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The effect of cassava mosaic geminiviruses on symptom severity, growth and root yield of a cassava mosaic virus disease-susceptible cultivar in Uganda

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Summary

A study was carried out to assess the effect of different cassava mosaic geminiviruses (CMGs) occurring in Uganda on the growth and yield of the susceptible local cultivar 'Ebwanateraka'. Plants infected with *African cassava mosaic virus* (ACMV), 'mild' and 'severe' strains of *East African cassava mosaic virus*-Uganda (EACMV-UG2) and both ACMV and EACMV-UG2 were grown in two experiments in Kabula, Lyantonde in western Uganda. The most severe disease developed in plants co-infected with ACMV and EACMV-UG2 and in those infected with the 'severe' form of EACMV-UG2 alone; disease was least severe in plants infected with the 'mild' strain of EACMV-UG2. ACMV-infected plants and those infected with the 'mild' strain of EACMV-UG2 were tallest in the 1999-2000 and 2000-2001 trials, respectively; plants dually infected with ACMV and EACMV-UG2 were shortest in both trials. Plants infected with 'mild' EACMV-UG2 yielded the largest number and the heaviest tuberous roots followed by ACMV and EACMV-UG2 'severe', respectively, whilst plants dually infected with ACMV and EACMV-UG2 yielded the least considering the two trials together. Reduction in tuberous root weight was greatest in plants dually infected with ACMV and EACMV-UG2, averaging 82%. Losses attributed to ACMV alone, EACMV-UG2 'mild' and EACMV-UG2 'severe' were 42%, 12% and 68%, respectively. Fifty percent and 48% of the plants infected with both ACMV and EACMV-UG2 gave no root yield in 1999-2000 and 2000-2001, respectively. These results indicate that CMGs, whether in single or mixed infections, reduce root yield and numbers of tuberous roots produced and that losses are substantially increased following mixed infection.

Key words: Cassava mosaic geminiviruses, cassava mosaic virus disease, *African cassava mosaic virus*, *East African cassava mosaic virus*-Uganda, yield loss

Introduction

Cassava is a key food security crop in sub-Saharan Africa and contributes significantly to the livelihood of millions of people. Its production faces constraints from a number of pests and diseases but, most significantly from cassava mosaic virus disease (CMD) that occurs in all cassava-producing areas in Africa, India and Sri Lanka. CMD is caused by cassava mosaic geminiviruses (CMGs) (*Geminiviridae*: *Begomovirus*) (Bock & Woods, 1983; Hong *et al*., 1993; Fauquet & Stanley, 2003) that can occur alone or in combination. Geminiviruses pose an increasing threat to world agriculture (Boulton, 2003; Varma & Malathi, 2003) and the CMGs are currently one of the most economically important members of this group of viruses (Varma & Malathi, 2003). *African cassava mosaic virus* (ACMV) and *East African cassava mosaic virus* (EACMV) appear to be the most prevalent CMGs in Africa. However, a recombinant virus referred to as UgV (Zhou *et al.*, 1997) or EACMV-UG2 (Deng *et al.*, 1997) was reported in Uganda and is associated with the pandemic of unusually severe CMD (Otim-Nape *et al.,* 2000) that continues to spread in neighbouring countries (Legg, 1999). In this paper, the acronym EACMV-UG2 is used throughout to refer to this recombinant virus. Two other related CMGs, EACMV-UG1 and EACMV-UG3 have also been reported from Uganda, but are infrequent (Pita *et al*., 2001; Sseruwagi *et al*., 2004).

Early studies of EACMV-UG2 suggested that where it occurred in single infections or in combination with ACMV, symptoms were severe (Harrison *et al.,* 1997). In contrast, plants infected with ACMV alone expressed mild or moderate symptoms. In the years following the passage of the pandemic, however, mild strains of EACMV-UG2 have also been shown to occur (Pita *et al.,* 2001).

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The mild (EACMV-UG2Mld) and severe (EACMV-UG2Svr) strains are virtually identical, however, sharing 99% nucleotide sequence identity in their DNA-A (Pita *et al*., 2001).

Yield losses attributable to CMD are highly variable ranging from insignificant to almost total loss (Thresh *et al.,* 1994). Thresh *et al.* (1997) estimated losses in Africa due to CMD of between 15% and 24% equivalent to 15-27 million tonnes per annum. Previously losses of 86% have been reported for the susceptible cultivar F279 in Kenya (Bock & Guthrie, 1978), 37% in cultivar CB in Ivory Coast (Fargette *et al.,* 1988) and 20-90% for susceptible cultivars (Beck & Chant, 1958; Thresh *et al.,* 1994). Yield losses ranging from 20% to 95% have also been reported in other countries (Fauquet & Fargette, 1990). In Uganda, various studies have assessed the yield effects of CMD (Otim-Nape *et al*., 1997; Byabakama *et al*., 1999; Osiru *et al*., 1999; Sserubombwe *et al*., 2001) and losses have been related to symptom severity (Osiru *et al*., 1999). In none of these studies, however, was loss related to the species of CMGs causing the disease. This is now possible following recent detailed molecular characterisation of CMGs and advances in diagnostic techniques (Harrison *et al*., 1997; Zhou *et al.,* 1997).

Although considerable efforts have been directed towards management of CMD, there is virtually no information on interactions between known CMGs and their effect on yield alone and in combination. The study reported here is the first to combine the use of nucleic acid-based diagnostics tests using the polymerase chain reaction (PCR) with traditional yield loss assessment methods with the overall objective of quantifying losses attributable to particular CMGs and CMG combinations.

Materials and Methods

Stem cuttings of the Ugandan cultivar 'Ebwanateraka' were collected in Soroti district in eastern Uganda from plants with no symptoms, mild symptoms (severity score 2) or very severe symptoms of CMD (severity score 4). 'Ebwanateraka' is one of the most widely grown local cultivars in Uganda and is highly sensitive to CMD (Sserubombwe *et al*., 2001). Leaf samples were collected from each plant for extraction of total DNA using the method of Dellaporta *et al.* (1983). Universal oligonucleotide primers UNIF and UNIR (Invitrogen, Life Sciences) were used for the amplification of near full-length fragments of DNA-A of CMGs from whole plant DNA extracts for each sample. DNA-A was then cut using the restriction enzymes *Eco*RV and *Mlu*I and digested products electrophoresed in a 1.5% ethidium bromide stained agarose gel in TAE buffer and visualised under UV light and Polaroid photographs taken. Virus diagnoses were then made following the approach of Sseruwagi *et al.* (2004). Primer pairs ACMV-AL1/F and ACMV-AR0/R specific for ACMV, and UV-AL1/F and ACMV-CP/R3 specific for EACMV-UG2 were used to confirm diagnoses (Zhou *et al.*, 1997).

ACMV-AL1/F	5'	GCGGAATCCCTAACATTATC 3'
ACMV-ARO/R	5'	GCTCGTATGTATCCTCTAAG GCCTG 3'
UV-AL1/F	5'	TGTCTTCTGGGACTTGTGTG 3'
ACMV-CP/R3	5'	GCCTCCTGATGATTATATGTC 3'
UNIF	5'	KSGGGTCGACGTCATCATCA ATGACGTTRTAC 3'
UNIR	5'	AARGAATTCATKGGGGCCC ARARRGACTGGC 3'

Where K = G + C, R = A + G and S = G + C

The test plants were identified through PCR (Zhou *et al.,* 1997) and random amplified fragment length polymorphism (RFLP) tests and comprised plants infected with the viruses ACMV, EACMV-UG2 'mild', EACMV-UG2 'severe' and ACMV + EACMV-UG2. 'Mild' and 'severe' EACMV-UG2 were separated based on symptom differences after identifying EACMV-UG2 in 'parent' plants from which cuttings were obtained. It was not possible to distinguish between mild and severe strains of EACMV-UG2, either in single or mixed infection (with ACMV), using molecular diagnostic techniques, since these two strains had greater than 99% nucleotide sequence homology in their DNA-A and differed only in point mutations (Pita *et al*., 2001). A virus-free control was included by collecting cuttings from symptomless plants that tested negative for CMGs with PCR. Experiments were planted in November 1999 and October 2000 at Lyantonde in Rakai District of western Uganda, an area characterised by little spread of CMD. An equal number of test plants (168 plants) comprising the different virus categories were randomly established in the field and spaced 1 m apart. PCR diagnostic tests were repeated at sprouting, 4 months after planting (MAP) and at harvest to confirm the virus status of the plants. Routine cultural practices such as weeding and trapping of mole rats were adopted and no fertiliser was applied. A systemic insecticide imidacloprid was applied at sprouting and again at 4 MAP to maintain integrity of the treatments by controlling the whitefly vector [*Bemisia tabaci* (Genn.)] and minimising the risk of virus spread. Additionally, the field was sprayed weekly with the contact insecticide cypermethrin to ensure the experiment remained free of whitefly infestation.

Starting from 1 MAP then monthly until 10 MAP, the severity of symptoms on each plant was recorded using a scale of 1-5 (Hahn *et al.,* 1980) where 1

represents no symptoms and 5 represents severe chlorosis, leaf distortion and stunting. At 4 and 8 MAP, the height of each plant was measured from ground level to the highest shoot tip. Plants were harvested at 10 MAP and yield data were recorded by harvesting each plant individually and taking records of the number of marketable and non-marketable tuberous roots and their weights. Marketable and non-marketable tuberous roots were separated based on size, where tubers more than 100 g were considered marketable and those less than this weight as non-marketable. Total weight and mean number of tuberous roots for each treatment were computed from this.

Based on PCR and RFLP results confirming the virus status of plants, those plants with consistent results were identified and used for subsequent analysis. Individual plants were selected for the analysis and those that gave negative PCR reactions were not included in the analysis. As a result, 20, 116, 122 and 20 plants for the 1999-2000 trial and 19, 95, 185 and 57 plants for the 2000-2001 trial infected with ACMV, ACMV + EACMV-UG2, EACMV-UG2 'mild' and EACMV-UG2 'severe' respectively were used during data analysis. Fourteen and 129 CMD-free plants for the 1999-2000 and 2000-2001 trials respectively sprouted CMD-free, remained disease free until the end of the trial and were used for data analysis. Transformations, square root for tuberous root numbers and logarithms for root weights were used to stabilise variance prior to statistical tests but actual mean values are presented in the tables of results. CMD symptom severity, height of plants and yield data were compared with ANOVA using SigmaStat software (Quinton *et al*., 1992). Yield loss was expressed as a percentage of yields of the unaffected controls.

Results

Severity of CMD symptoms

The disease symptom severities expressed were highly variable for the different CMG categories and ranged from mild to very severe (Table 1). Plants co-infected by ACMV and EACMV-UG2 had the most severe symptoms in the 1999-2000 trial while those infected with EACMV-UG2 'severe' alone and both ACMV and EACMV-UG2 had the most severe symptoms in the 2000-2001 trials. The least severe symptoms were recorded in plants infected with EACMV-UG2 'mild' followed by ACMV-infected plants in both trials.

Height of plants

Height of plants varied significantly ($P < 0.001$) for test plants at both 4 and 8 MAP in each trial. In 1999-2000, CMD-free plants were significantly taller than all virus-infected treatments (Fig. 1). Plants infected with both ACMV and EACMV-UG2 were significantly shorter than all other test plants. In 2000-2001, EACMV-UG2 'mild'-infected plants were tallest followed by the healthy controls and ACMV in that order. EACMV-UG2 'severe'-infected plants and those co-infected by ACMV and EACMV-UG2 were shortest at both 4 and 8 MAP but were not significantly different from each other. Consequently, there was a negative relationship between plant height and symptom score (Fig. 2).

Yields

Tuberous root number

There were statistically significant differences ($P < 0.001$) in the mean number of tuberous roots produced for the different test plants in each trial. In the 1999-2000 trial, control plants significantly out-yielded all the CMG-infected plants, by yielding more tuberous roots than EACMV-UG2 'mild', ACMV and EACMV-UG2 'severe'-infected plants (Table 2). Plants co-infected with ACMV and EACMV-UG2 produced significantly fewer tuberous roots than the other test plants. ACMV, EACMV-UG2 'mild' and EACMV-UG2 'severe' did not yield significantly different ($P = 0.06$) numbers of tuberous roots. In 2000-2001, EACMV-UG2 'mild'-infected plants produced the greatest number of tuberous roots, followed by control plants then ACMV-infected plants (Table 3). EACMV-UG2 'severe' and ACMV+EACMV-UG2-infected plants yielded the fewest tuberous roots. Fifty per cent and 48% of plants co-infected by ACMV and EACMV-UG2 in 1999-2000 and 2000-2001 produced no harvestable tuberous roots.

Tuberous root weight per plant

Tuberous root weights differed ($P < 0.001$) for the different CMG categories in both trials. Extreme weights of tuberous roots of individual plants ranged from zero in many of the plants co-infected with

Table 1. *Cassava mosaic virus disease (CMD) mean symptom severity scores of all values for plants at Lyantonde, western Uganda*

	Trial	
CMG category	1999-2000	2000-2001
EACMV-UG2 'mild'	2.44 (0.06)	1.77 (0.34)
EACMV-UG2 'severe'	3.60 (0.04)	3.53 (0.10)
ACMV	3.05 (0.12)	2.86 (0.20)
ACMV + EACMV-UG2	4.33 (0.06)	3.46 (0.06)
Healthy	1.00 (0.00)	1.00 (0.00)

Scale 1-5: 1 = symptom-free; 2 = mild chlorosis, little leaf distortion; 3 = moderate chlorosis, minor distortion; 4 = severe chlorosis, moderate leaf distortion, minor stunting; 5 = severe chlorosis, leaf distortion and stunting.
*Values in parentheses are the standard error of the mean (SEM)

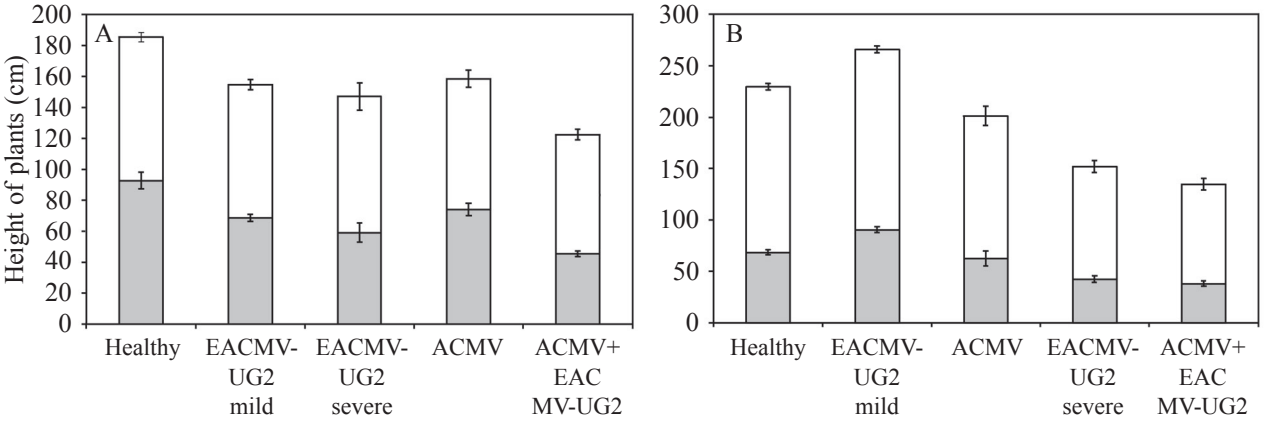

Fig. 1. Height of plants 4 and 8 months after planting (MAP) in 1999-2000 (a) and 2000-2001 (b) trials. Bars indicate the standard errors of the mean. White, 8 MAP; grey, 4 MAP.

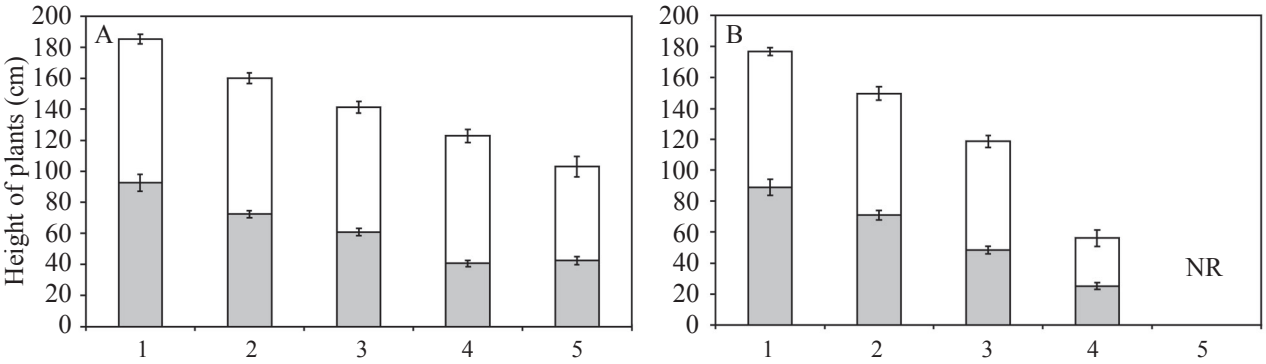

Fig. 2. Height of plants 4 and 8 months after planting (MAP) for the different CMG severity score classes: 1999-2000 (a) and 2000-2001(b) trials. Bars indicate the standard errors of the mean. White, 8 MAP; grey, 4 MAP.

Table 2. *Average yields per plant for the different health status categories 10 MAP: 1999-2000 trial*

Status	No. of plants in each category	No. of tuberous roots	No. of marketable tuberous roots	Weight of tuberous roots (kg)	Weight of marketable tuberous roots (kg)
E-UG2 'mild'	122	5.63 (0.50)	3.73 (0.24)	2.40 (0.13)	2.25 (0.33)
E-UG2 'severe'	20	3.95 (0.40)	2.20 (0.47)	1.68 (0.30)	1.34 (0.22)
ACMV	20	4.70 (0.80)	3.41(0.78)	2.26 (0.50)	2.10 (0.46)
ACMV+ E-UG2	116	1.34 (0.15)	0.53 (0.12)	0.46 (0.08)	0.39 (0.09)
Healthy	14	8.79 (1.50)	6.21 (1.00)	3.56 (0.50)	3.26 (0.48)

E-UG2 = EACMV-UG2
Values in parentheses are the standard error of the mean (SEM)

Table 3. *Average yields per plant for the different health status categories 10 MAP: 2000-2001 trial*

Status	No. of plants in each category	No. of tuberous roots	No. of marketable tuberous roots	Weight of tuberous root (kg)	Weight of marketable tuberous roots (kg)
E-UG2 'mild'	185	7.64 (0.43)	5.20 (0.36)	2.13 (0.15)	1.77 (0.14)
E-UG2 'severe'	57	1.53 (0.25)	0.86 (0.19)	0.35 (0.06)	0.24 (0.06)
ACMV	19	4.11(0.86)	2.56 (0.62)	1.04 (0.20)	0.84 (0.19)
ACMV+ E-UG2	95	1.97(0.34)	1.19 (0.27)	0.44 (0.08)	0.34 (0.07)
Healthy	129	6.23(0.35)	5.30 (0.32)	1.94 (0.12)	1.83 (0.11)

E-UG2=EACMV-UG2
Values in parentheses are the standard error of the mean (SEM)

both ACMV and EACMV-UG2 to 8 kg and 12 kg per plant for control plants in the 1999-2000 and 2000-2001 trials respectively. In 1999-2000, control plants had significantly greater mean tuberous root weights plant^{-1} than EACMV-UG2 'mild', ACMV and EACMV-UG2 'severe'-infected plants. Plants co-infected by ACMV and EACMV-UG2 yielded the least weight of tuberous roots (Table 2). Plants infected with EACMV-UG2 'mild' had the greatest mean tuberous root weight per plant followed by the control then ACMV, EACMV-UG2 'severe' and ACMV + EACMV-UG2 - infected plants in 2000-2001 (Table 3). However, tuberous root yields of ACMV, EACMV-UG2 'severe' and plants co-infected with ACMV and EACMV-UG2 were not significantly different.

Yield losses for the 1999-2000 trial were 87% for plants dually infected with ACMV and EACMV-UG2, 53% for EACMV-UG2 'severe' and 37% for ACMV alone. Comparable figures for 2000-2001 were 77%, 82% and 46%. EACMV-UG2 'mild'-infected plants sustained a 33% yield loss in 1999-2000 and an increase in yield of 9% in 2000-2001.

Discussion

CMD is arguably the most important constraint to cassava production in Africa, and as such, a considerable amount of research attention has been directed towards assessing losses. Many of these studies were reviewed by Thresh *et al.* (1994) who stressed the wide range of yield loss estimates in the published literature. The study reported here, however, is the first to make use of DNA-based virus detection techniques to determine the effects of particular viruses, virus strains and virus mixtures on growth and yield of cassava. PCR was used successfully to establish experimental treatments comprising plants infected with the most commonly occurring viruses and virus mixtures affecting cassava in Uganda. Different numbers of plants in different virus infection categories, however, meant that some comparisons were more efficient than others. Further studies using a similar approach should pay particular attention to the preparation of pure virus infection provenances prior to planting. Combining the identification and use of a low inoculum pressure location with whitefly management using the systemic insecticide imidacloprid provided an effective means of assuring the integrity of the virus-infected and healthy treatments. As such, the approach provides a useful model for similar future yield loss assessments.

Our studies show clearly that CMGs, whether in single or mixed infections, have significant negative effects on growth and yield of a CMD-sensitive cultivar. The only exception to this was where plants were infected by the mild strain of EACMV-UG2. Yield reductions are greatest in plants infected with the 'severe' form of EACMV-UG2 and those dually infected with ACMV and EACMV-UG2 but least in plants infected with the 'mild' form of EACMV-UG2. EACMV-UG2 'mild'–infected plants yielded significantly more and grew taller than CMD-free plants in the 2000-2001 trial. Comparable results of mildly infected plants out-yielding and growing better than CMD-free plants have been obtained (Cours, 1951). This observation has been attributed to the possibility that maybe slight or mild symptoms improve partitioning of assimilates between roots and aerial parts (Cours, 1951) hence the better growth and yield observed in the 2000-2001 trial. Results also confirm the intermediate losses which result from infection with a commonly occurring strain of ACMV; typically less than those resulting from severe EACMV-UG2 infection, but greater than those recorded for EACMV-UG2 mild infected plants. There are no directly comparable virus/strain-specific yield loss estimates from either Uganda or elsewhere in Africa. However, more severe symptoms for ACMV/EACMV virus mixtures have been reported for Uganda, Tanzania (Harrison *et al*., 1997) and Cameroon (Fondong *et al.,* 2000) and this is likely to be a general phenomenon. It is notable, however, that whilst mixed infections are an important and common feature of the CMD pandemic in East and Central Africa (Legg, 1999) they occur relatively infrequently in the cassava-growing areas of Africa that are as yet unaffected by the pandemic and where there is a relatively low incidence of infection (Legg & Fauquet, 2004).

The overall range in reductions of yield recorded in these experiments concurs with results of Terry & Hahn (1980) at IITA-Ibadan, Nigeria, comparing yields of susceptible and resistant cassava varieties in which significant reductions in yield were recorded in the susceptible variety. Also, yield losses recorded in this experiment fall between the ranges of 20-90% reported for susceptible varieties (Fauquet & Fargette, 1990). Previous yield studies in Uganda using the variety Ebwanateraka have provided contrasting results. Experiments conducted in southern Uganda between 1990 and 1992, prior to the onset of the severe CMD epidemic, gave yield loss figures of 20-40% (Otim-Nape *et al*., 1997). These reductions were substantially less than the 66% losses recorded from experiments conducted in 1993 which used Ebwanateraka planting material obtained from the epidemic-affected zone further to the north (Byabakama *et al*., 1999). It seems likely that whilst the earlier trials were recording the effect on yield of ACMV infection, planting material used for the later experiments was infected by EACMV-UG2, and mixed infections were also present, leading to the greater losses. The limited understanding of CMG diversity and the absence of

adequate diagnostics at the time, however, precluded the recognition of this possibility, and means that retrospective interpretations remain speculative.

The current study has provided clear field evidence that dual infected plants have the most severe symptoms, and there was a clear relationship between symptom severity and yield, as in previous studies (Otim-Nape *et al.,* 1994). Prior to this, only laboratory and screenhouse-based evidence had been presented in support of virus-virus synergism leading to the most severe symptoms (Harrison *et al.,* 1997; Fondong *et al.,* 2000; Pita *et al.,* 2001). It has been reported that reduction in yield as disease symptom severity increases may be related to the degree to which metabolic and photosynthetic processes are affected (Chant *et al.,* 1971; Cock, 1978; Otim-Nape *et al.,* 1994). This effect on photosynthesis and growth of the plant has a direct detrimental effect on tuberisation. The difference in yield demonstrated between plants infected with 'mild' and 'severe' forms of EACMV-UG2 is comparable to results of Fauquet & Fargette (1990) from Ivory Coast in which plants with mild disease yielded more than those of the same variety with severe disease. Mildly diseased plants have chlorotic areas that are more sparsely distributed, smaller and less intensely yellow than the most conspicuous symptoms of severely diseased plants (Storey & Nichols, 1938; Fargette *et al.,* 1987).

Preliminary observations have indicated a resurgence of local cultivars, especially in 'post-epidemic' areas of Uganda. It has also been reported that local susceptible cultivars continue to be widely grown despite the availability of resistant varieties (Calvert & Thresh, 2002). Plants of local cultivars that express mild symptoms are now common and they yield satisfactorily. Hence, they are retained especially if they have favourable taste or other desirable attributes. The low yield losses incurred by EACMV-UG2 'mild'-infected plants as indicated by results presented here may in part explain why local varieties continue to be grown if they withstand superinfection with more damaging virulent strains. The evident sustained success in cultivation of mildly-diseased cassava that this demonstrates raises questions about the mechanism by which mildly diseased plants avoid infection by more severe viruses or virus strains. This is an important topic for future research, since it may offer potential for exploitation as an additional CMD management approach.

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References

Beck B D A, Chant S R. 1958. A preliminary investigation on the effect of mosaic on *Manihot utilissima* Pohl. in Nigeria. *Tropical Agriculture (Trinidad)* **35**:59-64.

Bock K R, Guthrie E J. 1978. African mosaic disease in Kenya. In *Proceedings, Cassava Protection Workshop,* pp. 41-44. 7-12 November 1977. Eds T Brekelbaum, A Belloti and J C Lozano. Cali, Colombia: CIAT.

Bock K R, Woods R D. 1983. Etiology of African cassava mosaic disease. *Plant Disease* **67**:994-995.

Boulton M I. 2003. Geminiviruses: major threats to world agriculture. *Annals of Applied Biology* **142**:143.

Byabakama B A, Adipala E, Ogenga-Latigo M W, Otim-Nape G W. 1999. The effect of amount and disposition of inoculum on cassava mosaic virus disease development and tuberous root yield of cassava. *African Plant Protection* **5**:21-29.

Calvert L A, Thresh J M. 2002. The Viruses and Virus Diseases of Cassava. In *Cassava: Biology, Production and Utilization* pp. 237-260. Eds R J Hillocks, J M Thresh and A C Bellotti. Wallingford: CABI Publishing.

Chant S R, Bateman J G, Bates D C. 1971. The effect of cassava mosaic virus infection on the metabolism of cassava leaves. *Tropical Agriculture (Trinidad)* **48**:263-270.

Cock J H. 1978. Physiological basis of yield loss in cassava due to pests. In *Proceedings: Cassava Protection Workshop*, pp. 9-16. 7-12 November 1997. Eds T Brekelbaum, A Belloti and J C Lozano. Cali, Colombia: CIAT.

Cours G. 1951. Le manioc à Madagascar. *Mémoires de l'Institut Scientifique de Madagascar Série B Biologie Végétale* **3**:203-400.

Dellaporta S L, Wood J, Hicks J B. 1983. A plant DNA mini preparation: Version II. *Plant Molecular Biology Reporter* **1**:19-21.

Deng D, Otim-Nape G W, Sangare A, Ogwal S, Beachy R N, Fauquet C M. 1997. Presence of a new virus closely associated with cassava mosaic outbreak in Uganda. *African Journal of Root Tuber Crops* **2**:21-34.

Fargette D, Fauquet D, Thouvenel J C. 1987. Distribution and spread of African cassava mosaic in a cassava field. *CTA International Seminar on African Cassava Mosaic Disease and its Control*: 4-8 May 1987, Yamoussoukro, Côte d'Ivoire, pp. 105-108.

Fargette D, Fauquet C, Thouvenel J-C. 1988. Yield losses induced by African cassava mosaic virus in relation to mode and date of infection. *Tropical Pest Management* **34**:89-91.

Fauquet C, Fargette D. 1990. African cassava mosaic virus: etiology, epidemiology, and control. *Plant Disease* **74**:404-411.

Fauquet C, Stanley J. 2003. Geminivirus nomenclature: progress and problems. *Annals of Applied Biology* **142**:156-189.

Fondong V N, Pita J S, Rey M E C, de Kochko A, Beachy R N, Fauquet C M. 2000. Evidence of synergism between African cassava mosaic virus and double recombinant geminivirus infecting cassava in Cameroon. *Journal of General Virology* **81**:287-297.

Hahn S K, Terry E R, Leuschner K. 1980. Breeding cassava for resistance to cassava mosaic disease. *Euphytica* **29**:673-683.

Harrison B D, Zhou X, Otim-Nape G W, Liu Y, Robinson D J. 1997. Role of a novel type of double infection in the geminivirus-induced epidemic of severe cassava mosaic in Uganda. *Annals of Applied Biology* **131**:437-448.

Hong Y G, Robinson D J, Harrison B D. 1993. Nucleotide sequence evidence for the occurrence of three distinct whitefly-transmitted geminiviruses in cassava. *Journal of General Virology* **74**:2437-2443.

Legg J P. 1999. Emergence, spread and strategies for controlling the pandemic of cassava mosaic virus disease in east and central Africa. *Crop Protection* **18**:627-637.

Legg J P, Fauquet C M. 2004. Cassava mosaic geminiviruses in Africa. *Plant Molecular Biology.* (In press).

Osiru D S O, Sserubombwe W, Sseruwagi P, Thresh J M, Otim-Nape G W. 1999. Effects of cassava mosaic disease on growth and yield of cassava - some highlights of Makerere experiments. *African Crop Science Journal* **7**:511-522.

Otim-Nape G W, Shaw M W, Thresh J M. 1994. The effects of African cassava mosaic geminivirus on the growth and yield of cassava in Uganda. *Tropical Science* **34**:43-54.

Otim-Nape G W, Thresh J M, Shaw M W. 1997. The effects of cassava mosaic virus disease on yield and compensation in mixed stands of healthy and infected cassava. *Annals of Applied Biology* **130**:503-521.

Otim-Nape G W, Bua A, Thresh J M, Baguma Y, Ogwal S, Semakula G N, Acola G, Byabakama B, Colvin J, Cooter R J, Martin A. 2000. *The Current Pandemic of Cassava Mosaic Virus Disease in East Africa and its Control.* Chatham, UK: Natural Resources Institute. 100 pp.

Pita J S, Fondong V N, Sangare A, Otim-Nape G W, Fauquet C M. 2001. Recombination, pseudorecombination and synergism of geminiviruses are determinant keys to the epidemic of cassava mosaic disease in Uganda. *Journal of General Virology* **82**:655-665.

Quinton A, Lebedev G, Youtz P, Tuerke T, Sinkler R, Kuo J. 1992. *Sigmastat statistical software.* Jandel Scientific Software, Version 2.0.

Sserubombwe W S, Thresh J M, Otim-Nape G W, Osiru D S O. 2001. Progress of cassava mosaic virus disease and whitefly vector populations in single and mixed stands of four cassava varieties grown under epidemic conditions in Uganda. *Annals of Applied Biology* **138**:161-170.

Sseruwagi P, Rey M E C, Brown J K, Legg J P. 2004. The cassava mosaic geminiviruses occurring in Uganda following the 1990s epidemic of severe cassava mosaic disease. *Annals of Applied Biology* **145**:113-121.

Storey H H, Nichols R F W. 1938. Studies of the mosaic disease of cassava. *Annals of Applied Biology* **25**:790-806.

Terry E R, Hahn S K. 1980. The Effect of Cassava Mosaic Disease on the Growth and Yield of a Local and Improved variety of Cassava. *Tropical Pest Management* **26**:34-37.

Thresh J M, Fargette D, Otim-Nape G W. 1994. Effects of African cassava mosaic geminivirus on yield of cassava. *Tropical Science* **34**:26-42.

Thresh J M, Otim-Nape G W, Legg J P, Fargette D. 1997. African cassava mosaic disease: the magnitude of the problem. *African Journal of Root and Tuber Crops* **2**:13-19.

Varma A, Malathi V G. 2003. Emerging geminivirus problems: A serious threat to crop production. *Annals of Applied Biology* **142**:145-164.

Zhou X, Liu Y, Calvert L, Munoz C, Otim-Nape G W, Robinson D J, Harrison B D. 1997. Evidence that DNA-A of a geminivirus associated with severe cassava mosaic disease in Uganda has arisen by interspecific recombination. *Journal of General Virology* **78**:2101-2111.