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CASSAVA MOSAIC DISEASE: INCIDENCE AND YIELD PERFORMANCE OF CASSAVA CULTIVARS IN ZAMBIA

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SUMMARY

Cassava is the main food crop for an estimated 30% of the population in Zambia where yields of 5.8 t/ha are some of the lowest of any major cassava-producing country. A study was conducted to characterize yield responses of Zambian cassava genotypes to cassava mosaic disease (CMD), as well as the relative susceptibilities to the causal viruses. CMD-free planting material of four improved cultivars (Mweru, Chila, Tanganyika and Kampolombo), four officially-promoted landraces (Nalumino, Kapumba, Bangweulu and Katobamphunta) and a locally popular landrace (Manyopola) were evaluated at a field site in Rufunsa District, Lusaka Province (central-eastern Zambia). Manyopola and Bangweulu were found to be susceptible and had high foliar incidences of CMD (97.5% and 74.7%, respectively) based on visual CMD symptoms with high severity scores (3.5, 3.5), whilst cv. Kampolombo was resistant (incidence 0.7%, severity 2.0). Mweru had the highest root yield (17.6 t ha⁻¹) while Kapumba, the second most susceptible cultivar, had the lowest root yield (3.2 t ha⁻¹). Significant inverse regression relationships were demonstrated between CMD incidence and CMD severity with root yield. Using these regressions together with published data on cassava production and countrywide CMD incidence in Zambia, it was possible to estimate annual losses due to CMD at ca. US\$ 51.7 million. Evidence for resistance to CMD amongst several of the improved cassava cultivars tested suggests that there is great potential for the effective control and management of CMD in Zambia, if these materials could be widely disseminated.

Keywords: response, resistance, incidence, severity, Zambia.

INTRODUCTION

Cassava (*Manihot esculenta* subspecies *esculenta* Crantz) is a perennial shrub of the family Euphorbiaceae cultivated mainly for its starchy roots. It is one of the most important food staples in the tropics providing a means of livelihood for up to 500 million farmers and countless processors and traders around the world (Ntawuruhunga *et al.*, 2013).

Cassava is the mainstay for an estimated 30% of Zambians and is ranked the second most important food crop after maize (Chiona *et al.*, 2014). It is regarded as a staple in the five Provinces of Luapula, Northern, North-Western, Copperbelt and Western where it is mostly grown. Although there have been efforts to promote cassava productivity in Zambia, the average yield of 5.8 t/ha is low when compared to values for the whole of Africa (10.9 t/ha), South America (13.2 t/ha) and Asia (19.7 t/ha) (FAOSTAT, 2017). Low cassava yields in Zambia can be attributed to several factors, pests and diseases being some of the most important, among which, cassava mosaic disease (CMD) is a major constraint (Chikoti *et al.*, 2013). A pandemic of unusually severe CMD has been reported to affect an area of more than 2.6 million square kilometres in nine countries in East and Central Africa, resulting in an estimated annual economic loss of US\$ 1.9–2.7 billion (Legg *et al.*, 2006). The proximity of this pandemic to Zambia suggests a potential future threat of more severe CMD, but to date the pandemic-associated virus variant East African cassava mosaic virus-Uganda (EACMV-UG) has not been reported from Zambia (Chikoti *et al.*, 2013).

The national cassava breeding programme of Zambia has developed seven improved pest and disease resistant cultivars that are being promoted for production. In 1993, three local selections which had superior yield and quality traits compared with traditional local cultivars were released (Haggblade and Zulu, 2003). These were: Bangweulu (LUC55), Kapumba (LUC327), and Nalumino (LUC304). Four additional cultivars (Mweru, Chila, Tanganyika, and Kampolombo) were released in 2000. Each of these came from crosses between the best local cultivars and CMD-resistant germplasm obtained from the International Institute of Tropical Agriculture (IITA). Manyokola, popularly called Manyopola in Zambia, is a local selection

Table 1. Cassava varieties grown in Zambia and their major attributes.

Cultivar	Breeding line/Genealogy	Characteristics
Katobamphunta	Local germplasm (selected local landrace)	Late bulking and high yielding but susceptible to cassava mosaic disease (CMD).
Bangweulu	Local germplasm (selected local landrace)	Early bulking and high yielding (31 t/ha in 12 – 16 months). Moderately resistant to cassava mosaic disease (CMD) and cassava mealybug (CM).
Kapumba	Local germplasm (selected local landrace)	Sweet and preferred for eating raw or boiling. Non-branching plant type suitable for intercropping. Moderately resistant to CMD, cassava green mite (CGM) and CM (yield of 22 t/ha in 16 – 24 months).
Tanganyika	L9-304/139, Mother is <i>Nalumino</i>	High yielding (yield potential of 36 t/ha and 9 t/ha under farm conditions in 16 months), good root shape, short neck length, good plant architecture, moderately tolerant to major pests (CM, CGM) and diseases (CMD and cassava bacterial blight - CBB). It has wide adaptability but is susceptible to scale insects.
Manyopola	Introduced from Malawi	Sweet and fresh root quality is greatly preferred by farmers, tolerates CGM and cassava brown streak disease (CBSD) but is susceptible to CMD.
Chila	L9-304/9, Mother is <i>Nalumino</i> , half sib produced from crossing block with IITA improved introduced clones	Suitable for use as green leaf vegetable, flowers profusely, good dry matter content (DMC) and branching, moderately tolerant to pests and diseases and adaptable to a wide range of conditions. (yield potential of 35 t/ha and 24 t/ha under farm conditions in 16 months).
Mweru	L9-304/151, Mother is <i>Nalumino</i> , half sib produced from crossing block with IITA improved introduced clones	High yielding (yield potential of 41 t/ha and 26 t/ha under farm conditions in 16 months), sweet in most soils, limited branching, wide adaptability and tolerant to major pests (CM, CGM) and diseases (CMD and CBB).
Nalumino	Local germplasm (selected local landrace)	Profuse flowering, sweet, branching, high starch content, good quality flour, resistant to CM and CMD (29 t/ha in 24 months).
Kampolombo	L9-304/4, Mother is <i>Nalumino</i> , half sib produced from crossing block with IITA improved seed population	High yielding (yield potential of 38 t/ha and 16 t/ha under farm conditions in 16 months), sweet, highly branched, medium Hydrocyanic Acid (HCN) content, moderately tolerant to major pests and diseases, and has wide adaptability.

from Malawi which has been widely adopted throughout the region because of its preferred quality characteristics, in spite of the fact that it is considered to be susceptible to CMD (Alene *et al.*, 2013). Katobamphunta is a local selection from Zambia and has been observed to be susceptible to CMD (M. Chiona, personal communication).

Evaluation of the existing released varieties in Zambia was based on symptom expression rather than indexing of the genotypes to the viruses that cause CMD to compare their performance with those of popular local cultivars. In this study, improved and popular local cassava genotypes were therefore evaluated under some of the severest inoculum pressure conditions occurring in the country, in order to evaluate their response to CMD and to compare their yields. This is particularly important in view of the currently limited adoption of improved cultivars by Zambian farmers (Alene *et al.*, 2013), and the likely future scenario of increased CMD severity associated with the predicted arrival of the CMD pandemic.

MATERIALS AND METHODS

Experimental design and layout. The cassava stock plants, from which the nine cassava cultivar cuttings were obtained for planting, were collected from the Root and Tuber Improvement Programme at Mansa Research Station. Prior to planting in the field the genotypes were tested for virus presence/absence at the Mount Makulu virology laboratory using the primers CMBrep/F, ACMVrep/R

and EACMVrep/R (Alabi *et al.*, 2008) and found to be free of the viruses causing CMD. The genotypes included four improved cultivars (Mweru, Chila, Tanganyika and Kampolombo) tolerant to pests and diseases, and five local landraces (Nalumino, Kapumba, Bangweulu, Katobamphunta and Manyopola) (Table 1). The experiment was laid out in a randomized complete block design (RCBD) with four replications. The stem cuttings, each measuring 30 cm long and having at least four nodes, were planted in plots measuring 11 m × 6 m at spacing of 1 m between plants and 1 m between rows. Out of the 84 plants, 48 plants were the healthy treatment plants consisting of 4 rows in each plot whilst 36 plants were CMD-infected plants of the highly CMD-susceptible genotype (Bangweulu) collected from farmers' fields within Rufunsa district, which were used as spreaders to ensure high CMD inoculum pressure. Healthy and spreader rows were planted alternately. The experiment was carried out under rainfed conditions without applying pesticides and fertilisers and was kept weed-free by regular hand-weeding.

Data collection and analysis. The observation for CMD foliar symptoms and whitefly abundance was done as soon as visible leaves were produced by the cassava cuttings. However, data on CMD foliar symptoms and whitefly abundance were collected starting at two months after planting (map) and continued at monthly intervals until 6 map. Twenty plants out of the 48 in each plot were systematically sampled by skipping the first and last plants in each row and then picking every

Table 2. CMD severity scores on different cassava (*Manihot esculenta*) genotypes at different growth stages: Rufunsa, Lusaka Province - 2012/2013.

Genotypes	Age (months)					Mean
	2	3	4	5	6	
Bangweulu	3.3	3.5	3.3	3.5	3.7	3.5
Chila	2.2	2.1	2.1	2.2	2.5	2.2
Kampolombo	1.0	2.0	2.0	2.0	1.0	2.0
Kapumba	2.4	2.8	2.6	2.8	2.4	2.6
Katobamphunta	2.8	3.3	3.1	3.2	3.3	3.1
Manyopola	3.0	3.6	3.5	3.7	3.7	3.5
Mweru	2.2	2.0	2.1	2.2	2.4	2.2
Nalumino	2.0	2.0	2.0	2.1	2.3	2.1
Tanganyika	2.4	2.4	2.1	2.2	2.4	2.3
Mean	2.6	2.6	2.5	2.6	2.6	2.5
LSD _(0.05)	0.3	0.3	0.3	0.3	0.3	
CV %	23.2	6.7	5.3	7.5	23.2	

Values are mean scores on a scale of 1-5 where 1=no symptoms and 5=severe mosaic with marked leaf distortion (Hahn *et al.*, 1980).

third plant in a row. The plants were measured for symptom severity and whitefly abundance. CMD incidence was assessed by counting the number of diseased plants with typical symptoms and expressed as a proportion of the total number of plants in each plot. CMD severity was scored for each plant in the plot using the 1-5 scale described by Hahn *et al.* (1980) where: 1) no symptoms observed; 2) mild chlorotic pattern over entire leaflets or mild distortion at the base of leaflets only, with the remainder of the leaflets appearing green and healthy; 3) moderate mosaic pattern throughout the leaf, narrowing and distortion of the lower one-third of leaflets; 4) severe mosaic, distortion of two thirds of the leaflets and general reduction of leaf size; 5) severe mosaic distortion of the entire leaf. The number of whiteflies was counted on the five top fully-expanded leaves of the tallest shoot. Leaves were held and gently turned to count the whiteflies on the undersides of sampled leaves. At 12 map, the height of each plant was measured from ground level to the highest shoot tip and the number of stems per plant was counted. Plants were harvested at 12 map and yield data were recorded by harvesting each plant individually and taking records of the tuberous roots and their respective weights. The total weight and mean number of tuberous roots for each treatment were determined and computed. Root yields in each plot were converted to t/ha values by dividing the total root yield for the plot by its area (including a 0.5 m wide border area to recognize that each plant occupies an area of 1 m²), and then converting the kg/m² value obtained in this way to t/ha.

CMD symptom severity, height of plants, number of stems and yield data were analysed with the ANOVA function in GenStat (16th edition, VSN International 2013). Regression analyses were run using SigmaPlot 11.0 (Systat Inc., Chicago, USA) to compare the relationships between cassava yields per plant and CMD incidence and severity over the period from 2 to 6 map.

Table 3. CMD incidence on different cassava (*Manihot esculenta*) genotypes at different growth stages: Rufunsa, Lusaka Province - 2012/2013.

Genotypes	CMD Incidence (%)					
	Age (months)					Mean
	2	3	4	5	6	
Bangweulu	55.0	72.5	80.0	81.2	85.0	74.7
Chila	31.2	55.0	48.8	41.2	25.0	40.2
Kampolombo	0	1.2	1.2	1.2	0	0.7
Kapumba	51.2	87.5	93.8	87.5	71.2	78.2
Katobamphunta	70.0	83.8	90.0	90.0	91.2	85.0
Manyopola	88.8	98.8	100	100	100	97.5
Mweru	41.2	68.8	66.2	65.0	40.0	56.2
Nalumino	21.2	36.2	56.2	38.8	15.0	33.5
Tanganyika	75.0	86.2	78.8	81.2	71.2	78.5
Mean	48.2	65.6	68.3	65.1	55.4	60.5
LSD _(0.05)	20.9	24.2	13.5	22.9	26.9	
CV %	6.2	6	9.1	9.5	7.3	

DNA extraction and PCR-based detection of viruses.

Leaflets from 10 symptomatic or non-symptomatic plants per genotype were randomly sampled directly into microfuge tubes, stored in a coolbox, and then transported to the Plant Virology Laboratory at the Zambia Agriculture Research Institute (ZARI), Mt. Makulu Central Research Station in Chilanga, for virus testing. Total DNA was extracted from the cassava leaf samples using the modified method described by Dellaporta *et al.* (1983). A multiplex-PCR assay was used to test all the samples for ACMV and EACMV-like viruses using the primers CMBrep/F, ACMVrep/R and EACMVrep/R (Alabi *et al.*, 2008). PCR was performed in a Techne 500 PCR System using one cycle of 94°C for 1 min; 52°C for 2 min, and 72°C for 3 min, followed by 36 cycles, in which each cycle consisted of 94°C at 1 min, 52°C for 2 min and 72°C for 1 min 30s with a final extension cycle at 72°C for 5 min. A 25 µl reaction mixture was used, made up of 5 µl of PCR reaction buffer (10x), 2.4 µl of 25 mM magnesium chloride, 0.5 µl of 10 mM dNTP mix, 0.5 µl of 10 mM CMBRep-F, 0.5 µl of 10 mM ACMVRep-R, 0.5 µl of 10 mM EACMVRep-R, 0.12 µl (0.8 Units) Taq DNA Polymerase, 4 µl DNA template and 11.48 µl sterile distilled water. The PCR amplified products were resolved by agarose gel electrophoresis, stained with ethidium bromide, and visualized under UV light using a gel documentation system (Gel Doc XR, Bio-Rad, USA).

RESULTS

CMD symptoms, whitefly abundance and plant growth observed on field-grown cassava genotypes. The cassava cuttings sprouted 10 days after planting and observation for symptom development commenced shortly afterwards. Varied symptoms of disease were observed on young as well as mature leaves, i.e. mild chlorosis, mild distortions at the basis of most leaves, while the remaining



Fig. 1. Cassava genotypes showing (a) mild chlorosis, (b) pronounced mosaic pattern on most leaves, (c) severe mosaic distortion of two thirds of most leaves and general reduction of leaf size, (d) very severe mosaic symptoms on all leaves, distortion, twisting, misshapen and severe leaf reductions of most leaves.

parts of the leaves and leaflets appeared green and healthy (Fig. 1a). In other cases, symptoms comprised: a pronounced mosaic pattern on most leaves, narrowing and distortion of the lower one-third of the leaflets (Fig. 1b), severe mosaic and distortion of two-thirds of most leaves, a general reduction of leaf size and stunting of the shoots (Fig. 1c) and very severe mosaic on all leaves, including distortion, twisting and severe leaf reduction of most leaves accompanied by severe stunting of the plants (Fig. 1d).

The average number of adult whiteflies recorded differed among cultivars ($P < 0.001$) (Table 5). Whitefly abundance was generally high for all cultivars at 2 map and progressively declined except for cvs Chila and Katobamphunta (4 map). The highest average number of adult whiteflies was recorded on cv. Mweru (32.1) and the lowest on cv. Tanganyika (12.0). No correlation was found between above-ground biomass and the numbers of adult whitefly, nor was there any relationship between above-ground biomass and CMD severity (Table 4).

CMD symptom severity of cassava cultivars. CMD symptoms were observed on both the local and the improved genotypes. Highly significant differences ($P < 0.001$) in symptom severity were recorded among cassava cultivars (Table 2). The highest CMD mean severity

was recorded in cvs Manyopola and Bangweulu (3.5), while the lowest was recorded in cv. Kampolombo (2.0), although symptoms were infrequent in it. However, some plants from cvs Manyopola and Bangweulu showed severe symptoms above 4. In general, the trend in symptom severity was consistent throughout the growing period with no significant change (Table 2).

Incidence of CMD in cassava genotypes. Analysis of variance (ANOVA) showed significant differences ($P < 0.001$) in the incidence of CMD among genotypes (Table 3). The lowest average CMD incidence (0.7%) was recorded in cv. Kampolombo while the highest (97.5%) was recorded in cv. Manyopola (Table 3). At the outset, there was a gradual increase in the number of plants developing symptoms of CMD, however, incidence declined from 4 to 6 map. In cv. Kampolombo, there was complete recovery at 6 map, whereas in cvs Bangweulu, Katobamphunta and Manyopola, more plants continued to develop CMD symptoms (Table 3).

Tuberous root yield. There were significant differences ($P < 0.001$) in tuberous root weights of the evaluated cultivars. In particular, there were significant negative correlations between root weight with CMD symptom severity

Table 4. Correlation of root weight, above-ground biomass, CMD incidence, CMD severity and *B. tabaci* abundance.

Parameter 1	Parameter 2	Coefficient	P value
Above-ground biomass	Weight of tuberous roots	0.60	<0.001*
Root weight	CMD severity	-0.16	<0.001*
Above-ground biomass	CMD severity	-0.51	0.528 ^{ns}
Root weight	CMD incidence	-0.57	<0.001*
Above-ground biomass	<i>B. tabaci</i> abundance	0.12	0.226 ^{ns}

ns = not significant at 5% level.

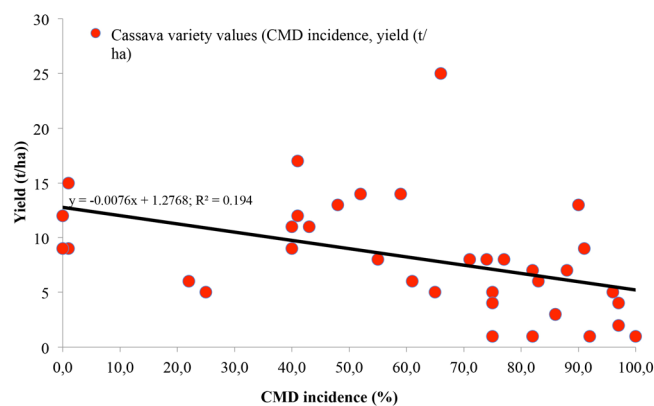
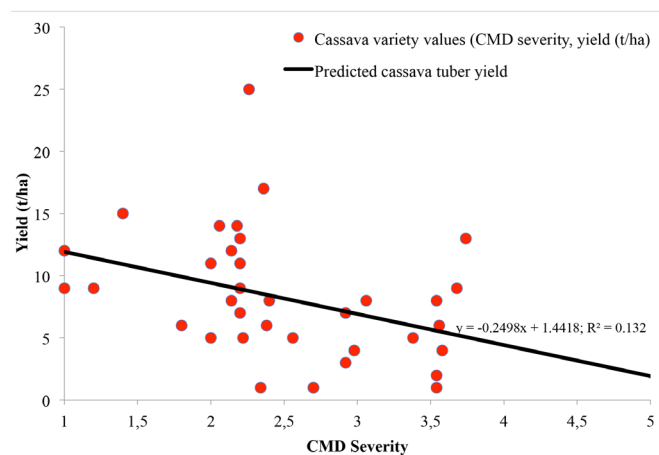
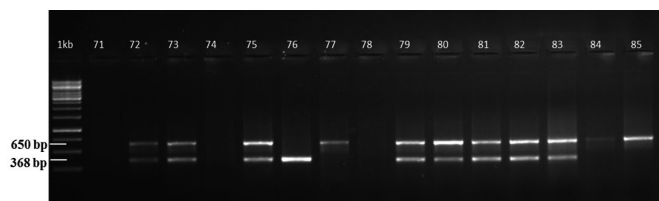
* = Significant at $P < 0.05$ level.

and CMD incidence (Table 4). Regression analyses testing the associations between cassava yield and CMD incidence and severity showed negative relationships for the period from 2 up to 6 map. The inverse relationship between cassava yield and CMD incidence (Fig. 2) ($P = 0.007$) was more strongly significant than that between yield and CMD severity (Fig. 3) ($P = 0.029$). Mweru had the highest root yield (17.6 t ha^{-1}) while cv. Kapumba had the lowest (3.2 t ha^{-1}) (Table 5). Lower root yields were recorded in the CMD-susceptible cultivars. Only cvs Mweru (17.6 t ha^{-1}), Chila (12.6 t ha^{-1}) and Kampolombo (11.9 t ha^{-1}) produced significantly ($P < 0.001$) higher root yields than the overall average root yield of 8.9 t ha^{-1} .

Viruses detected in cassava genotypes. When using the multiplex PCR diagnostic method described, the CMBrep/F, ACMVrep/R and EACMVrep/R primers amplified DNA fragments of the expected sizes: 368 bp for ACMV and 650 bp for EACMV (Fig. 4). The genotypes responded differently to infection by the different viruses (Table 6). Viruses were detected most frequently in cv. Manyopola (10), and least frequently (2) in cv. Kampolombo. In general, there was a higher proportion of mixed infections in the tested cultivars than in the cv. Bangweulu spreader plots.

DISCUSSION

This study complements the work done in Mansa (Zambia) in 2009/10 and 2010/11 seasons by Chikoti *et al.* (2016). Cassava mosaic disease is the most important biotic constraint to cassava production in Zambia, but the deployment of resistant cassava cultivars offers a potentially effective means of addressing the problem (Thresh *et al.*, 1994a; Dixon *et al.*, 2003). This study has described an important first step in the implementation of this control strategy, which comprised the evaluation of the responses to CMD of some of the most promising cassava genotypes in Zambia. The cultivars that were tested differed significantly in their responses, when evaluated at a CMD 'hotspot'. Manyopola showed the highest level of susceptibility. Manyopola is one of the preferred varieties by most farmers due to its sweet taste but was not evaluated by Chikoti *et al.* (2016). By contrast, cv. Kampolombo

**Fig. 2.** Relationship between yields of cassava (*Manihot esculenta*) genotypes and CMD incidence from 2 map to 6 map for all of the experimental plots.**Fig. 3.** Relationship between yield of cassava (*Manihot esculenta*) genotypes and CMD severity from 2 map to 6 map for all of the experimental plots.**Fig. 4.** Gel electrophoresis of multiplex PCR amplified DNA fragments of African cassava mosaic virus (368 bp) and East African cassava mosaic virus (650 bp) using the primers CMBrep/F, ACMVrep/R and EACMVrep/R with 1 kb ladder

was almost unaffected by CMD, indicating its resistance under high disease pressure conditions. These results are consistent with the work done by Chikoti *et al.* (2016) highlighting the low CMD severity scores of cv. Kampolombo. The higher level of resistance demonstrated by this cultivar is not surprising, since it is a progeny from crosses between cv. Nalumino and an IITA male improved clone with a background of CMD resistance. The results are also consistent with other observations of Tropical *Manihot* Series (TMS) genotypes from IITA, showing

Table 5. Average yields at 12 months after planting and whitefly abundance for the different cassava (*Manihot esculenta*) genotypes: Rufunsa, Lusaka Province - 2012/2013.

Genotypes	No. of tuberous roots/plant	Weight of tuberous roots (t/ha)	Whitefly abundance
Bangweulu	5.5	9.1	17.6
Chila	8.6	12.6	27.5
Kampolombo	6.2	11.9	23.0
Kapumba	1.7	3.2	13.9
Katobamphunta	5.9	5.9	20.7
Manyopola	2.6	4.0	18.1
Mweru	9	17.6	32.1
Nalumino	6.8	8.5	25.2
Tanganyika	7.2	6.8	12.0
Mean	5.9	8.9	21.2
LSD _(0.05)	1.4	2.7	7.7
CV %	4.8	11.5	38.4

considerable resistance to infection by CMD (Otim-Nape *et al.*, 1998; Ogbé *et al.*, 2003). Many of the TMS cultivars that are resistant to CMD have inherited resistance genes derived from the Amani (north-eastern Tanzania) breeding programme of the 1950s in which inter-specific crosses were made between cassava and its wild relative *Manihot glaziovii* Müll. Arg. (Jennings, 1957). Early resistant cassava genotypes generated through this programme were taken to Nigeria from which they were subsequently incorporated into the breeding work of IITA in Ibadan. Chila, another improved cultivar crossed with IITA parent material, was moderately susceptible, although symptoms expressed were always mild and plants recovered as they matured. Reversion (CMD-free plants sprouting from cuttings derived from infected parent stems) and recovery are frequent characteristics of CMD resistance (Fargette *et al.*, 1994). The other cultivars derived from IITA parent materials – Mweru and Tanganyika – were susceptible, but both had only moderate symptoms. The cassava cultivars also differed in whitefly infestation as observed in previous findings elsewhere (Otim-Nape *et al.*, 1998). However, there was no clear association between whitefly abundance on the tested cultivars and CMD symptoms observed; cv. Kampolombo had the least CMD incidence with more than the average number of whitefly adults, whilst cv. Manyopola had the highest incidence of CMD but fewer than average whitefly numbers. These findings equally indicate cultivar preferences of whiteflies, as reported previously (Otim-Nape *et al.*, 1996). Other studies have noted the lack of association between whitefly abundance and CMD infection (Legg *et al.*, 2003; Sserubombwe *et al.*, 2001). It is therefore clear that resistance to inoculation and/or suppression of virus soon after establishment are likely to be more important determinants of CMD resistance (Hahn *et al.*, 1980). Although research in Uganda suggested that plant vigour plays a key role in the colonization of plants by whiteflies and consequent patterns of CMD infection (Sserubombwe *et al.*, 2001), our results showed no association between plant vigour (determined

from measurements of above-ground biomass) and whitefly abundance.

Diagnostic testing indicated that there was a significantly higher proportion of mixed virus infection (73%) amongst the initially healthy test plants compared with the infected spreader plots of cv. Bangweulu. It is known that mixed virus infections comprising ACMV and EACMV-like species result in synergistic interactions that lead to increased virion concentrations of both species (Fondong *et al.*, 2000), and that these raised virus titres result in increased whitefly transmission of the mixed virus populations (Legg, 2010). These published findings provide a direct explanation for the pattern of virus occurrence in the cultivars evaluated here.

In this study, as observed previously (Thresh *et al.*, 1994b; Owor *et al.*, 2004a), there was a linear and negative relationship between CMD symptom severity and tuberous root weight. The cassava cultivars evaluated gave yields of between 3.2-17.6 t/ha. The yield ability for cassava depends on several factors including the yield potential of the genotype, the genotype/temperature interaction, soil moisture and soil fertility. The low yields obtained could have been therefore due to several reasons including insufficient rainfall, low temperature and low soil fertility. The trial was planted in Agroecological Region 1 which is characterized by rainfall below 800 mm per year. The rainfall distribution varies from year to year and within a season. Although the genotypes used yield better in favourable environments, in Rufunsa, the yields were relatively low due to low rainfall, low soil fertility, as well as the effects of CMD demonstrated from our experimental data. The annual rainfall in Rufunsa for the 2012/2013 season was 350.6 mm. The low temperatures experienced between June and August 2013 could have also contributed to the low yields obtained. Mean minimum and maximum temperatures were 10.4 and 26.3°C, respectively. At low temperatures (< 16°C) cassava growth is inhibited (Hillocks *et al.*, 2002). Research conducted elsewhere has shown that higher yields were obtained at different temperatures according to the cultivar indicating that temperature has a significant bearing on yield (Irikura *et al.*, 1979). Our results showed low yields for all the genotypes evaluated given the low temperatures even with the improved cvs Mweru, Tanganyika and Chila, which yielded 17.6, 6.8, and 12.6 t/ha, respectively. Although cassava performs better than most crops on infertile soils, higher yields can be obtained when adequate amounts of nutrients are available (Howeler, 2002). Results for soil samples analysed at Mount Makulu Soil Chemistry Laboratory showed low levels of organic carbon (0.2 %), nitrogen (0.02%) and phosphorus (4 ppm). The soil was classified as a sandy loam. Nitrogen deficiency is usually observed in very sandy soils low in organic matter (Howeler, 2002). In addition to low soil nitrogen, the genotypes at Rufunsa were harvested early (12 map) and this might also have contributed to the low yields obtained. Similar low yields (<5 t/ha) were obtained

Table 6. Cassava mosaic geminiviruses detected in the cassava genotypes and spreader plots four months after planting.

Genotype	Viruses					Spreader (Bangweulu)			
	ACMV	EACMV	ACMV + EACMV	Negative reactions	Total	ACMV	EACMV	ACMV + EACMV	Total
Bangweulu	1	0	5	4	10	2	1	1	4
Chila	2	0	6	2	10	2	0	2	4
Kampolombo	0	1	1	8	10	1	1	2	4
Kapumba	1	2	4	3	10	1	0	3	4
Katobamphunta	1	2	6	1	10	1	1	2	4
Manyopola	0	1	9	0	10	1	1	2	4
Mweru	1	2	5	2	10	2	1	1	4
Nalumino	0	2	6	2	10	1	1	2	4
Tanganyika	1	0	3	6	10	2	0	2	4
Total	7	10	45	28	90	13	6	17	36

in Nigeria in a cassava trial harvested at 11 map (Kang and Okeke, 1984). Cassava is usually harvested between 16 and 24 map to achieve maximum yields. Research has shown that cassava cultivars harvested at 16–24 map gave higher yields (Barratt *et al.*, 2006). Tuberisation in cassava starts at 75 days after planting (dap) (Alves, 2002) and some of the improved cultivars have been bred to reach maximum bulking at 18–24 map (Cock, 1984). Low temperatures, such as those occurring at Rufunsa in July and August, slow down the tuberisation process in cassava.

Improved cultivars generally had higher yields than the CMD-susceptible cv. Manyopola. Results indicated the negative effect of CMD infection on the yield for all the tested cultivars. However, the findings also revealed that relatively high yields can be obtained from improved cultivars even though the plants become infected with CMD. Many CMD-resistant cultivars can be infected by CMD but express mild symptoms that have little significant impact on yield (Thresh *et al.*, 1994b). Mildly diseased plants are characterized by chlorotic areas that are smaller, less intensely yellow and distributed more sparsely than the mostly conspicuous symptoms of severely CMD-diseased plants (Fargette *et al.*, 1987). Associated with this, differing degrees of yield loss resulting from varied CMD severities have been attributed to the degree to which the metabolic and photosynthetic processes are affected (Chant *et al.*, 1971). It is this effect on photosynthesis and growth of the plant that has a detrimental effect on tuberisation (Owor *et al.*, 2004b).

Regression results can be used to predict the effects of CMD on yield. The CMD incidence *vs* yield regression shows that at an incidence of 0%, the expected yield would be 12.8 t ha⁻¹, whilst at an incidence of 100%, the yield would be 5.2 t ha⁻¹. In a situation where 100% of plants were infected, this equates to a yield loss of 59.2%. Similarly, for the CMD severity *vs* yield regression: where all plants are symptomless (mean severity of 1), the predicted yield is 11.9 t ha⁻¹ whilst for 100% severely infected plants (score 5) the anticipated yield would be 1.9 t ha⁻¹, representing a potential yield loss under the most severe disease conditions of 83.9%. Using country-level production data for 2014 (FAOSTAT, 2017), we can estimate

countrywide losses due to CMD, on the assumption that the average for the mixed selection of the cultivars evaluated in this study is representative of the diversity of the cultivars grown in the country. Total production in Zambia for 2014 was 919,497 tons (FAOSTAT, 2017), and the most recent countrywide estimate of CMD incidence is 57.4% (Chikoti *et al.*, 2015). Since the yield loss at 100% infection is 59.2%, the predicted loss for an overall incidence of 57.4% would be 34.0%, which would equate to an annual countrywide loss of 473,275 tons. In Zambia, the average cassava price was estimated at US\$110 per ton dry weight after removing the effect of inflation (Alene *et al.*, 2013). Based on this value, the predicted financial loss for the effects of CMD in Zambia is more than US\$ 52 million.

Most of the cultivars showed varying levels of recovery from CMD symptoms over the course of the season. Recovery and reversion occur commonly amongst CMD-resistant cultivars that normally express only mild to moderate symptoms (Fargette *et al.*, 1994). The occurrence of such a phenomenon among the cultivars tested offers options for their deployment in different epidemiological backgrounds. For instance, the highly resistant cv. Kampolombo could be deployed in both low and high disease pressure areas, whilst the moderate recovery types such as cvs Chila, Kapumba, Mweru and Nalumino would be suitable for low to moderate CMD pressure areas. However, cvs Bangweulu, Katobamphunta, Manyopola and Tanganyika should only be grown using disease-free planting material in low CMD pressure areas. In view of its high level of resistance, cv. Kampolombo has great potential for use as a CMD-resistant parent in cassava breeding programmes.

In addition to the cassava cultivars evaluated in the present study, there is a need for more genotypes to be included for evaluation across the three major agro-ecological regions of Zambia and over a period of two or more seasons in order to confirm the levels of CMD resistance. This is because expression of CMD in different cassava genotypes is known to be dependent on the environment, host and the virus species. New knowledge on the diversity of virus species causing CMD in Zambia (Mulenga

et al., 2015) should also be exploited in efforts to screen new germplasm against virus isolates of known species, as well as virus mixtures, using grafting techniques. CMD continues to cause significant losses to cassava production in Zambia, but based on the findings described here, there is considerable potential to deploy resistance sources that will greatly reduce these losses in future years. The potential for cassava cultivars that showed low or moderate CMD infection to be used as sources of resistance in the cassava breeding programme needs to be explored.

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