominated by *Chromolaena odorata* and *Triumphetta cordifolia* and prepared by slashing and burning the biomass as practiced by farmers in the area. The varieties TME 419 and TMS 92/0326 were obtained from IITA-Cameroon while 8034 and Zaebomadje was obtained from the cassava collection of the National Institute of Agricultural Research for Development (IRAD). Each of the four variety shows different level of susceptibility to CMD infection.

Experimental design and treatments

We considered cassava varieties and phytosanitation as factors with each variety subjected to all phytosanitation methods. All plots were set in a complete randomized block design with four repetitions. Cuttings were planted in a 7 × 4 array, with 1 m between plants. Phytosanitation methods included (1) cassava cuttings selected from plant without visible CMD symptoms (TS); (2) cassava cuttings from plant chosen using a random table of numbers with removal of plant showing CMD symptoms at 4 weeks after planting (TR), (3) combination of selection and removal (TSR), and cassava cuttings from plants chosen using a random table of numbers, but with no removal of plant showing CMD symptoms (TC). The trial was planted in September 2015.

Parameters recorded

CMD severity was assessed in each plot using a 1 to 5 scale scores where 1 means “no visible CMD symptoms on leaves” and 5 means “severe CMD symptoms with stunted and distorted leaves” (IITA,1990). Field evaluation started at 1 month after planting (MAP), then at 3, 6 and 9 MAP. CMD incidence was calculated as the proportion of the number of plant infected to the total number of plant evaluated in the plot. We also score the abundance of the whiteflies in the various treatment by counting the number of whiteflies under the apical leaves of the plants. The field was harvested 12 MAP and we measured the fresh weight of 10 selected plants excluding the border rows.

Data analysis

Chi square test was used to evaluate the relationship between the CMD incidence and score and the phytosanitation method applied. We used the linear regression model to evaluate the relationship between the number of whitefly and the CMD incidence on plants. We also used the T-test to compare CMD mean CMD incidence of each phytosanitation sanitation method with the control. Whiteflies abundance was compared between treatment using ANOVA after log transformation Analysis was blocked by variety and was done using the Statistical software JMP®8.0.2 (SAS, 2009).

RESULTS

Phytosanitation had effect on CMD incidence on all varieties except the local variety in which more than 90% of the plants were infected. No CMD symptoms were observed in the selection and selection plus removal treatment for the varieties 92/0326 and TME 419. When all the varieties were considered, greatest CMD incidence was observed in the control treatment (33.7%) followed by the selection

Table 1. Average CMD-incidence on varieties under different phytosanitation practices

Variety	Phytosanitation				Average
	TC	TR	TS	TSR	
8034	12.5	5.0	0.0	2.5	5.0
92/0326	7.5	2.5	0.0	0.0	2.5
Local	97.5	92.5	95.0	95.0	95.0
TME 419	17.5	12.5	0.0	0.0	7.5
Average	33.7	28.1	23.8	24.4	27.5

(TS) Cassava cuttings selected from plant without visible CMD symptoms; (TR) cassava cuttings from plant with removal of plant showing CMD, (TSR) combination of TS and TR; (TC) cassava cuttings from random plants with no removal of plant showing CMD symptoms (control). Each treatment was replicated four times.

Table 2. Pairwise comparison of CMD incidence between treatments

Method	TC	TR	TSR
TR	3.06		
TSR	6.81	1.18	
TS	7.43	1.81	-1.94

Positive values show significant differences. (TS) Cassava cuttings selected from plant without visible CMD symptoms; (TR) cassava cuttings from plant with removal of plant showing CMD, (TSR) combination of TS and TR; (TC) cassava cuttings from random plants with no removal of plant showing CMD symptoms (control). Each treatment was replicated four times.

plus removal (24.4%), the removal treatment (28.1%) and the selection (23.8%) (Table 1).

The local variety was the most affected (95%) compared to 92/0326 (2.5%). The pairwise comparison of CMD incidence showed that the control differed from the other phytosanitation methods (Table 2).

The distribution of CMD severity score differed between treatments for all the tested varieties Chi-square test: $\chi^2 = 126.7$, $p < 0.0001$, df = 12). Greater severity score (score 5) was recorded only in the removal treatment. Severity in the other treatment ranged from 2 to 4 (Figure 1).

There was no difference in whitefly population distribution between treatment ($F_{(3,255)} = 0.17$; $p = 0.918$) when all varieties were pooled and when we considered each variety separately ($p > 0.05$). However, variety 92/0326 had more whiteflies than other varieties ($F_{(3,255)} = 25.7$; $p < 0.0001$) while variety TME 419 had the lowest whitefly count (1.3 ± 0.3) (Table 3). The number of whiteflies was negatively correlated with CMD incidence (Linear regression: Incidence = 33.1 - 1.3*Mean(whitefly), $F_{(3,254)} = 9.25$, $p = 0.003$)

At harvest, there was a significant difference in the fresh root yield between the phytosanitation methods ($F_{(3,63)} = 2.94$; $p = 0.041$). Plant in the removal and selection plus removal treatment yielded more than plants in the selection and control treatments. The main difference was observed between the removal and the selection treatments (t = 2.45, df = 57, p = 0.0172) and between the removal and the control treatments (t = 2.59, df = 57, p = 0.0119) (Table 4). Greater difference could be observed between varieties with the local variety producing less than the other varieties ($F_{(3,63)} = 87.7$; $p = 0.0001$).

DISCUSSION

Several studies had discussed the CMD management strategy in farmer's fields (Atiri et al., 2004; Thresh and Cooter 2005; Vanderschuren et al., 2007, 2012; Mallowa et al., 2011). Some farmers used chemical to control the vector, but this has not been widely practiced because of the cost associated to the methods, the danger to farmer's health and the negative impact of pesticide on the environment. Our study showed that the selection of cuttings and the use of improved varieties in the field can contribute to the reduction of CMD incidence in the cassava farm. By using selected cassava cuttings with no visible CMD symptoms, there are more chances to get clean plants at germination. Also, the removal of infected plant after germination reduces the source of inoculum in the field, thus reducing in incidence of CMD on neighboring plants. This method is however controversial as it has been

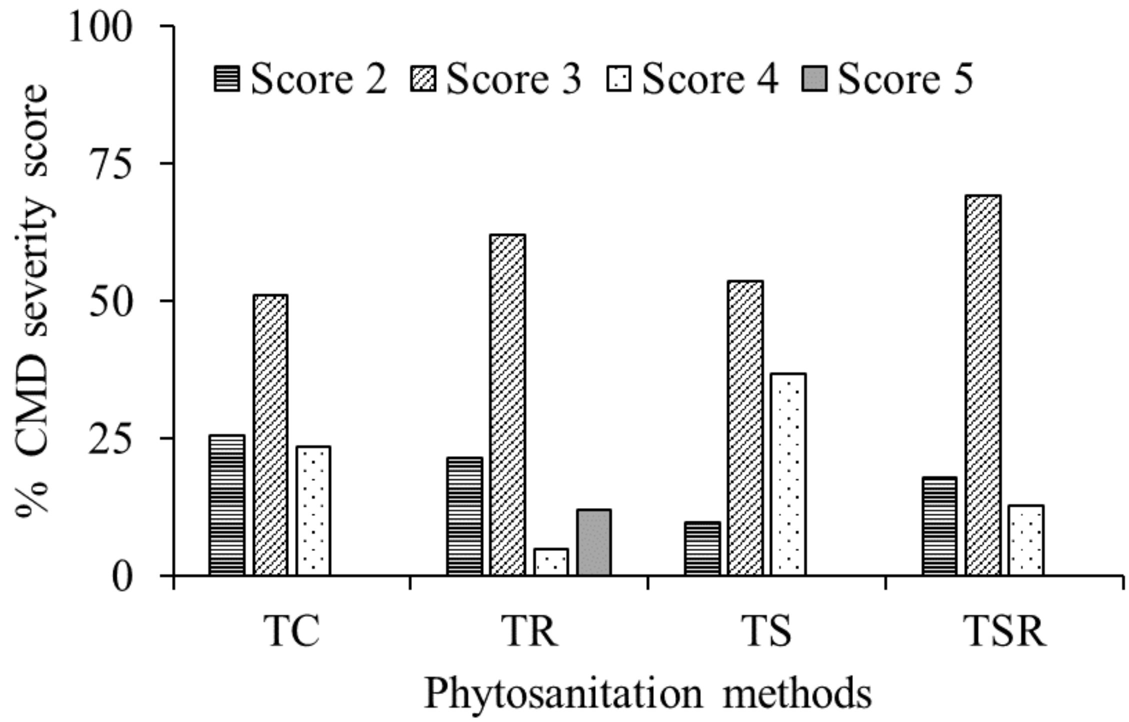

Figure 1: Distribution of CMD severity scores in the various (Chi-square test: $\chi^2 = 126.7$, $p < 0.0001$, df = 9)

Table 3. Average number of whiteflies per plant on varieties under different phytosanitation practices

Variety	Phytosanitation				Average
	TC	TR	TS	TSR	
8034	3.4	5.3	4.6	4.9	4.5[b]
92/0326	9.5	8.7	8.4	9.0	8.9[a]
Local	3.0	1.3	2.5	3.7	2.6[c]
TME 419	0.8	1.6	1.5	1.5	1.3[d]
Average	4.2[a]	4.2[a]	4.3[a]	4.8[a]	

Values sharing same letters in a row or column are not significantly different (p > 0.05). (TS) Cassava cuttings selected from plant without visible CMD symptoms; (TR) cassava cuttings from plant with removal of plant showing CMD, (TSR) combination of TS and TR; (TC) cassava cuttings from random plants with no removal of plant showing CMD symptoms (control). Each treatment was replicated four times.

Table 4. Fresh root yield (kg/plant) of varieties under different phytosanitation practices

Variety	Phytosanitation				Average
	TSR	TS	TR	TC	
8034	21.3	21.5	15.5	18.0	19.1[b]
92/0326	25.3	22.9	27.0	23.0	24.6[a]
Local	11.5	12.0	16.0	13.5	13.3[d]
TME 419	16.5	14.5	19.5	16.8	16.8[c]
Average	18.7[ab]	17.7[b]	19.5[a]	17.8[b]	18.4

Values sharing same letters differ non-significantly (p > 0.05) (TS) Cassava cuttings selected from plant without visible CMD symptoms; (TR) cassava cuttings from plant with removal of plant showing CMD, (TSR) combination of TS and TR; (TC) cassava cuttings from random plants with no removal of plant showing CMD symptoms (control). Each treatment was replicated four times.

demonstrated CMD infection spread more rapidly from neighboring fields compared to the spread with the field (Fargette et al., 1994). This is mainly due to the viruliferous whitefly coming from neighboring field, thus reducing the effect of the removal of infected plant within the field as observed in our field, unless the removal is combined with initial cutting selection (Thresh and Cooter, 2005).

For a sanitation method such as cutting selection to be practical and efficient, there should be plant with no visible CMD symptoms and the variety must show some delay to the infection by whiteflies (Thresh and Cooter, 2005). When there is a delay in infection after cutting has been selected, plants establish and grows faster compared to plants that sprout from infected cuttings. Even if selected varieties get infected later during its growth, the yield will be greater than that of plant grown from infected cuttings (Fargette et al., 1994; Thresh et al., 1994; Thresh and Cooter, 2005). However, because of latent period during which the virus develops and spread into the plant, cutting selection from asymptomatic plants does not guarantee the absence of virus into those plants, and should be done repeatedly to eliminate plant with latent infection.

We also noted that the effect of cassava variety was highly significant for CMD infection. The local check remains consistently infected at higher incidence despite the sanitation methods applied showing that CMD infection is more dependent on the variety than the environment or the sanitation method applied (Muengula-Manyi et al., 2013). Usually, most of the improved cassava varieties show less amount of latent infection especially when cuttings are in areas where the CMD incidence is low the vector population is less active. They could then be used in conjunction with phytosanitation methods to lower the spread of CMD (Thresh and Cooter, 2005). Highest severity index (score 5) was not recorded in the selection treatment even for the local check. This suggest that delaying the infection in young plant by selecting CMD symptomless plant may reduce the severity of the disease on the plant.

Some studies have argued that more whiteflies abundance results in higher transmission rate of viral diseases (Fargette et al., 1985; Fargette et al., 1990, 1994; Maruthi et al., 2005; Adjata et al., 2012). These observations contrast with our findings as we observed that the variety with more whiteflies has the lowest CMD incidence. It has been proven that vectors that align on a suitable host plant, feed and reproduce there are not likely to spread the disease from plant to plant (Stansly and Naranjo, 2010). Based on this theory, we can affirm that high whiteflies population does not invariably lead to higher CMD incidence if the variety can oppose initial resistance as demonstrated with the variety 92/0326. Similar observations were recently done in Kenya (Njoroge et al., 2016).

Difference in the fresh root yield were not observed between the phytosanitation methods although plant in the removal and selection plus removal treatment yielded more than plants in the selection and control methods. By selecting cassava cuttings at planting, the farmer could get highest weight of tuberous roots per plot, but this was function of the variety (Mallowa et al., 2011). The removal on infected plants in our experiment may have allowed the remaining plants to thrive on the free space and produce more roots. However, at the level of yield per hectare, the removal of plant reduces the plant density and the yield may not outweigh the plants removal., It is therefore advisable to remove infected plant early and replace with cuttings from healthy plants to maximize the root yield. Removal could be encouraged in cassava seed production farms to limit the propagation through planting material.

This study has shown that phytosanitation can contribute to the reduction of CMD incidence and severity on cassava. It success will be more perceptible if the cassava variety can display initial resistance to CMD infection. There was negative correlation between vector abundance and CMD incidence. Therefore, the combination of phytosanitation with cassava varieties that have initial level of CMD resistance can reduce the transmission of CMD in cassava fields in the humid tropical forest. Farmer should make effort to identify and mark asymptomatic plant to collect their cuttings from those plants even if the plants become leafless.

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Conflict of interests

The authors declare that they have no conflicting interests.

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