

## VIRUS/VECTOR/PLANT RELATIONSHIPS

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### INTRODUCTION

The relationships between African Cassava Mosaic Virus (ACMV), the symptoms, the vector, and the host plant were investigated at the plant level. The concentration of virus was evaluated by the Elisa test, the intensity of the symptoms was recorded on a scale from 0 to 5, the size of the whitefly populations was estimated, and the growth of the cassava plant was taken into account by observing the size of the leaflets. The role of wild species as a reservoir of virus was also investigated.

### RESULTS

#### Cassava

##### - Localization of the virus in the leaves:

Leaves in stage 2, displaying symptoms of intensity 1 and 2, were sampled. The yellow and green parts of each leaf were detached separately and the extracts, sometimes clarified, were tested by the Elisa method to estimate their virus content. Without treatment with chloroform, ACMV was detected in the yellow parts of nine out of thirteen samples. The four other samples gave optical densities slightly higher than background level, but the significance of these results is uncertain. However, after clarification, the virus was then clearly detected in all the samples from yellow areas. The observed optical densities lay between the background reaction and 0.27 for the untreated extracts, and from 0.24 to 0.54 for the treated ones.

##### - Intensity of the symptoms and virus content:

A relationship exists between the intensity of the symptoms and the optical density of the unclarified extracts, the leaf having been sampled at stage 2. ACMV was present in most leaves with symptoms, even of class 1. The virus could also be sometimes detected, among those leaves which did not show external signs. There was a close relationship ( $r = 0.98$ )

between the intensity of the symptoms and the mean optical density of the samples of each class. Within each class, however, the observed optical density was very dispersed, and there was an important overlapping of classes.

### Evolution

The development of symptoms on the leaves, from the "apex" stage until the fall of the leaf, were observed on 25 diseased cassava plants, naturally infected in the field. The symptoms were clear and could be classified according to Cours's scale once the leaflets reached a mean length of 9 cm. Thereafter the symptoms did not change any further, though the leaf continued to grow. The correlation calculated between the intensity of the symptoms when first observed and then 20 days later reached 0.96. However, along a given stem, the intensity of the symptoms varied greatly, and two successive leaves might have intensities ranging from 0 to 4.

### Age of the leaf and virus content

The measurements of optical density were performed on the extracts from leaves sampled in the same conditions on a given stem:

- each of the first 12 leaves was tested separately.
- leaves from 1 to 4, then leaf 6, then every 4th leaf up to the 34th was also tested separately.

In all the plants the virus was detected in the extracts of the first three leaves (Leaf 1 = "first" leaf = stage 1: leaf having, on average, leaflet length of 12 cm). In three out of eight cases no virus was detected until the 4th leaf, and in one case not until the 6th. The highest optical density was obtained, in six out of eight cases, for leaf 1, in one case for leaf 2, and in another case for leaf 3. The level of virus was always highest in the younger leaves.

The growth of the leaf was estimated as a function of the length of the largest leaflet, a measurement closely related to the leaf's surface area. The maximum length was generally reached at stage 4 of the leaf, but sometimes also at stages 3 or 5. The most rapid rate of growth came between stages 1 and 2. With leaves before stage 1, we could sometimes detect the virus by the Elisa method, sometimes even with young leaves showing no apparent symptom of mosaic disease.

- Susceptibility to infection

The susceptibility of cassava leaves of various ages was estimated by Storey and Nichols. They placed groups of 100 whiteflies on leaves of increasing age and observed the number of plants showing symptoms afterwards. They observed that young, growing leaves were more susceptible to the disease than "adult" leaves, and that the oldest leaves were no longer susceptible.

## Identification of the vector

The great majority of puparia identified belonged to the species Bemisia tabaci. A few B. hancocki were sometimes seen.

## Distribution on the cassava plants

On 25 cassava plants we counted adult whiteflies and the number of larvae present on each leaf, from the apex to the oldest leaves. The count was made on plants aged 4, 5, or 6 months. The great majority of the whiteflies were found on growing leaves (numbers 2 and 3) and their number decreased with the age of the leaf. No whiteflies were observed on leaves 9 to 25. The distribution of the larvae followed the distribution of the adults, but shifted slightly.

## Virus-carrying ability

The virus-carrying power of the whiteflies in the fields of virus-infected cassava was evaluated periodically by collecting groups of 50 whiteflies and placing them on test plants. The percentage of whiteflies which transmitted the virus was always very low (0.45 on average), with the 90% confidence interval between 0.1 and 1.6%. The percentage varied little from one sample to another (from 0 to 1.7). The level of transmission observed depended also on the variety of plant used in the test; the highest level reached was observed with the susceptible variety H58. A very approximate upper-limit estimate of the level of virus-carrying flies was obtained, in the field, by comparing the size of the whitefly populations and the amount of contamination observed afterwards. This crude approach indicates that the virus-carrying power of the whiteflies present on cassava is, at the most, a few per cent.

## Adventitious plants

Several tens of plant species currently encountered in and around cassava fields were tested by Elisa. The presence of whiteflies was looked for. The results obtained were not unequivocal, given the presence of artefactual reactions with several species. All the same, the results of the Elisa tests, of the symptomatology, and the mechanical transmission test all agree and confirm what has already been published: two euphorbiaceae closely related to M. esculenta, M. glaziovii, and Jatropha multifida, and one Convolvulaceae, Hewitia sublobata, are reservoirs for ACMV. It appears, however, that the number of wild species that actually function as reservoirs is low and that their epidemiological role is limited, considering their limited diffusion and the small number of whiteflies that they harbour.

## DISCUSSION

A reservoir plays an effective role in the propagation of a pathogen, if the vector is present, if it acquires the pathogen and if it then inoculates other plants. The location of most B. tabaci on young, growing leaves favours at the same time the acquisition, the inoculation, and therefore the propagation of the

pathogen agent in the field, because of the high content of virus in young leaves and of their greater susceptibility to infection. This distribution of the vector and of the pathogen agent favours the spread of the disease. However, the virus-carrying power of the whiteflies present on cassava is much lower than that of other geminiviruses, particularly cowpea golden mosaic virus, where 70% of individuals are vectors.

In order to evaluate the actual role of a species as a source of infection, its range, the proportion of plants infected, their virus content, and the presence and abundance of the vector all must be taken into account. Two euphorbiacea, J. multifida and M. glaziovii, may well play a role in the epidemiology of the disease, because of their virus content and of the presence of B. tabaci, sometimes in large numbers. However, J. multifida is used as an ornamental plant in urban environments and its role as a source of infection in the field seems doubtful. On the contrary, M. glaziovii is present in the vegetation, although sparsely. We monitored the recontamination by B. tabaci of fields planted with healthy cassava cuttings, which was bordered at several points by groups of diseased M. glaziovii harbouring whiteflies. No focus of infection from these wild cassavas was observed. The absence of propagation of the disease from these glaziovii indicates that the role of M. glaziovii in ACMV field spread is probably very limited.

The virus content, the presence of the vector, and the large areas under cultivation make M. esculenta the principal reservoir of virus all year round and the most dangerous potential source of infection by whiteflies, especially in regions of heavy cassava culture. To assess the extent of transmission by whiteflies, it is necessary to investigate the recontamination of fields planted with healthy cuttings. This has been done, at the field and region levels, and is included in the communications which follow.

Fargette Denis, Fauquet Claude, Thouvenel Jean-Claude.  
(1987)

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In : African cassava mosaic disease and its control.

Wageningen : CTA, 105-108. International Seminar on African  
Cassava Mosaic Disease and its Control

Yamoussoukro (CIV), 1987/05/04-08