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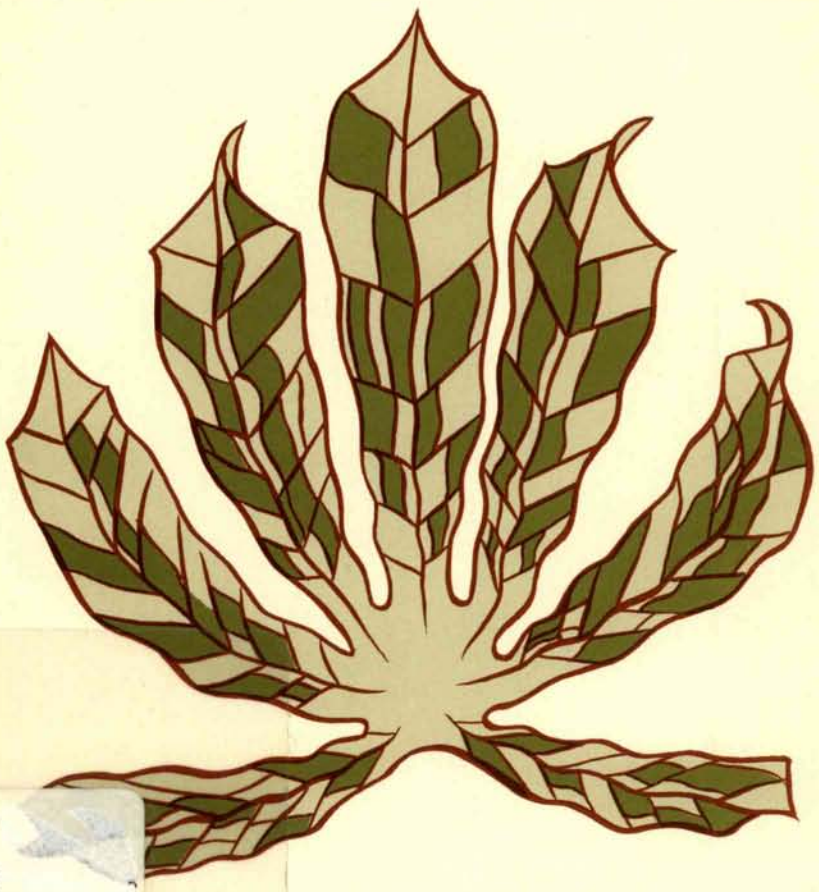
African Cassava MOSAIC



Report of an Interdisciplinary Workshop held at Muguga, Kenya, 19-22 February 1976

Editor: Barry L. Nestel

Cosponsored by the East African Agriculture and Forestry Research Organization
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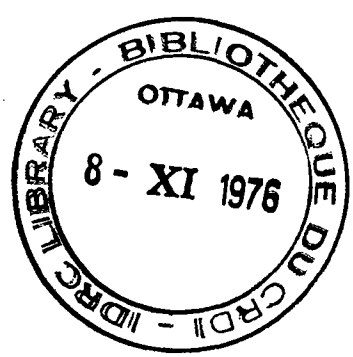

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Recent Advances in Research on Cassava Viruses in East Africa

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Introduction

WE are studying three different aspects of cassava viruses at EAAFRO and in the field at the Coast Agricultural Research Station, Mtwapa, where we have collaborated with A. A. Seif, Plant Pathologist, Kenya Department of Agriculture, and his colleagues. The aspects are: (1) The establishment, under glass, of a mosaic-free collection of cassava varieties of direct or indirect importance to East African agriculture; (2) Studies on the epidemiology of cassava mosaic in the field; and (3) The identification and characterization of cassava mosaic and cassava brown streak viruses.

The EAAFRO mosaic-free collection

At the conclusion of a 25-year cassava breeding program undertaken by EAAFRO, H. H. Storey selected approximately 90 lines which either were promising commercially or would be of outstanding importance in further breeding. When we surveyed cassava collections in East Africa in 1969–70, it was clear that much of this material was in danger of being lost, or of becoming totally infected with mosaic.

We therefore collected apparently healthy material of as many of the EAAFRO varieties as possible, whenever this was opportune, during visits to various field stations in East Africa. We also included material of the popular varieties of the different areas visited.

The reason for initiating the collection was both to safeguard the existence of what is obviously valuable breeding material, and also to enable EAAFRO to supply mosaic-free cuttings on request to agronomists and breeders.

While most of the work involved routine horticultural techniques, of interest is our observation that mosaic infection can on occasion remain latent through two propagation cycles. Material collected in the field as apparently free of mosaic remained so for two successive cycles of propagating by cuttings, only manifesting itself in the third cycle. One possible explanation is that we collected material of very recent infection, and that infection remained sub-clinical because of the comparatively rapid succession (2–3-month intervals) of propagating cycles.

Studies on the epidemiology of cassava mosaic

In our field studies we set out first to compare the rate of spread of mosaic within plots which spread into plots planted initially with mosaic-free material. We would stress that all this work has been of an observational, as distinct from a statistical, nature.

For studying the rate of spread within plots, seven centrally placed, mosaic-infected cuttings of cv. 46106/27 were surrounded by concentric hexagons of a total of 156 mosaic-free cuttings of the same variety, which is described as moderately tolerant, but not resistant, to mosaic (Plot 1). Plants which became infected during the experiment were not rogued. Spread from infected to healthy plants was rapid and at the time of harvest 14 months after planting, 84 of the 156 plants (54%) were infected.

In contrast, the amount of spread into an isolated (300 m from nearest cassava) mosaic-free plot (Plot 2) was very small. Here, 100 mosaic-free plants each of cv. 46106/27 and the highly susceptible F279 were planted in 10 alternate rows of 20 plants; plants were 1 m apart with 2 m between the double rows. Infected plants were rogued. At the time of harvest (14 months) only two of the 100 plants of 46106/27, and 15 of the F279 (15%) plants, had become infected. Of the latter, there is evidence that at least eight were infected before transplanting to the field; 7% infection of F279 for the 14-month period is possibly a more accurate figure.

These results suggest that satisfactory field control of mosaic might be achieved by the use of mosaic-free propagation material resistant cultivars planted in reasonable isolation, with rigorous roguing of infected plants.

In order to ensure that these results were not attributable to site ecology, four further plots, each similar to the second experiment (Plot 2) described above, were initiated in 1975 in areas of widely differing ecologies. These were:

Plot 3: on same site as plot 1, in open grass fields and with some degree of isolation from other infected cassava. Shortly after initiation of this trial several plots of cassava, in which incidence of mosaic was moderately high, were planted within 50 m of plot 3;

Plot 4: surrounded by cashew trees;

Plot 5: sheltered from the prevailing southeast monsoon wind by citrus, cashew, and coconut;

Plot 6: on a farm near Mtwapa Research Station, surrounded by widely spaced coconut palms.

Spread of mosaic into these plots was very slow and the disease did not build up at any time during the season. Results are summarized in Table 1.

TABLE 1. Incidence of mosaic in "mosaic-free" plots.

	46106/27	F279	
Plot 3	0/100	0/100	
Plot 4	2/100	5/100	
Plot 5	4/100	2/100	
Plot 6	0/100	2/100	
Total:	6/400	9/400	15/800
Percent:	1.5	2.3	1.9

Crop-loss assessment trial To estimate the effect of planting infected cuttings on yield of 46106/27 and F279, a line of 35 plants derived from infected cuttings was planted between two lines of 35 plants derived from mosaic-free cuttings. Rows were 2 m apart with 1.5 m between plants. The trial was established in May 1975 and lifted in February 1976 (at 10 months).

Analysis of variance showed the results to be highly significant in level of loss between the varieties: the drop in yield due to disease was greater for F279 than for 46106/27 (Table 2).

TABLE 2. Mean yield (untransformed data) per plant (in kilograms) of plants derived from infected and healthy cuttings.

	46106/27	F279
Healthy	3.86	3.67
Diseased	1.19	0.52
Percent loss	70	86

Identification of CMD and brown streak viruses

We have isolated by sap transmission from cassava to *Nicotiana clevelandii* two morphologically similar and serologically related but distinct viruses. In spite of repeated attempts, we have been unable to infect cassava with either; until this has been done their identity must remain open to question.

We have tentatively concluded that one of the two viruses is likely to be cassava brown streak (CBSV) for reasons which we now give, and we refer to it throughout the text as CBSV. The second virus is either a strain of CBSV, and the symptoms of brown streak are masked by cassava mosaic virus or infection in the cultivars studied is symptomless, or it is in fact cassava mosaic virus (CMV). We have some evidence to support the latter hypothesis, and refer to the virus in the text as CMV.

Particle morphology Purified preparations of both viruses contain numerous similar isometric particles, 20 nm in diameter, which are usually paired (30 × 20 nm). They are indistinguishable from particles of maize streak virus, but are apparently unrelated to MSV serologically.

Host range and symptomatology (Fig. 1–3) In 10 days CBSV induces in *Nicotiana clevelandii* a systemic vein clearing followed by an extremely severe systemic leaf crinkling and curling, with reduction in size of the leaves. Subsequently the leaves develop a fine necrotic vein etch. CBSV also infects *Petunia hybrida*, *Datura stramonium*, *N. glutinosa*, *N. rustica*, and *N. tabacum* (hosts also infected by Lister (1959) in his work on CBSV), *D. ferox*, *Solanum nigrum*, and *Salpiglossis sinuata*.

CMV induces systemic leaf curling and crinkling in *N. clevelandii* in 12–14 days, which is markedly less severe than that induced by CBSV. Leaf size is only moderately affected; coarse irregular yellow vein banding and yellow areas

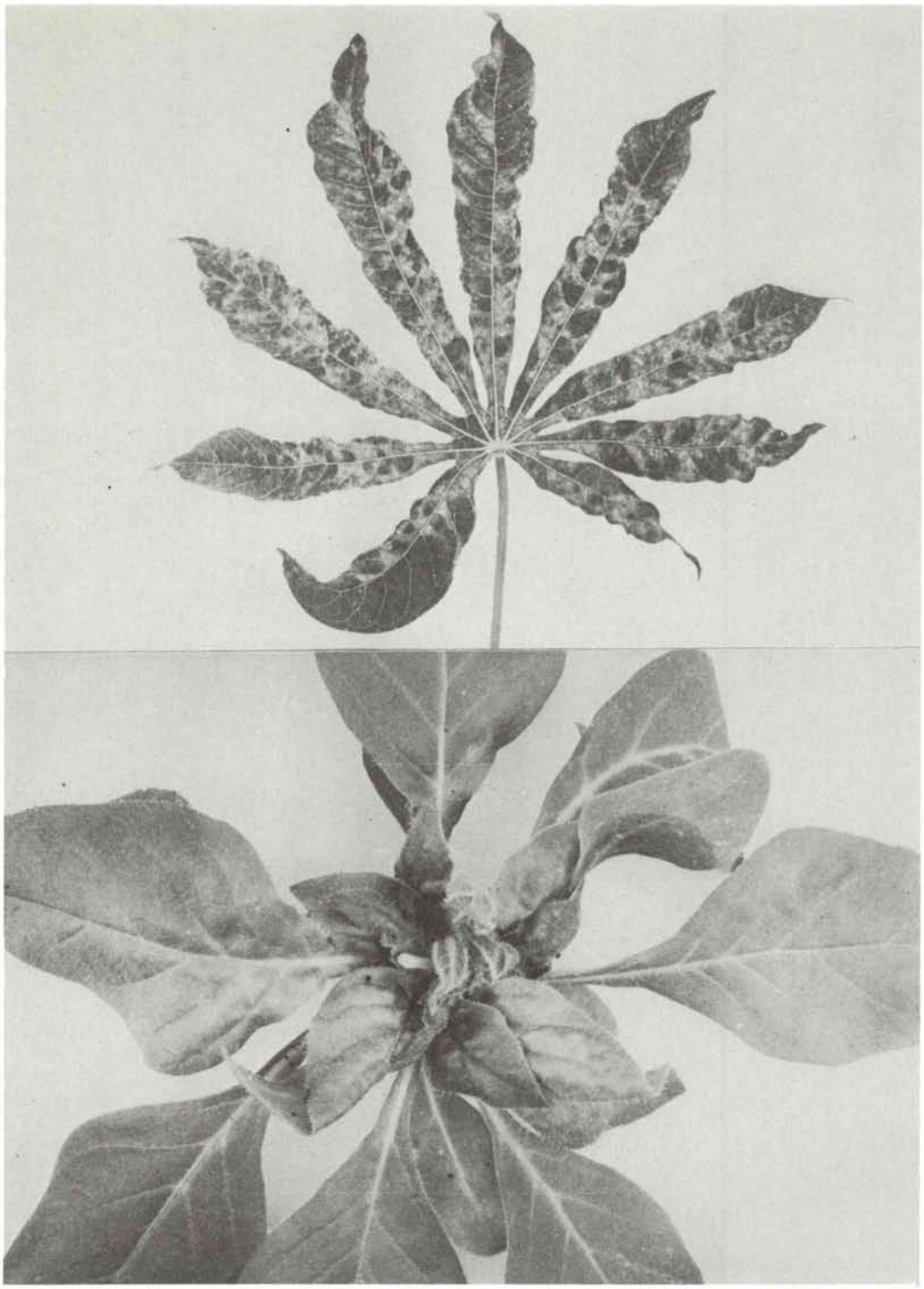

Fig. 1. Top Symptoms of African cassava mosaic in cassava; *Bottom Nicotiana clevelandii* systemically infected with a virus isolated from African cassava mosaic material.

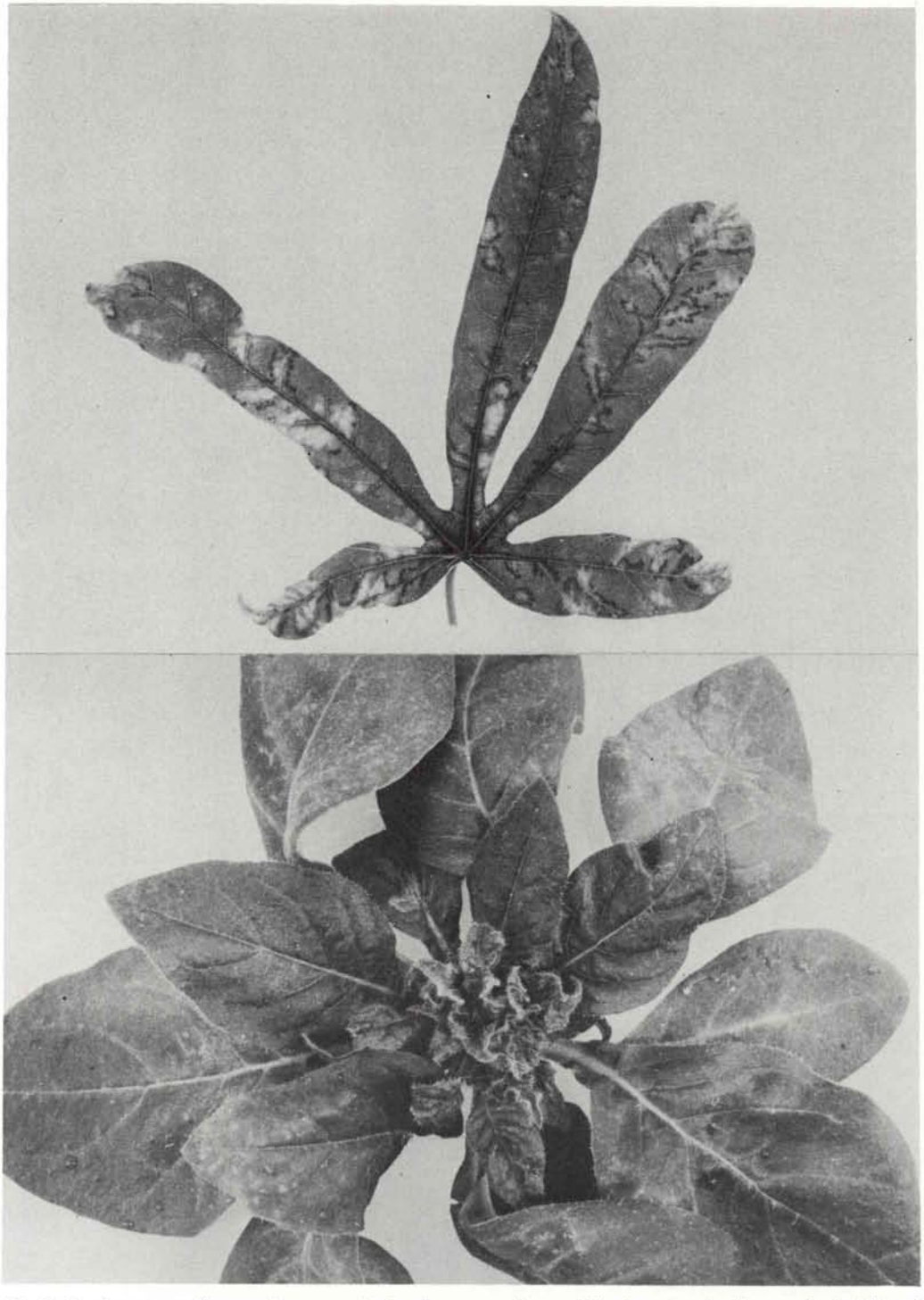

Fig. 2. Top Symptoms of cassava brown streak virus in cassava; *Bottom Nicotiana clevelandii* systemically infected with a virus isolated from cassava brown streak virus material.

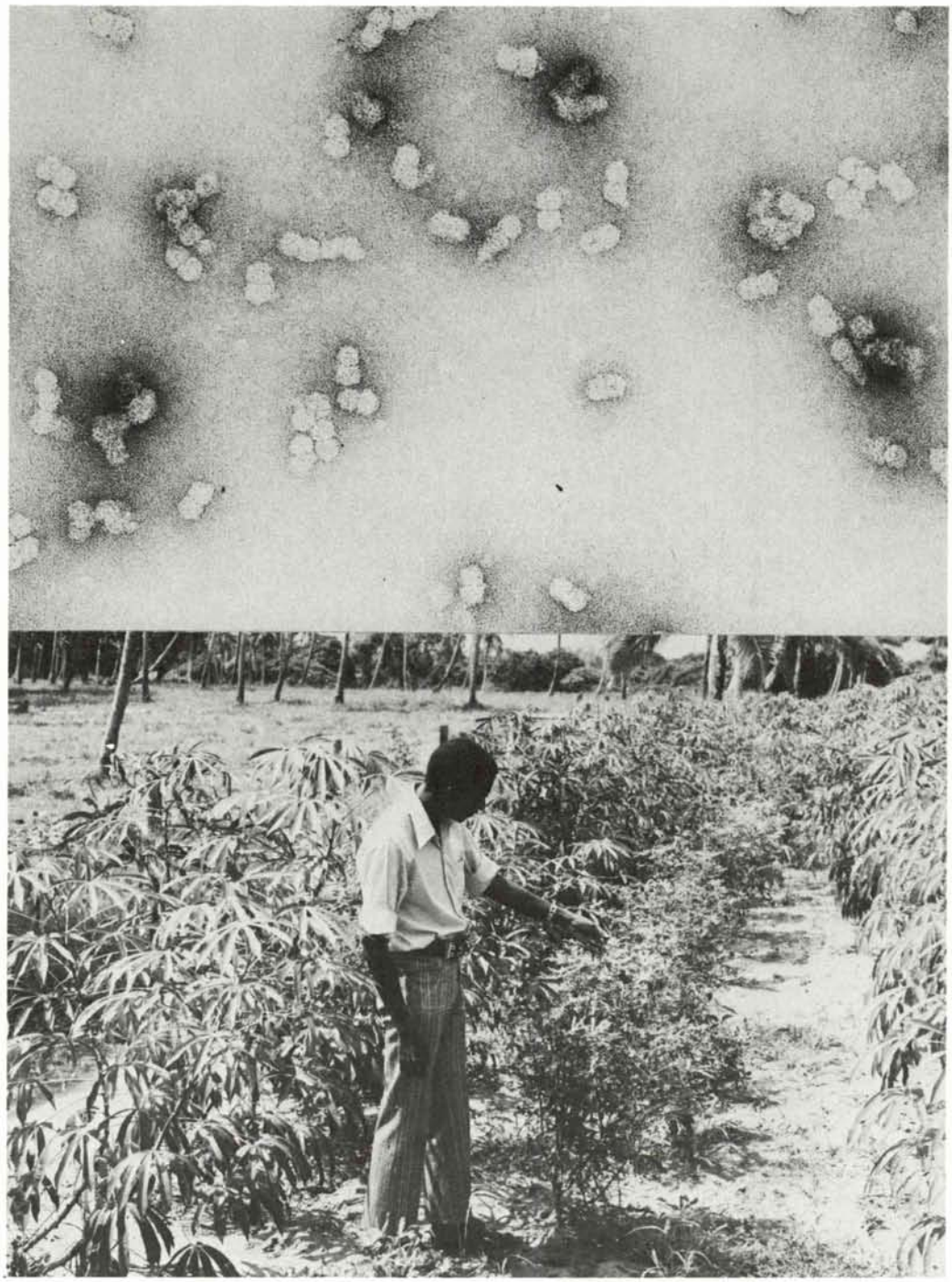

Fig. 3. Top Particles of the virus (approx. 19 × 33 nm) isolated from African cassava mosaic. The particles of brown streak are morphologically identical (Micrograph by Scottish Horticultural Research Institute, Invergowrie, Dundee, Scotland); *Bottom* Reaction of the highly susceptible variety F279 to cassava mosaic. Infected line at centre, healthy F279 to right and left.

develop and are typical of infection. Host range is essentially similar to that of CBSV, but we were unable to infect *S. nigrum* or *S. sinuata*.

Purification CMV is easily purified from systemically infected *N. clevelandii* by homogenizing leaves in 0.1 M borate buffer containing 1% mercapto-ethanol at pH 8.1, and clarifying with equal volumes of a 1:1 mixture of *n*-butanol and chloroform. For biochemical assays for RNA and protein entity determination, the pellet derived from the first ultracentrifugation of clarified extracts was resuspended in 0.005 M borate containing 0.005 M ethylenediaminetetraacetic acid and 0.2% formalin; pellets derived from the two subsequent ultracentrifugation cycles were resuspended in 0.005 M borate containing 0.005 M EDTA. Polyacrylamide gel electrophoresis studies by Dr H. Barker (Scottish Horticultural Research Institute) indicated that CMV, like maize streak virus, contains one species of protein and two of RNA (mol wt 34000, 1.7×10^6 and 1.3×10^6, respectively).

This method of purification did not prove satisfactory for antiserum preparation; an antiserum with a comparatively low titre (1/64 in agar gel diffusion tests) was prepared, however, and was used to test the relationship of CMV to CBSV. Purification methods suitable for CMV are unsuitable for CBSV, thus underlining the host reaction and serological differences between the two.

Serology We tested purified CBSV and CMV viruses against CMV antiserum. CMV reacted to a titre of 1/64, and there was spur formation between CBSV and CMV, suggesting that the viruses are closely related. The antiserum did not react with crude clarified sap of either CBSV or CMV.

Geographic origin of isolates and association with cassava virus symptoms We have isolated CMV from mosaic-infected material (seven isolates) collected from widely separated areas throughout East Africa (in Kenya, Uganda, and Tanzania), but we have not isolated the virus from CBSV-infected or apparently healthy material.

CBSV has been isolated from cassava showing classic CBSV symptoms but without mosaic (four isolates) and from plants with a dual infection of CBSV and CMV, all but one originating from coastal areas of Kenya and Tanzania. The exception (variety No. 20, from Ukiriguru, W. Tanzania) was collected by Dr D. L. Jennings as CBSV-infected material.

Summary

We have isolated two serologically related but distinct viruses from cassava infected with brown streak and with mosaic. Our working hypothesis, which is admittedly based at the moment on tenuous data, and which can only be verified by re-inoculation to cassava, is that one is cassava brown streak virus and the other mosaic virus.

References

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