African Crop Science Journal, Vol. 15, No. 1, pp. 1 - 9
ISSN 1021-9730/2007 $4.00

INCIDENCE AND SEVERITY OF CASSAVA MOSAIC DISEASE IN THE REPUBLIC OF CONGO

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(Received 29 December, 2006; accepted 19 March, 2007)

ABSTRACT

Diagnostic surveys were conducted in 2002 and 2003 in order to provide a comprehensive and detailed assessment of the status of cassava mosaic disease (CMD) in the Republic of Congo (ROC) and to determine if the disease was spreading. In 2002, 105 farmers' fields were assessed in the four major cassava-producing regions of the country. In 2003, 163 fields were sampled in nine regions and Brazzaville Commune. Incidence of cassava mosaic disease was generally high, averaging 80 and 86 % for 2002 and 2003, respectively; while damage was moderate to severe. In 2002, disease incidence was moderate in Pool (73%) but high in Brazzaville (81%), Cuvette Centrale (82%) and in Plateaux Region (84%). Pool region still had the lowest incidence (78%) in 2003, while Sangha (95%) had the highest incidence. The greatest disease severity was recorded in Niari region in the south and Cuvette Ouest region in the north in 2003. East African cassava mosaic virus Uganda variant (EACMV-UG) occurred virtually throughout the country, commonly in dual infections with *African* cassava mosaic virus. The high incidence of disease in plants considered to be the results of cutting infection (74% in 2002 and 82% in 2003), relatively low incidence of disease in plants considered to be infected by whiteflies and wide distribution of EACMV-UG points to the fact that the CMD pandemic is a chronic in the country and the areas sampled are currently in a stable post-epidemic phase. This situation is comparable to that in areas of East Africa affected by a pandemic during the 1990s, including Uganda, parts of western Kenya and north-western Tanzania. These findings clearly verify the assertion that the CMD pendemic has expanded across Central Africa and provide a basis for designing interventions and control strategies for the entire region.

Key Words: East African, geminiviruses, *Manihot esculenta*

RÉSUMÉ

Les enquêtes diagnostiques étaient faites en Janvier 2002 et en Février 2003 en vue d'une évaluation compréhensive et détaillée et mettre à la portée de tous les statuts de la maladie mosaïque du manioc (MMM) en République du Congo (RC) et de déterminer si la maladie pouvait se répandre. Pendant la première année, 105 champs de cultivateurs étaient examinés dans les quatre régions produisant le manioc dans le pays. En 2003, un échantillon de 163 champs étaient sélectionnés dans neuf régions y compris la commune de Brazzaville. L'incidence de la MMM était généralement élevée moyennant respectivement 80 et 86% pour l'année 2002 et 2003, pendant que le dommage s'aggraver de plus en plus. En 2002, l'incidence de la maladie était modérée dans la région du Pool (73%) mais élevée en Brazzaville (81%), dans la cuvette centrale (82%) et la région des plateaux (84%). L'incidence dans la région du Pool demeurait encore plus faible (78%) en 2003 pendant que Sangha avait l'incidence plus élevée (95%). En 2003 la plus grande gravité de la maladie était enregistrée dans la région de Niari au Sud et dans la région Ouest de la cuvette au Nord. La variante du virus de la mosaïque du manioc de l'Afrique de l'Est en Ouganda (VVMMAEO) s'était virtuellement manifestée à travers le pays; les infections s'étaient couplées avec le virus de la mosaïque du manioc africain. La grande incidence de la maladie des plantes considérée être les résultats de la contagion des boutures était de 74% en 2002. Relativement, la basse incidence dans les plantes considérées être contaminées par les mouches blanches et la grande part du VVMMAEO était de 82% en 2003. Ceci a abouti à la conclusion selon laquelle la pandémie de la MMM avait contaminé le pays quelques années auparavant et que les régions ayant fait l'objet de l'échantillon sont actuellement dans une phase post-endémique. Cette situation est comparable à celle des régions de l'Afrique de l'Est qui étaient contaminées par une pandémie les années 1990 y compris l'Ouganda, les parties Ouest du Kenya et le Nord- Ouest de la Tanzanie.

Mots Clés: Afrique de l'Est, germinivirus, *Manihot esculenta*

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a dietary staple and significant cash crop throughout the tropical world; serving as a food security crop for more than 300 million people in sub-Saharan Africa (Nweke, 1996). The capacity of the plant to produce, even under adverse climatic conditions and in poor soils, makes it an ideal food security crop. Cassava is grown in most parts of the Republic of Congo (ROC), where 95,700 ha are under cultivation with a total production of 861,500 t. Infact, per capita consumption is the highest here on the continent (FAO, 2003).

Production of cassava in Africa is greatly constrained by pests and diseases, particularly cassava mosaic disease (CMD), estimated to cause losses of 15-27 million tonnes (Thresh *et al*., 1997). The disease is caused by cassava mosaic geminiviruses (CMGs) (Family, *Geminiviridae*: Genus, *Begomovirus*) transmitted by the whitefly vector, *Bemisia tabaci* (Gennadius) and through the cuttings used routinely for vegetative propagation (Storey and Nichols, 1938; Dubern, 1994). The most commonly occurring CMGs in Africa are *African cassava mosaic virus* (ACMV), *East African cassava mosaic virus* (EACMV) and a recombinant hybrid designated the Uganda variant of EACMV (EACMV-UG) (Hong *et al*., 1993; Zhou *et al*. 1997). Effects on yield vary from no reduction to total yield loss, depending on cassava variety and environmental conditions (Muimba-Kankolongo and Phuti, 1987, Otim-Nape *et al*., 1993). Dual infections of EACMV and ACMV or EACMV-UG and ACMV commonly lead to synergistic interactions resulting in more severe symptoms (Harrison *et al*., 1997; Fondong *et al*., 2000). Losses of 80-90% have been reported for plants infected by both ACMV and EACMV-UG in Uganda (Owor *et al*., 2004).

Cassava mosaic virus has been recognised in East Africa for more than a century, but for much of this time it has been considered a minor problem with limited impact on the region's cassava production. Since the late 1980s, this situation changed dramatically. An epidemic of unusually severe CMD, initially reported from north-central Uganda, expanded to cover a large part of East and Central Africa with devastating effects on cassava production in the affected zones (Legg, 1999; Legg and Fauquet, 2004). Diagnostic tests of CMD-diseased samples collected in the Plateaux region in a 1999 survey in ROC revealed the presence of EACMV-UG, suggesting the possibility that the pandemic of severe CMD had expanded across Central Africa (Neuenschwander *et al*., 2002). The most severely diseased plants were infected with both EACMV-UG and ACMV, a characteristic of severe CMD associated with the pandemic in East Africa (Harrison *et al*., 1997). The objective of the study reported here was to provide a comprehensive and detailed assessment of the status of CMD in ROC and to determine whether the disease was spreading in the characteristic manner reported for the pandemic in East Africa (Legg, 1999; Otim-Nape *et al*., 2000).

MATERIALAND METHODS

This study comprised of a two-stage survey process. The first survey in January 2002 was conducted in 105 fields in the regions of Pool, Plateaux, Cuvette Centrale and Brazzaville Commune. Twenty-one districts of the three regions were sampled: 39 fields in Cuvette Centrale, 38 fields in Plateaux, 22 fields in Pool and six fields around Brazzaville. In February 2003, 163 fields were sampled in 35 districts in nine regions and Brazzaville Commune. Eleven fields were sampled in Bouenza, 29 in Cuvette Centrale, 20 in Cuvette Ouest, 10 in Lekoumou, 12 in Likouala, 15 in Niari, 40 in Plateaux, 10 in Pool North, 10 in Sangha and 6 in Brazzaville. Fields were sampled at 10-15 km intervals along roads in surveyed areas. Only fields with crops between three and six months old were considered. Thirty plants were selected along two diagonals across each field. Data collected included CMD incidence and severity, major cassava cultivars grown and the abundance of *B. tabaci* adults. The longitude and latitude for each sampling site were recorded using a global positioning system device.

Incidence of cassava mosaic disease was calculated as the percentage of plants with symptoms. Severity was assessed using the 1 to 5 severity scale of the International Institute of Tropical of Agriculture (IITA) (1990), where 1 represents no symptoms and 5 the most severe disease symptoms including severe mosaic, leaf deformation and general plant stunting. Two categories of infection were recognised and recorded, namely, ‘cutting infection’ resulting from the use of diseased cuttings and recognised by the presence of symptoms in the lowermost first-formed leaves; and ’whitefly infection’, recognised by the presence of symptoms on upper leaves only. Leaf samples for subsequent determination of the presence of virus were collected from a single plant in each sampled field. Selected plants were, in all cases, of the predominant variety and had conspicuous CMD symptoms.

DNA was extracted from leaf samples during the course of the survey in ROC using the procedure of Dellaporta *et al*. (1983); and final diagnoses were made using polymerase chain reaction (PCR) techniques in the laboratory of IITA-Uganda. Near full length DNA-A fragments were amplified using universal primers (Briddon and Markham, 1994). Virus diagnoses were then made following digestion of DNA-A amplicons with the restriction enzymes *Mlu*I and *Eco*RV (Okao-Okuja *et al*., 2004; Sseruwagi *et al*., 2004a) and comparison of the resulting banding patterns with predicted results based on published sequence data. Adults of *B. tabaci* were counted on the top five leaves of the tallest shoot of each of the 30 plants sampled per field.

The general linear model was used to analyse data with SAS software (Littel *et al*., 1996), and the chi-square test was used to compare CMD severity scores. Means were calculated for CMD incidence, severity and adult whitefly population. Incidence was transformed for statistical comparison. To allow for the effect of multiple infections, values for whitefly-borne CMD were transformed using the multiple infection transformation (Gregory, 1948), in which W is equal to the proportion of plants with whitefly-borne CMD, C is the proportion of plants with cutting-borne CMD, and the final calculated value for CMD change ‘y’ was converted to multiple infection units by multiplying by 100:

$$y = \ln(1/(1-C-W)) - \ln(1/(1-C))$$

Where appropriate data were transformed using the multiple infection transformation (Gregory, 1948). Means separation was done to compare the difference between significant effects.

RESULTS

Cassava was the predominant food crop grown by all farmers in ROC. Cassava mosaic disease was present in all fields of all regions in the two years. Disease incidences were significantly different ($P<0.005$) between regions for each year (Table 1). In 2002, CMD incidence was moderate in Pool, but high in Brazzaville and Cuvette Centrale with the highest incidence observed in Plateaux Region (84.7%). Overall mean incidence for all regions was 80.3%. Incidence in 2003 was 86.2%, with the lowest incidence still in the Pool Region (78.3%). This was significantly ($P<0.05$) higher than that of the previous year. The

TABLE 1. Incidences of cassava mosaic disease (CMD) and number of adult whiteflies (*B. tabaci*) per region in 2002 and 2003 in the Republic of Congo

Year	Region	CMD incidence (%)			Adult *B. tabaci* per plant
		Cutting-borne	Whitefly-borne (transformed[b])	Total	
2002	Brazzaville	78.3	2.8(13.8)	81.1	2.4
	Cuvette C.	79.3	3.3 (17.4)	82.6	3.8
	Plateaux	76.1	8.6(44.6)	84.7	2.0
	Pool	60.6	12.7(38.9)	73.3	2.4
	Mean (total)	73.6	6.9	80.4	2.8
	SE	0.40	0.13	0.35	0.12
2003	Brazzaville	80.6	2.2(12.0)	82.8	0.3
	Cuvette C.	76.7	7.8(40.8)	84.4	3.6
	Plateaux	76.9	5.6(27.8)	82.5	1.6
	Pool	69.0	9.3(35.7)	78.3	0.7
	Bouenza	85.8	2.4(18.5)	88.2	2.1
	Cuvette O.	84.7	2.2(15.5)	86.9	1.2
	Lekoumou	88.0	2.7(25.5)	90.7	1.6
	Likouala	89.2	0.8(7.7)	90.0	2.1
	Niari	88.9	2.9(30.3)	91.7	2.8
	Sangha	93.7	1.0(17.3)	94.7	2.0
	Mean (total)	81.8	4.3	86.2	2.0
	SE	0.24	0.11	0.20	0.07

[b] Transformed using the multiple infection transformation (Gregory, 1948) (multiple infection units); SE = Standard Error

incidence in 2003 was highest in Sangha (94.7%). Most infection was attributed to the planting of diseased cuttings (74.2% in 2002 and 81.8% in 2003) while the levels of whitefly-borne infection were relatively low, 6.2% and 4.2%, respectively (Table 1). Whitefly-borne infection was generally low in both years, but was greatest in Pool, with incidences of 12.7% in 2002 and 9.6% in 2003. Mean adult whitefly populations varied greatly both between and within regions, but were very low in 2003. Whiteflies were most abundant in Cuvette Centrale for both 2002 and 2003. Fields with highest populations were often those grown within forests.

Disease severity varied significantly between regions in 2002 (x^2=65.5, df=9, P<0.001) and also in 2003 (x^2=485.6, df=27, P<0.001) (Fig. 1). No significant difference was observed over time between the four regions covered in both 2002 and 2003. However, there was a significant (x^2=37.5, df =3, P<0.001) difference between both years for Pool region when considered alone. In both cases, the greatest number of plants had moderate (3) or severe (4) symptoms. In 2002, symptoms were most severe in Plateaux in which 15.3% of plants expressed the most severe symptoms (5). Sangha had the highest incidence in 2003, but severity was relatively low ($\leq$3). Overall CMD severity in 2003 was highest ($\geq$4) in Niari and Cuvette Ouest.

The most frequently and widely grown cultivars in the country were susceptible to CMD (Table 2). Nzete ya Mbongo was the most frequently grown cultivar and the incidence of disease in this cultivar was 90.8%. The cultivar with the highest CMD incidence was Oke-Ola (95.4%), which also expressed severe symptoms (3.4). Cultivar MM 86, which had earlier been selected for bacterial blight disease tolerance, was also susceptible to CMD (68.4%).

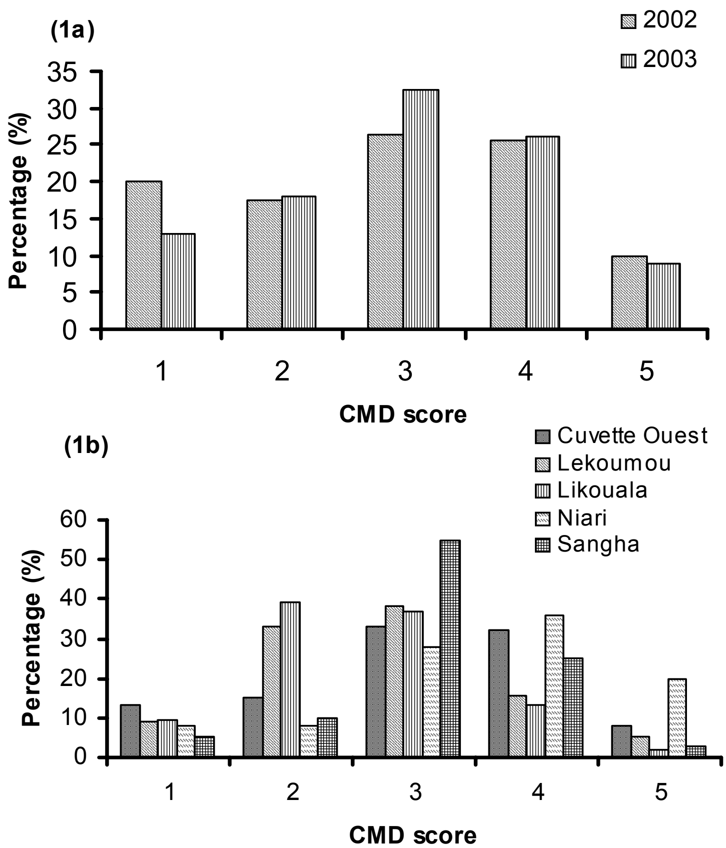

Figure 1. Comparison of CMD severity between 2002 and 2003 (1a) and among regions.

TABLE 2. Cassava mosaic disease (CMD) incidence and severity of the most frequently-grown cultivars in the Republic of Congo, combining data for 2002 and 2003

Cultivar	Number of fields	CMD incidence (%)	CMD severity
Nzete ya Mbongo	40	90.8	2.9
MM 86	34	68.4	2.9
Ongana	24	92.0	3.2
Ewur' Oyeba	15	82.0	2.9
Limbwana	15	92.4	3.3
Onganyinga	12	95.2	3.5
Mwebale Okisi	8	85.3	2.8
Oke-Ola	8	95.4	3.4
Mopoukou	7	89.2	3.0
Oke-Ofi	7	65.2	2.4
Ebobo	6	83.3	3.0
Omanyinga	5	75.6	2.7
Opepembe	5	90.0	3.1
Muduma	4	88.3	3.4
Mean (n=268)		83.9	2.9
SE		0.18	0.1

PCR analysis of samples collected in 2002 (Table 3) gave amplified products for 82 out of 105 samples using universal primers. Based on RFLP analysis, 45.1% of the samples that provided products were infected by ACMV alone, 19.5% had EACMV-UG alone and 35.4 % were infected by both ACMV and EACMV-UG. Plants infected by EACMV-UG were distributed throughout the areas sampled with a high frequency of occurrence in Plateaux region and frequent mixed infections explaining the severe symptoms. Based on results from 2003, 136 (83.4%) of the 163 cassava mosaic virus infected samples gave products with universal primers (Table 4).

EACMV-UG and ACMV infected 51.9% of the positive samples , 26.7% were infected by ACMV alone and 21.4% by EACMV-UG alone. An EACMV strain that gave an RFLP pattern distinct from that of EACMV-UG was identified from the sample collected from a single site in Sangha. More detailed characterization involving sequencing would be required to determine the identity of this isolate. Dual infections of ACMV

TABLE 3. Occurrence of cassava mosaic geminiviruses in regions of the Republic of Congo, January-February, 2002

Region	Virus types			Total	CMD severity
	ACMV	EACMV-UG	ACMV+EACMV-UG		
Brazzaville	2	1	2	5	2.9
Cuvette C.	8	8	10	26	2.3
Plateaux	12	6	13	31	3.2
Pool	15	1	2	18	2.6
Total	37	16	29	82	
Percentage	45.1	19.5	35.4	100	

ACMV = African cassava mosaic virus; EACMV-UG = East African cassava mosaic virus-Uganda; ACMV+ EACMV-UG = ACMV and EACMV-UG both present in the same plant

TABLE 4. Occurrence of cassava mosaic geminiviruses in the regions of the Republic of Congo, January-February, 2003

Region	Virus type			Total	CMD severity
	ACMV	EACMV-UG	ACMV +EACMV-UG		
Bouenza	3	3	4	10	3.0
Brazzaville	1	2	1	4	2.6
Cuvette C.	7	5	16	28	2.8
Cuvette O.	0	4	14	18	3.1
Lekoumou	5	2	1	8	2.7
Likouala	0	1	7	8	2.6
Niari	1	1	9	11	3.5
Plateaux	10	8	13	31	3.0
Pool	8	1	1	10	3.1
Sangha	1	2	4	7	3.1
Total	36	29	70	135	
%	26.7	21.4	51.9	100	

ACMV = African cassava mosaic virus
EACMV-UG = East Africa cassava mosaic virus - Uganda
ACMV+ EACMV-UG = ACMV and EACMV-UG both present in the same plant

and EACMV were also detected in two samples from Cuvette Centrale. Considering both single and dual infections, EACMV-UG was present in 71% and ACMV in 77.7% of plants.

DISCUSSION

Based on results of this study, a chronic and severe CMD situation exits in the Democratic Republic of Congo. In 2002 and 2003, more than 80% of all plants assayed were diseased; an incidence that exceeds that reported for most other countries in Africa (Thresh *et al*., 1997). Although there were small differences in incidence and severity between the regions of ROC, there was a rather uniform disease status, distinct from the conditions reported from countries where epidemics have occurred such as Uganda (Otim-Nape *et al*., 2000) and neighbouring western Kenya and north-western Tanzania (Legg, 1999). This study confirmed the occurrence of the pandemic associated recombinant virus, EACMV-UG, throughout the country and confirms and extends the earlier report of this virus in ROC (Neuenschwander *et al*., 2002). The present results show that the dominant viruses in the Republic of Congo are ACMV and EACMV-UG. The predominance of cutting-borne infection throughout areas surveyed in both years could mean that the CMD pandemic has covered the entire country. This could have been completed is now in a mature phase that is relatively stable. If the pandemic is mature, the similarity of environments in neighbouring Gabon to the west, could mean that the western 'front' of the pandemic may well lie in this country. Surveys conducted in Gabon in 2003 confirmed the presence of EACMV-UG and rapidly spreading CMD, a characteristic of the pandemic 'front', in the eastern plateaux region of Gabon (Legg *et al*., 2004). By contrast, only ACMV occurred in the western two-thirds of Gabon.

There was a clear relationship between whitefly abundance and whitefly-borne infection, as has been demonstrated from studies conducted elsewhere in Africa (Legg and Raya, 1998). However, such relationships are often difficult to substantiate due to the high degree of temporal variability in whitefly populations (Fishpool *et al*., 1995), and the latent period of 3-5 weeks between transmission and first symptom expression (Fargette *et al*., 1993). Surveys conducted at more frequent intervals or experimental population dynamics studies would be required to improve understanding of the relationship between vector populations and CMD spread in ROC.

There were differences in symptom severity between years and regions. These differences were not very pronounced, and the general disease situation was of relatively severe disease when comparing data with averages for other countries (Sseruwagi *et al*., 2004b). This could be attributed to the high frequency of occurrence of mixed virus infections, known to elicit more severe symptoms through synergistic interactions (Harrison *et al*., 1997; Fondong *et al*., 2000; Pita *et al*., 2001). Although ACMV was the most frequently occurring virus in both years, the greatest proportion of sampled diseased plants had mixed ACMV+EACMV-UG infections. Significantly, this proportion increased in 2003. There was also a concomitant decrease in plants infected with ACMV alone, dropping from 45 to 27%. These results are consistent with patterns of epidemic development observed in East Africa (Harrison *et al*., 1997), where ACMV has been shown to be the originally occurring virus and EACMV-UG the invasive, epidemic-associated species.

Substantial genetic variability exists within cassava germplasm cultivated throughout the regions surveyed. However, virtually all the landraces encountered were heavily diseased with CMD. The fact that much of the disease was cutting derived means that it is difficult to draw firm conclusions on the resistance or susceptibility of these landraces. These will need to be tested under known inoculum pressure conditions to determine relative levels of resistance/susceptibility. Because of the generally high incidence levels, resistance is unlikely to be found amongst these landraces. No improved clone was recorded during the survey apart from the locally-selected and cassava bacterial blight-resistant MM 86 that was widely grown in Plateaux region. It was apparent from the two surveys that CMD is the dominant pest/disease constraint in the ROC.

Highly successful CMD pandemic management programmes have been implemented in East Africa (Legg *et al*., 1999; Otim-Nape *et al*., 2000). These have been based on the dissemination and multiplication of CMD-free planting material of resistant varieties in combination with a rigorous phytosanitation programme. Similar approaches have been initiated in ROC. Concerted efforts will be required in the immediate future, combining the deployment of host plant resistance with training and extension programmes, if comparable successes in CMD mitigation are to be achieved in ROC.

ACKNOWLEDGEMENTS

This study was conducted under the auspices of the International Institute of Tropical Agriculture (IITA) Project, 'Cassava Mosaic Disease Pandemic Mitigation in East and Central Africa', funded by the Office for United States Foreign Disaster Assistance (OFDA). Thanks to the former USA Ambassador to the ROC, Mr. D. H. Kaeuper, and to the Food and Agriculture Organisation Representative to the ROC, Mr. A. Ouattara, for their support. The Ministry of Agriculture and Husbandry and the Ministry of Research, through its General Delegation for Scientific and Technological Research (DGRST), are acknowledged for their assistance.

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