EFFECTIVENESS OF PHYTOSANITATION IN CASSAVA MOSAIC DISEASE MANAGEMENT IN A POST-EPIDEMIC AREA OF WESTERN KENYA

S. O. Mallowa[1], D. K. Isutsa[1], A. W. Kamau[1] and J. P. Legg[2]
[1]Egerton University, Egerton, Kenya
[2]International Institute of Tropical Agriculture, Dar es Salaam, Tanzania
E-Mail: dorcaski@yahoo.com

ABSTRACT

The effectiveness of phytosanitation in managing cassava mosaic disease (CMD) was studied in a post-epidemic area of western Kenya. Four varieties [MM96/4466 (resistant), TMS 30572 (Migyera, moderately resistant), Bukalasa 11 (Serere, moderately susceptible) and Karemo (susceptible)] and four phytosanitation approaches (roguing, cutting selection, roguing plus cutting selection, and none) were studied at two sites (Alego and Ugunja) for two seasons. With no phytosanitation, resistant varieties had very low levels of CMD infection, and infected plants expressed very mild symptoms. By contrast, the most susceptible variety became heavily diseased, with incidences of > 90%, whether phytosanitation measures were applied or not. Tuberous root yields were highest for CMD-resistant varieties. Roguing susceptible varieties resulted in significantly reduced root yields compared to the 'do nothing' control, largely as a result of the greatly reduced plant population. However, plots of CMD-susceptible varieties where selection was applied gave tuberous root yields that exceeded those of the control and that were comparable to those of the resistant variety with the equivalent treatment. These data clearly demonstrate the value of selection as an approach for maintaining the production of local varieties under the CMD post-epidemic conditions that are now prevalent across large areas of East and Central Africa. Conversely, the results show that roguing provides no tuberous root yield benefit under any of the tested circumstances. Raising awareness amongst farming communities of the potential advantages of selection of healthy planting material should be an important component of CMD management programmes, in tandem with efforts to multiply and disseminate planting materials of resistant varieties.

Keywords: cassava mosaic disease, phytosanitation, M*anihot esculenta*, resistant, roguing, selection, severity, susceptible.

INTRODUCTION

Cassava mosaic disease (CMD) is the most widespread and economically important disease of cassava in Africa (Legg, 1999; Thresh *et al*., 1994a). The disease is caused by cassava mosaic Gemini viruses (CMGs), is propagated by the use of infected cuttings and CMGs are transmitted by the whitefly *Bemisia tabaci* (Gennadius) (Storey and Nichols, 1938). A novel recombinant CMG (East African cassava mosaic virus-[Uganda]) was associated with the severe CMD pandemic, which was first reported in Uganda in the late 1980s and has since spread to affect nine countries in East and Central Africa (Legg, 1999; Legg *et al*., 2006). Management efforts for CMD have focused mainly on multiplication and dissemination of CMD-resistant varieties with considerable success (Otim-Nape *et al*., 2000). However, the spread of the pandemic exceeds the pace of implementation of these measures (Legg and Fauquet, 2004). Therefore, alternative means of CMD management using local varieties available in 'post-epidemic' areas are needed. Proving that phytosanitation is unwarranted when varieties are suitably resistant in 'post-epidemic' areas will greatly facilitate extension work through direct targeting of scarce resistant varieties for planting in areas most recently affected by the CMD pandemic.

Phytosanitation involves measures such as selection and roguing that improve the health-status of cassava planting materials, thereby enhancing growth and reducing sources of inoculum (Thresh, 1987). Selection of disease-free materials for all new plantings results in rapid establishment, faster growth and higher yields (Fargette *et al*., 1990). Virus-free materials eliminate the initial source of virus within crops during the early and most vulnerable stages, thus delaying subsequent infection. It is possible to maintain a CMD-susceptible cultivar free of CMG infection by growing virus-free plants in a low-spread area treated with insecticide against vector insects (Owor *et al*., 2004b). Roguing diseased plants, especially during early months of growth (Guthrie, 1987), eliminates initial sources of inoculum from which further infection can occur. Additionally, it has been shown that the rate of CMD spread is greater in un-rogued than in rogued plots (Fargette *et al*., 1990). Severe epidemics reported in Uganda in the 1940s were controlled using CMD-resistant varieties and phytosanitation (Jameson, 1964). This success was only achieved, however, through the robust area-wide enforcement of roguing in affected parts of Uganda. Although it is unlikely that such an approach could be replicated under current socio-political conditions, the application of phytosanitation in CMD control merits further research, since resistant variety dissemination programmes are costly and slow. This paper reports results of a study undertaken to investigate the effectiveness of selection and roguing phytosanitation in a CMD 'post-epidemic' area of western Kenya, with a view to identifying cheap and readily applied CMD

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management measures that can complement resistant variety dissemination.

MATERIALS AND METHODS

Experimental sites, design and layout

The experimental site was located in Alego and Ugunja Divisions of Siaya District, which is a 'post-epidemic' area of western Kenya. 'Post-epidemic' areas are characterized by low CMD incidence (<30%), low CMD severity (<3.0), spread of CMD primarily through the planting of infected cuttings and moderate *B. tabaci* vector populations (Legg, 1999). The experiment was set up as a 4 x 4 factorial arrangement in a completely randomized design with four treatments and four replicates at each site. At each site there were 16 plots and each plot was planted with 25 plants spaced at 1 m x 1 m in a 5 x 5 array. All plots were planted with CMD-free material in the first year. The four cassava varieties planted were selected on the basis of their relative susceptibility to CMD as: MM96/4466 (resistant), TMS 30572 (moderately resistant), Bukalasa 11 (moderately susceptible) and Karemo (susceptible). The management practices were: roguing once at 12 weeks after planting (WAP), selection of CMD-free cuttings, combination of roguing and selection, and none (control).

The trials were first planted in October 2002 to June 2003 (season 1). All plants that sprouted while showing CMD symptoms were removed at 6 WAP in all plots. In season 2 (October 2003 to June 2004), cuttings obtained from the first planting were used to replant the experiment. In selection plots, five CMD-free stems were selected at random. Where CMD-free plants were not present, the most vigorous of the diseased stems were selected. In non-selection plots, five stems were selected using random numbers. Plants expressing CMD symptoms at sprouting were recorded and removed in the roguing treatment only.

Variables assessed

Starting at 1 MAP, then once every month until 8 MAP, CMD symptom severity was scored on the whole plant, for all plants in all plots using a 1 to 5 scale (Hahn *et al*., 1980). Incidence of CMD was determined indirectly from severity data and expressed as a percentage of the total number of plants per experimental treatment. Tuberous root yield data recorded in both seasons 1 and 2 was based on the fresh weight of tuberous roots for each plant and then converted to tonnes per hectare. Tuberous roots were separated on the basis of size into marketable and non-marketable ones.

Data analysis

Whole plant CMD severity data were subjected to the Chi-square test to determine differences in distribution of severity scores between treatments. Chi-square analysis was done using the Statistical Analysis System software (SAS, Cary-North Carolina, USA, 1999). Tuberous root fresh weight data were subjected to 4 x 4 factorial analysis of variance using Genstat software (Lawes Agricultural Trust, Rothamsted, UK, 2003) to determine effects of variety and phytosanitation. T-tests were done using Genstat to make comparisons between specific pairs of phytosanitation and variety combinations.

RESULTS

Disease (symptom) severity

In season 1, plants were initially healthy and severity score was 1 at 1 MAP (Figure-1). Later on TMS 30572, Karemo and Bukalasa 11 plants expressed CMD symptoms, although only a small number of TMS 30572 plants became diseased. MM96/4466 plants did not become infected at either site in both seasons. In season 1 at Alego, only Karemo was affected. Symptom severity for Karemo and Bukalasa 11 ranged from 2 to 3 at both sites regardless of treatment (Figure-1).

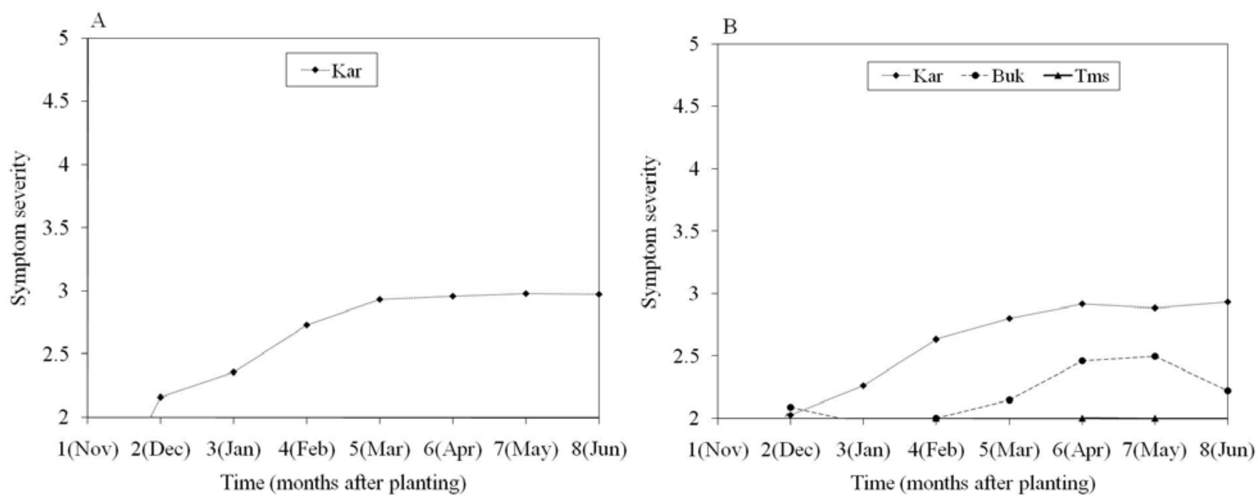

Figure-1. Cassava mosaic disease severity in November 2002 to June 2003 for Alego Division (A), Ugunja division (B). Kar = Karemo, Tms = TMS 30572, Buk = Bukalasa 11.

Symptom severity in TMS 30572 remained around 2 to the end of experimentation or disappeared in some plants over time. In season 2, severity score was between 2 and 3 for Karemo at both sites and this score was highest in the control plots. Severity score for Bukalasa 11 was between 2 and 3 for all treatments, except the control, which was slightly greater than 3, starting at 6 MAP at both sites. Symptom severity in rogued plots in season 2 decreased after the roguing exercise and thereafter gradually increased (Figure-2).

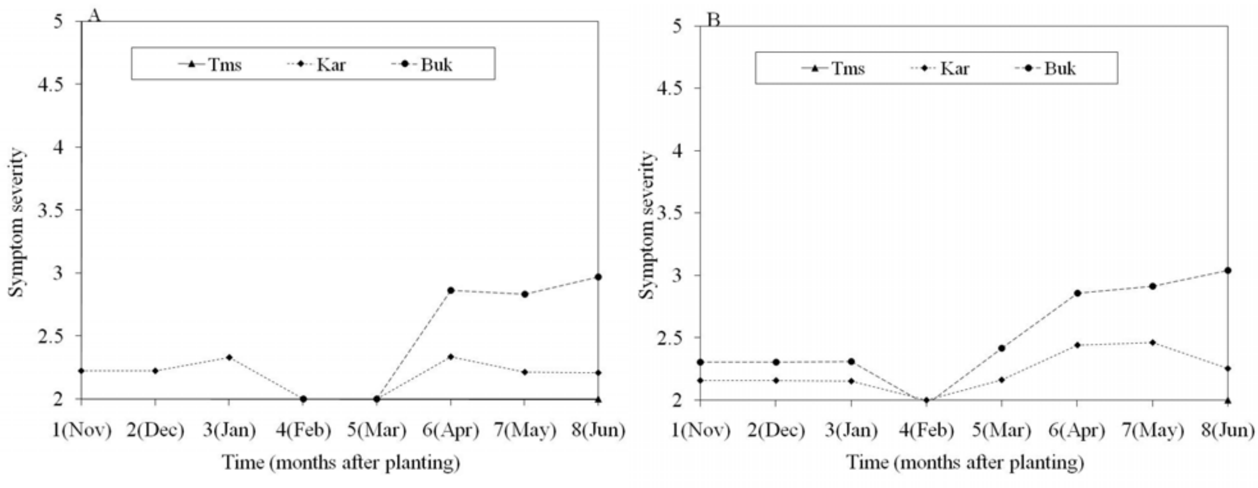

Figure-2. Cassava mosaic disease severity in November 2003 to June 2004 season for Alego Division (A), Ugunja Division (B). Kar = Karemo, Tms = TMS 30572, Buk = Bukalasa 11.

Disease incidence

At Alego CMD affected only Karemo in season 1, while in season 2 TMS 30572, Karemo and Bukalasa 11 were affected. The final incidence in TMS 30572 was less than 10% in each season. At Ugunja, Bukalasa 11 and Karemo were affected in season 1, whereas TMS 30572 was additionally affected in season 2. The final CMD incidence was generally higher at Ugunja than at Alego. It was also higher in season 2 than in season 1 (Table-1).

First infections in Karemo were recorded at 2 MAP for both sites in season 1. In season 2, Karemo started out diseased in all treatments at both sites. In season 2, Bukalasa 11 started out diseased in the non-selection treatments at Ugunja, while at Alego first infections were recorded at 5 MAP. In TMS 30572, first infections were observed at 4 MAP in season 1 and at 3 MAP in season 2 at Ugunja, and at 8 MAP in season 2 at Alego. Disease spread was fastest in Karemo at both sites, while MM96/4466 was not affected by CMD at either of the two sites. The incidence in varieties that were affected by CMD increased progressively over time. The cumulative percentage of diseased Karemo and Bukalasa 11 plants decreased immediately after the roguing exercise undertaken at 4 MAP of season 2 at both sites, but increased again thereafter (Table-1).

Fresh weight of tuberous roots

The weight of tuberous roots varied significantly among varieties ($P<0.05$) at each site in both seasons (Tables 2 and 3). The highest weight of marketable tuberous roots was for MM96/4466, whereas the lowest was for Karemo. When considering all varieties together, the effect of phytosanitation on weight of tuberous roots was only significant ($P<0.05$) at Ugunja in season 2 (Tables 2 and 3).

For the CMD-susceptible varieties, specific analysis showed phytosanitation having a significant ($P<0.05$) effect on the weight of tuberous roots for Karemo, except at Alego in season 1 (Table-4). Selection resulted in the highest weight of tuberous roots per plot. A similar analysis for Bukalasa 11 showed phytosanitation having no significant ($P>0.05$) effect.

There was also a greater weight of tuberous roots in the combined selection than in the combined non-selection plants for the two seasons at Alego (Table-5). Paired t-test comparisons showed a greater weight of tuberous roots in the selection than in the control plants of Karemo in season 2. Tuberous root weight was significantly less ($P<0.01$) for rogued than for control plants of Karemo in season 2. When compared to the resistant varieties, tuberous root weight for the Karemo selection treatment was greater than that for the moderately resistant TMS 30572 and equivalent to that for the resistant MM96/4466.

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Table-1. Final disease incidence (%) for varieties and phytosanitation practices at Alego and Ugunja divisions during seasons 1 and 2.

Site	Season	Variety	Phytosanitation practice ^{z}			
			1	2	3	4
Alego	1	MM96/4466	0	0	0	0
		TMS 30572	0	0	0	0
		Bukalasa 11	0	0	0	0
		Karemo	100.0	100.0	100.0	100.0
	2	MM96/4466	0	0	0	0
		TMS 30572	6.2	8.2	1.3	3.6
		Bukalasa 11	37.6	35.9	41.0	45.0
		Karemo	100.0	100.0	97.2	100.0
Ugunja	1	MM96/4466	0	0	0	0
		TMS 30572	3.6	0	0	12.5
		Bukalasa 11	22.0	9.4	30.3	7.4
		Karemo	98.1	100.0	93.8	100.0
	2	MM96/4466	0	0	0	0
		TMS 30572	6.0	19.3	6.7	9.8
		Bukalasa 11	65.1	67.1	60.4	86.6
		Karemo	91.7	98.4	95.8	100.0

^{z}1 = roguing, 2 = selection, 3 = roguing + selection, and 4 = none

Table-2. Effect of variety and phytosanitation practice on tuberous root weight (t ha-1) at Alego and Ugunja during season 1.

Site	Variety	Phytosanitation practice ^{z,y}				Mean
		1	2	3	4	
Alego	MM96/4466	25.6	21.4	22.9	21.3	22.8a
	TMS 30572	18.5	17.0	15.2	10.7	15.4b
	Bukalasa 11	13.1	11.8	12.5	5.6	10.8bc
	Karemo	14.3	10.0	6.8	7.8	9.7c
	Mean	22.8	15.0	14.4	11.4	
	Variety $LSD_{0.05}$	5.0				
	Phytosanitation $LSD_{0.05}$	NS				
	Interaction $LSD_{0.05}$	NS				
	CV (%)	27.8				
Ugunja	MM96/4466	25.1	20.6	27.7	24.6	24.5a
	TMS 30572	16.2	19.0	17.1	13.8	16.5b
	Bukalasa 11	11.4	12.3	13.0	12.8	12.3c
	Karemo	9.2a	10.2a	4.7b	6.5b	7.7d
	Mean	15.5	15.5	4.7	6.5	
	Variety $LSD_{0.05}$	3.2				
	Phytosanitation $LSD_{0.05}$	NS				
	Interaction $LSD_{0.05}$	NS				
	CV (%)	22.0				

^{z}1 = roguing, 2 = selection, 3 = roguing + selection, and 4 = none.
^{y}Values not followed by a letter or followed by the same letter within each factor and site are not significantly different at $P \leq 0.05$.

Table-3. Effect of variety and phytosanitation practice on tuberous root weight (t ha^{-1}) at Alego and Ugunja during season 2.

Site	Variety	Phytosanitation practice ^{z,y}				Mean
		1	2	3	4	
Alego	MM96/4466	13.2	13.1	12.1	11.9	12.6a
	TMS 30572	6.9	6.7	6.9	5.9	6.6b
	Bukalasa 11	8.4	9.4	8.8	6.7	8.3b
	Karemo	3.4b	11.6a	6.1b	5.7b	6.7b
	Mean	8.0	10.2	8.4	7.5	
	Variety $LSD_{0.05}$	3.0				
	Phytosanitation $LSD_{0.05}$	NS				
	Interaction $LSD_{0.05}$	NS				
	CV (%)	24.8				
Ugunja	MM96/4466	9.8	9.0	8.5	7.3	8.6a
	TMS 30572	8.2	14.0	6.4	10.0	9.6a
	Bukalasa 11	2.8	7.0	3.2	4.1	4.2b
	Karemo	1.5c	9.8a	1.9c	6.1b	4.8b
	Mean	5.6b	9.8a	5.0b	7.0b	
	Variety $LSD_{0.05}$	2.7				
	Phytosanitation $LSD_{0.05}$	2.7				
	Interaction $LSD_{0.05}$	NS				
	CV (%)	22.9				

^{z}1 = roguing, 2 = selection, 3 = roguing + selection, and 4 = none.
^{y}Values not followed by a letter or followed by the same letter within each factor and site are not significantly different at $P \leq 0.05$.

Table-4. Paired comparison of different varieties and phytosanitation practices for tuberous root weight (t ha^{-1}) at Alego and Ugunja during season 2.

Variety	Phytosanitation practice ^{z}	Tuberous root weight	Phytosanitation practice	Tuberous root weight	t-value	Significance ^{y}
Karemo	1	2.5	4	5.9	-3.56	**
Karemo	2	10.7	4	5.9	5.12	***
Karemo	3	4.0	4	5.9	-1.37	NS
Phytosanitation practice	Variety	Tuberous root weight	Variety	Tuberous root weight	t-value	Significance
2	Karemo	10.7	MM96/4466	10.3	0.23	NS
2	Karemo	10.7	TMS 30572	6.6	2.52	*
4	Karemo	5.9	MM96/4466	9.6	-0.33	NS
4	Karemo	5.9	TMS 30572	7.9	0.1	NS

^{z}1 = roguing, 2 = selection, 3 = roguing + selection, and 4 = none.
^{y}NS, *, **, ***, indicate: not significant, significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

Table-5. Paired comparison of bukalasa variety and phytosanitation practices for tuberous root weight (t ha^{-1}) at Alego and for both sites combined.

Variety	Phytosanitation practice [z]	Tuberous root weight	Phytosanitation practice	Tuberous root weight	t-value	Significance [y]
Bukalasa 11	2 and 3	16.2	1 and 4	8.4	3.3	**
Bukalasa 11	2	10.6	4	6.1	2.3	*

[z]1 = roguing, 2 = selection, 3 = roguing + selection, and 4 = none.
[y]NS, *, **, ***, indicate: not significant, significant at *P*<0.05, *P*<0.01, and *P*<0.001, respectively.

DISCUSSIONS

There were marked differences in CMD symptom expression among varieties, with CMD-resistant varieties showing less conspicuous symptoms than CMD-susceptible ones. The CMD-resistant MM96/4466 consistently expressed no symptoms most likely because it did not get infected at all. TMS 30572, though moderately resistant, showed mild symptoms at different times, and the proportion of plants that became infected depended on the site, probably because of the difference in prevailing inoculum pressures (Mallowa *et al*., 2006). Diseased TMS 30572 plants were mainly in the severity score category 2. However, there was a lot of recovery in TMS 30572 and most of the diseased plants eventually showed no symptoms. This result concurred with that of Rossel *et al.* (1992), who demonstrated that recovery was a common feature of improved CMD-resistant varieties.

The phenomena of recovery and reversion (where cuttings taken from infected parent plants sprout without symptoms) have also been reported for CMD-susceptible varieties (Fauquet and Fargette, 1990; Fargette *et al*., 1994; Owor *et al*., 2004a). Reversion was also used to explain the occurrence of some CMD-free plants in a very susceptible variety in an area where disease incidence was expected to be high in Cameroon (Fondong *et al*., 2000). Both recovery and reversion are the result of the incomplete systemicity of CMGs in cassava plants. This phenomenon could explain the slight drop in symptom severity of Bukalasa 11 and Karemo at different times during the course of the present study. Karemo became mildly infected and symptom severity remained fairly constant. Consequently, there was no marked change in the distribution of plants in the different severity score categories over time.

The severity score distribution of Bukalasa 11 changed markedly over time. It was mostly 2 at the beginning and ranged from 3 to 5 at the end of assessment. These responses resulted in big differences in symptom expression of diseased plants for the two varieties over time at the two sites, especially in the second season. At Alego in season 2, Karemo plants were initially diseased, but the symptoms expressed were mild. By contrast, plants of Bukalasa 11 were mainly symptomless at the start of season 2, but subsequently became infected and expressed severe symptoms. These contrasting outcomes suggest that initially mildy-diseased plants of the highly susceptible variety Karemo may be benefiting from mild strain protection, or cross protection, as has been demonstrated elsewhere for the CMGs (Owor *et al*., 2004a/b).

Disease incidence was highest in CMD-susceptible varieties and lowest in CMD-resistant varieties. These resistant varieties have proved resistant to all CMG species, strains and mixtures identified to date (Legg, 1999). The same CMGs elicit rapid spread and high final disease incidence in local landraces, as was the case with Karemo and Bukalasa 11 in the present study.

In season 1, in contrast to season 2, initially virus-free cuttings were used for all plantings and plants were not infected early during crop growth. This result was especially evident when final disease incidences for the two seasons were compared for the CMD-susceptible varieties Bukalasa 11 and Karemo. In the second season, incidences were much higher in the control plots than in the selection plots. This observation is consistent with results of earlier experiments in Kenya, showing that planting virus-free cuttings lowered disease incidence (Bock, 1983; Mallowa *et al*., 2006). Roguing also reduced disease incidence compared to plots in which it was not done. However despite this reduction, final disease incidences in Karemo were similar to those reported by Colon (1984) in Ivory Coast for local CMD-susceptible varieties, which exhibited high (77.5%) final disease incidence in rogued and non-rogued plots.

Tuberous root yields were generally greater for the CMD-resistant than for CMD-susceptible varieties. However the moderately CMD-susceptible Bukalasa 11 consistently yielded less than the other varieties, probably due to its inherent genetic yield potential. Conversely, the CMD-resistant MM96/4466 consistently yielded higher than all the other varieties. The effects of the phytosanitation treatments on yield became most apparent during the second season. Although no significant differences among treatments were observed for the CMD-resistant varieties, significant effects were noted for the two CMD-susceptible varieties.

Roguing had a particularly severe negative effect on the tuberous root yield of the highly susceptible Karemo, since the plant population was greatly reduced in rogued plots. Conversely, selection alone gave greater yields than the 'do nothing' control. Most importantly, the selection treatments of both Karemo and Bukalasa 11 gave yields that were statistically equal to the yield of the equivalent treatment of MM96/4466 at both sites. This is a remarkable result, as it suggests that under post-epidemic conditions, the effective application of selection alone, using locally available CMD-susceptible varieties, can deliver yields that are as good as those of 'improved' CMD-resistant varieties. This is particularly significant in view of the fact that a critical constraint to the use of CMD-resistant varieties is the slow pace with which they can be multiplied and distributed to farmers (Thresh *et al*., 1998).

The yield gain for CMD-susceptible varieties derived from the selection treatment can be attributed to several factors. These include: the slightly lower (albeit non-significant) incidence of CMD, the reduced effect of CMD arising from delayed infection (Thresh *et al*., 1994b) and the likely cross-protective effect of mild CMG strains (Owor *et al*., 2004a). The combined action of these factors probably explains why there is an apparent amelioration in the impact of CMD over time, following the passage of the epidemic 'front' (Legg, 2010).

CONCLUSIONS

The CMD pandemic has had a devastating impact on cassava producers in East and Central Africa since its emergence in the late 1980s, and has spread to affect more than 2.6 million square kilometres across nine countries (Legg *et al*., 2006). There has been great success in developing and disseminating resistant varieties (Dixon *et al*., 2001; Manyong *et al*., 2000), yet their widespread adoption continues to be hindered by the logistical difficulties of the large-scale multiplication and distribution of bulky planting materials. In view of this situation there has been considerable debate amongst researchers and other cassava stakeholders about whether or not local landraces have any utility in CMD management programmes.

The results presented here provide some very clear answers. It has been unequivocally demonstrated that for CMD-susceptible local landraces, the selection of CMD-free stems can lead to yields that are equivalent to those produced by CMD-resistant varieties. Conversely, roguing was shown not to be beneficial for any variety under any circumstance. The best practical approach to achieving effective selection in rural subsistence farming communities still needs to be determined. However, the systematic provision of guidance on selection by CMD management initiatives has enormous potential to enhance cassava production in all but the most recently pandemic-affected countries and regions. Such efforts should be integrated with work to disseminate virus-resistant varieties.

This study has shown that roguing is inappropriate for cassava producers where maximizing yield is the key goal. However, removing symptomatic plants will continue to be an important component of disease control in large-scale schemes for the multiplication of 'healthy' planting material where producing disease-free stems is more critical than obtaining high root yields. In fact, roguing within multiplication schemes is becoming increasingly important with the recent outbreak of cassava brown streak virus disease (CBSD) in the hitherto unaffected Great Lakes region of East and Central Africa (Alicai *et al*., 2007). Although CBSD remains less widespread than CMD, an important target of future research should be the evaluation of the effectiveness of phytosanitation measures against this second major disease.

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