MONITORING AND DIAGNOSTIC SURVEY OF CASSAVA MOSAIC VIRUS DISEASE (CMD) IN EASTERN DEMOCRATIC REPUBLIC OF CONGO

Obonyo R., Tata H., Koffi Tete M., Asiimwe P., Legg J.P.

March, 2007

Introduction

Cassava mosaic disease (CMD) is the major constraint to cassava production in Africa. CMD is caused by cassava mosaic geminiviruses (CMGs). An unusually virulent recombinant strain, *East African cassava mosaic virus*-Uganda (EACMV-UG) has been associated with the severe CMD pandemic in East and Central Africa. Both severe CMD and EACMV-UG have been recorded from the Democratic Republic of Congo (DRC) (Neuenschwander *et al.,* 2002). However, these results were obtained from the western provinces of Kinshasa and Bas Congo. There is currently no published information on the occurrence of CMGs in eastern DRC. A diagnostic survey was therefore carried out in order to establish the CMD situation in selected provinces of Eastern DRC. The survey was funded by the IITA/CRS Crop Crisis Control Project (C3P) in collaboration with IITA-Uganda and the Ministry of Agriculture in DRC.

Objectives

The primary objectives of the surveys were to:
(i) Assess the incidence, severity, abundance and effect of the major pests and diseases affecting cassava.
(ii) Assess the impact of CMD, pests and other diseases affecting cassava production and also on cultivation intensity and the frequency of occurrence of newly introduced improved cassava varieties.
(iii) Provide data for incorporation into a GIS food security model, being developed by IITA that will facilitate improved targeting of CMD mitigation efforts

Methodology

i) *Field sampling.*
A survey was carried out in the cassava-growing districts of South Kivu and North Kivu provinces in eastern DRC. A total of 126 cassava fields were visited. Cassava fields aged three to six months were selected at regular intervals of at least 5-10 km on average along the main roads and occasionally traversing to the feeder roads during the survey. Young cassava crops (3-6 months old) were selected to permit a clear distinction between cutting and whitefly-borne infections by observing the lower first-formed leaves. Where all leaves show symptoms, infection is considered to be cutting-borne but where only upper leaves show symptoms, infection is taken to be as a result of current-season whitefly-borne infection. In each field selected, thirty plants were examined along two diagonal transects. CMD severity, CMD infection type (whitefly or cutting–borne) and the number of adult whiteflies on the five topmost fully-formed apical leaves of the tallest shoot were recorded for each sampled plant. CMD severity was assessed using the standard 1-5 scale in which 1 represents CMD symptom-less plants and 5, the most severe symptoms including severe chlorosis, leaf distortion and plant stunting (Hahn *et al.,* 1980). Cassava green mite (CGM), cassava mealybug (CM), cassava bacterial blight (CBB) and root rots (RR) were also taken into consideration. Altitudes, longitudes and latitudes were recorded at each sampling site using a global positioning system (GPS) handset. Mean values were calculated for all factors measured both for each field, as well as for each district. At least

two 1cm^2 leaf samples showing CMD symptoms were picked per field and each put in an eppendorf tube for subsequent virus diagnostics. However, for fields with unusual CMD characteristics, more than two samples were collected. During the survey, a total of 134 samples were collected from 126 sites, in both provinces. The samples were each put in a separate eppendorf tube, labelled and stored in a cool box containing ice blocks in order to preserve them. DNA was extracted from the cassava leaf tissues at the end of each day using the protocol of Dellaporta *et al*. (1983). DNA extracts were preserved for virus diagnoses using PCR techniques for virus characterization at the IITA-Uganda laboratory following the completion of the survey.

ii) PCR amplification

Primer pairs specific for ACMV (AL1/F and ARO/R) and EACMV-UG (AL1/F1 and ACMV-CP/R3) were used to amplify fragments of DNA-A of cassava mosaic geminiviruses (CMGs) in the reaction mixture using; water 10.6µl; PCR buffer 2.5µl; Tween-20 2.5µl; 25mM Magnesium Chloride 1.5µl; 2.5 mM DNTPs 1.0µl; 20 µM of forward and reverse primers 0.2µl; Taq polymerase (1 unit) at 0.25µl and DNA Template of 5µl. Two drops of mineral oil were layered on top of each tube to stop evaporation. The viral DNA was amplified in Techne and Hybaid thermal-cyclers at the conditions below:

Initially: 94ºC for 2:00 min, 55ºC for 1:00min and 72ºC for 2:00 min, for 1 cycle
Then: 94ºC for 1:00min, 55ºC for 1.30min and 72ºC for 2:00min for 30 cycles
Finally: 94ºC for 1:00 min, 55ºC for 1:00 min and 72ºC for 10 min for 1 cycle
And thereafter the samples were held at 4ºC until the agarose electrophoresis gel was ready

The amplified DNA fragments were electrophoresed in a 1.2 % agarose gel with ethidium bromide stained at 10mg/ml and run at 80 volts for 60 minutes in x1 Tris-Acetate-EDTA (TAE) buffer of pH 8. The gel was then visualized under UV light and photographed using an Olympus digital camera with the Digi Doc-IT gel imaging system.

Results

For all areas surveyed, the mean age of cassava fields surveyed was 4.8, and fields had an average altitude of 1399m above sea level. CMD incidence was low to high, ranging from 35%-95.3%, with the highest incidence being 95.3% in Uvira and lowest 35% in Goma (Table 1). The overall average incidence for all the districts was 58.8%. Cutting infection was highest in Masisi (70.5%) and lowest in Goma (31.7%) with an overall average of 51.4%. Current-season whitefly-borne infection was highest in Uvira (38.6%) and lowest in Masisi (0.5%). CMD mean severity for the area surveyed was high (3.4). Whitefly number (mean) was highest in Rutshuru (8.2) and lowest in Goma (0.02), while mean nymph abundance was highest in Rutshuru (7.5) with an overall mean of 2.0. The green mite natural enemy, *T. aripo*, was observed near the border between DRC and Uganda in the districts of Rutshuru (0.07%) and Beni (0.03%). CGM incidence was generally high in the two provinces, and was highest in Kalehe (86.7%) and least in Uvira (28.6%). Overall mean severity of CGM was low (2.5). CM was only observed in Kabare

(0.9%), Uvira (4.8%), Fizi (17.6%) and Walungu (0.7%) giving a total incidence of 2.4%. CM mean severity was moderate (2.5) in the four districts where it occurred. CBB occurred in the far north of Kivu province and its incidence was greatest in Lubero (55.3%) and least in Rutshuru (10.3%). Overall incidence of CBB was 10.7% and mean severity was 2.5 for the three districts where it occurred. Sooty mould and whitefly damage mean severities were low in each of the five districts with an overall mean of 2.3. The frequency of cassava production was moderate throughout the districts with the lowest mean field size in Walungu (539) and the highest in Fizi (1464) giving an overall average of 891. Nearby plots were most frequent in Walungu (21) and least in Goma (2) with an overall mean of 7.5. The intensity of cassava cultivation, assessed by recording the number of fields per km along surveyed routes, varied greatly, being least in Rutshuru (1.1) and greatest in Walungu (11.5). There was little production of improved varieties in surveyed areas. These were only noticed in the north of Kivu Province. Percentages of improved varieties recorded as the predominant variety in sampled fields ranged from 20% in Rutshuru down to 1.9% in Beni. Improved varieties predominated in 8.9% of fields in the four districts where they occurred as predominant varieties (Table 1), but overall incidence in the surveyed areas was 3.6%.

Many local varieties were encountered during the survey, with varying numbers per district. Beni district had more varieties with the most frequently-cultivated being Balulu (23.1%) and the majority occurring only occasionally. By contrast, Goma had only two varieties that predominated - Muchikunzi (66.7%) and Nambiobio (33.3%) (Table 2). However, production of improved varieties was too low and restricted to limited areas with only two varieties noted in the northern provinces of Kivu. Production of improved varieties ranged from 1.9% to 20% for Nase 3 and MM/96/028 in Beni, Lubero, Masisi and Rutshuru districts (Table 3).

Of the 134 samples analyzed, 109 gave positive results, of which 5, 43 and 61 samples were identified with ACMV, EACMV-UG2 and ACMV+EACMV-UG2 respectively. EACMV was not detected in any of the samples. Mixed ACMV and EACMV-UG2 infections were most frequent in Beni, Lubero and Rutshuru (Table 5). Comparison of these survey results with results obtained from similar studies in eastern DRC show a strong trend towards reduced ACMV incidence and increased EACMV-UG incidence. This is a pattern that has characterized all areas affected by the CMD pandemic.

CMD severity varied considerably amongst varieties from the least affected, which were ‘other-improved’ and Neude (2.9), to the most affected, which was Ndoliro (4.0) (Table 5). CMD incidence varied from 50% to 70% in most varieties with an overall average of 59%. CGM severity was greatest for Naihunde (3.0) and least for Kinyoka (1.4). Prevalence of *T. aripo* was almost negligible for all the varieties. Mean whitefly abundance was very high for ‘other-improved’ (18.3) and least for Nabizonza (0.3) (Table 5).

Comparison of the 2006 data with earlier surveys in eastern DRC in 2003 shows a general increase in CMD severity (3.0 to 3.5) but a decrease in incidence from 78% to

60%. Although *T. aripo* was very infrequent in 2006, this still represented a significant change from previous years where it had not been recorded at all (Table 6).

Conclusions

The pest and disease survey of ten districts of eastern DRC showed that cassava mosaic disease (CMD) is the most important constraint affecting cassava production. Moderate to severe symptoms occurred virtually throughout sampled areas. Spread of the disease was most rapid in Uvira district of South Kivu. Virus diagnoses showed that the pandemic-associated virus, EACMV-UG, occurred throughout the surveyed area, and was the predominant virus form, commonly occurring in mixed infection with ACMV. Comparison with data from previous years showed that the frequency of ACMV in single infection has greatly decreased. The general pattern of spread inferred from the data confirms the previous thinking that most rapid of the CMD pandemic in eastern DRC is occurring from South Kivu towards north Katanga, in areas neighbouring the shores of Lake Tanganyika. The most frequently observed pest was cassava green mite (CGM). The predatory mite, *T. aripo*, which has been widely released across the cassava-growing areas of Africa, was found to be present in eastern DRC, but it was rare. The limited distribution indicates that it would be useful to make additional releases on cassava in the different parts of eastern DRC to enhance CGM control.

For CMD control, some farmers had been able to obtain CMD-resistant varieties. However, these were only recorded from four of the ten districts surveyed, and these varieties were the predominant variety in only 3.6% of surveyed fields. This fact highlights the strong requirement for expanding CMD-resistant variety multiplication and dissemination efforts.

Acknowledgements

This survey was financed through the United States Agency for International Development (USAID) as part of the joint CRS-IITA Crop Crisis Control Project (C3P). The work was implemented by IITA-Uganda and IITA-DRC. Contributions to the implementation of the survey were also made by: Henry Ugendo, provincial inspectors and government extension staff. The technical assistance and support from the IITA-Uganda virology team is highly acknowledged.

References

Dellaporta SL, Wood JJ, Hicks JB. 1983. A plant DNA minipreparation: version II. *Plant Molecular Biology Reporter* **1**, 19-21.

Hahn SK, Terry ER, Leuschner K (1980). Breeding cassava for resistance to cassava mosaic disease. *Euphytica,* **29**, 673-683.

Neuenschwamder P, Hughes J d'A, Ogbe F, Ngatse JM, Legg JP. 2002. The occurrence of the Uganda Variant of East Africa cassava mosaic virus (EACMV-UG) in western Democratic Republic of Congo and the Congo Republic defines the westernmost extent of the CMD pandemic in East/Central Africa. *Plant Pathology,* **51**(3), 384.

Table 1: Summary of parameters assessed by districts in the DRC survey, 2006

	DISTRICTS										
Parameters	**Kabare**	**Uvira**	**Fizi**	**Walungu**	**Kalehe**	**Goma**	**Masisi**	**Rutshuru**	**Lubero**	**Beni**	**Overall**
Number of fields surveyed	11	7	11	5	12	2	7	11	27	33	**126**
Cutting infection (%)	42.7	56.7	54.8	46.0	58.9	31.7	70.5	52.4	47.2	52.9	**51.4**
Whitefly infection (%)	1.8	38.6	1.0	0.7	1.4	3.3	0.5	9.1	11.4	6.5	**7.4**
CMD total incidence (%)	44.5	95.3	55.8	46.7	60.3	35.0	71.0	61.5	58.5	59.4	**58.8**
CMD symptom severity	3.2	3.2	2.9	3.3	3.4	3.2	3.4	3.3	3.9	3.7	**3.4**
T. aripo incidence (%)	0	0	0	0	0	0	0	0.06	0	0.03	**0.01**
CGM incidence (%)	58.5	28.6	81.8	74.7	86.7	58.3	78.1	66.1	44.1	33.9	**61.1**
CGM severity	2.4	2.3	2.8	2.8	2.8	2.7	2.2	2.7	2.4	2.3	**2.5**
CM incidence (%)	0.9	4.8	17.6	0.7	0	0	0	0	0	0	**2.4**
CM severity	2.3	2.9	2.9	2.0	-	-	-	-	-	-	**2.5**
CBB incidence (%)	0	0	0	0	0	0	0	10.3	55.3	41.5	**10.7**
CBB severity	-	-	-	-	-	-	-	2.1	2.7	2.5	**2.4**
W/F adult number (mean)	0.75	2.0	2.4	0.43	0.37	0.02	1.7	8.2	1.9	0.98	**1.9**
Nymph (mean)	0.25	2.0	1.1	1.3	0.24	0	1.6	7.5	4.6	3.0	**2.0**
Sooty mould severity	-	-	2.1	-	2.4	-	2.3	2.6	2.3	-	**2.3**
W/F damage severity	-	2.0	2.5	-	-	-	2.3	2.4	2.5	-	**2.3**
Field size (m^2)	950	947	1464	539	808	625	914	868	1005	793	**891**
Nearby plots	9	11	6	21	5	2	5	4	9	3	**7.5**
No. fields/km	6.9	4.8	5.5	11.5	7.9	2.1	2.1	1.1	7.6	5.7	**5.5**
Improved Variety (%)	-	-	-	-	-	-	10	20	3.7	1.9	**3.6**
Altitude (m)	1804	893	925	1641	1564	1555	1567	1165	1735	1143	**1399**
Age (Months)	4.5	5.3	5.0	4.6	4.7	3.5	5.1	5.3	4.7	5.0	**4.8**

Table 2: Summary of cassava varieties encountered in the DRC survey, 2006

DISTRICTS									
Kabare	**Uvira**	**Fizi**	**Walungu**	**Kalehe**	**Goma**	**Masisi**	**Rutshuru**	**Lubero**	**Beni**
Cibongodoka (9.1%)	Kitamise (6.25%)	Kalulu (20.8%)	Cibongodoka (27.3%)	M'bailo (23.5%)	Muchikunzi (66.7%)	D'Liyayi (10%)	Kaude Kasai (60%)	Kabumba (1.9%)	Babitolo (1.9%)
Mbachunguri (9%)	Nakaigere (6.25%)	Naihunde (37.5%)	Malula (9.1%)	Muchikunzi (5.9%)	Nambio-bio (33.3%)	MM96/028 (10%)	Madame (13.3%)	Kachiro (1.9%)	Balulu (23.1%)
M'bailo (18.2%)	Nakakwindi (12.5%)	Nakaigere (16.7%)	M'Mizinzi (9.1%)	Nambio-bio (58.8%)		Muchikunzi (50%)	Mwaka Mmoja (6.7%)	Kaude Kasai (14.8%)	Bamboo (9.6%)
M'Kanyanyi (13.6%)	Nakarasi (6.25%)	Nakanyemwa (8.3%)	Mwesa (18.2%)	Namusukuzi (11.8%)		Nakatumbwe (10%)	Nase 3 (20%)	Kibututu (1.9%)	Kabulwazi (1.9%)
M'Mizinzi (4.5%)	Nakatumbi (6.25%)	Namasikini (4.2%)	Nakabumbu (9.1%)			Nambio-bio (20%)		Kitenge (1.9%)	Kalinga (1.9%)
Nabizonza (13.6%)	Nakibembe (6.25%)	Unkown 1 (4.2%)	Nakarasi (9.1%)					Kivhwuto (3.7%)	Kambole (1.9%)
Nabwikwendeke (4.5%)	Namasikini (6.25%)	Unkown 2 (4.2%)	Nambio-bio (9.1%)					Kyikombora (3.8%)	Kanyamunyu (1.9%)
Nambio-bio (22.7%)	Namugendanyi (6.25%)	Unkown 3 (4.2%)	Nyamwero (9.1%)					Mercy (24.1%)	Katinamor (1.9%)
Nyabizonza (4.5%)	Namwero (6.25%)							Muchikunzi (1.9%)	Kaude Kasai (7.7%)
	Naihunde (25%)							Mwadako (11.1%)	Kibalabala (1.9%)
	Naulugembe (6.25%)							Mwaka Mmoja (9.3%)	Kinyoka (19.2%)
	Ngunganyoge (6.25%)							Nase 3 (3.7%)	Kipehe (1.9%)
								Ndoliro (11.1%)	Kipese (1.9%)
								Pesa (5.5%)	Kivarua (7.7%)
								Serengeti (1.9%)	Mercy (1.9%)

Table 2: Summary of cassava varieties encountered in the DRC survey, 2006 (cont.)

DISTRICTS									
Kabare	**Uvira**	**Fizi**	**Walungu**	**Kalehe**	**Goma**	**Masisi**	**Rutshuru**	**Lubero**	**Beni**
								Unkown (1.9%)	Mukalasi (1.9%)
									Mukarasa (1.9%)
									Mutundi (3.8%)
									Nase 3 (1.9%)
									Ndoliro (1.9%)

Table 3: Summary of improved cassava varieties encountered in the DRC survey, 2006

	DISTRICTS									
Varieties	**Kabare**	**Uvira**	**Fizi**	**Walungu**	**Kalehe**	**Goma**	**Masisi**	**Rutshuru**	**Lubero**	**Beni**
MM 96/028	-	-	-	-	-	-	10	-	-	
Nase 3	-	-	-	-	-	-	-	20	3.7	1.9

Table 4: Virus occurrence in the ten districts of Eastern DR Congo

District	ACMV	EACMV-UG	ACMV+EACMV-UG	Total
Kabare	2 (20%)	6 (60%)	2 (20%)	10 (100%)
Uvira	0 (0%)	1 (20%)	4 (80%)	5 (100%)
Fizi	0(0%)	4 (66.7%)	2 (33.3%)	6 (100%)
Walungu	0(0%)	2 (66.7%)	1 (33.3%)	3 (100%)
Kalehe	1 (11.1%)	5 (55.6%)	3 (33.3%)	9 (100%)
Goma	0 (0%)	0 (0%)	2 (100%)	2 (100%)
Masisi	0 (0%)	4 (57.1%)	3 (42.9%)	7 (100%)
Rutshuru	1(8.3%)	5 (41.7%)	6 (50%)	12 (100%)
Lubero	1 (4.2%)	5 (20.8%)	18 (75%)	24 (100%)
Beni	0 (0%)	11 (35.5%)	20 (64.5%)	31 (100%)
Total	**5 (4.4%)**	**43 (42.4%)**	**61 (53.2%)**	**109(100%)**

Table 5: Predominant varieties in the Eastern DR Congo survey, Sept/Oct 2006

Varieties	CMD sev.	CMD Inc.	Wfad Mn	CGM sev.	*T. ar* Mn
Balulu	3.8	50	1.5	2.4	0.0
Kaude Kisai	3.6	60	4.1	2.7	0.0
Kinyoka	3.7	70	1.6	1.4	0.1
Kivarua	3.8	70	0.7	2.1	0.0
M'bailo	3.3	60	0.4	2.6	0.0
Mercy	3.9	60	0.7	2.3	0.0
Mucikunzi	3.4	70	1.1	2.2	0.0
Nabizonza	3.2	50	0.3	2.3	0.0
Nambio-bio	3.4	60	1.2	2.6	0.0
Ndoliro	4.0	60	1.2	2.1	0.0
Neunde	2.9	50	1.5	3.0	0.0
Others (local)	3.4	60	1.9	2.2	0.0
Other (Improved)	2..9	50	18.3	2.0	0.0

Table 6: Three year comparison of variety prevalence to disease and pests with virus presence in Eastern DR Congo

Parameters	2003	2004	2006
CMD severity	3.0	3.0	3.5
CMD Inc. (%)	50	78	60
CGM severity	—	2.6	2.5
***T. aripo* (%)**	—	0.0	1.4
ACMV	66.7	49.5	4.4
EACMV-UG	8.9	19.8	42.4
ACMV+ EACMV-UG	24.4	30.7	53.2
EACMV	0	0	0