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Yam mosaic, a new potyvirus infecting *Dioscorea cayenensis* in the Ivory Coast

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SUMMARY

The major disease affecting *Dioscorea cayenensis* in the Ivory Coast is caused by a virus which was transmitted by mechanical inoculation to some *Dioscorea* spp. and *Nicotiana benthamiana*. In extracts of *D. cayenensis* leaves infectivity was lost after 10 min at 60 °C but not 55 °C and after dilution to 10^{-3} but not 10^{-2}. A purification procedure is described. The virus particles are flexuous filaments *c.* 785 nm long. The virus was transmitted by four aphid species in the non-persistent manner, and is serologically related to four African potyviruses. The name yam mosaic virus is proposed; the present cryptogram is: */*:*/6:E/E:S/Ve/Ap, potyvirus group.

INTRODUCTION

Yams are staple food crops widely cultivated in tropical areas. In the Ivory Coast, production of tubers was 1 880 000 t in 1975, and is planned to reach 2 170 000 t in 1980 and 2 550 000 t in 1985. Yam plants are relatively resistant to pests and diseases when compared with many other tropical crops; nevertheless serious virus diseases have been reported, mainly from West Africa and Puerto Rico (Coursey, 1967) and from the Caribbean (Harrison & Roberts, 1973; Migliori & Cadilhac, 1976). Miège (1957) reported symptoms, mainly stunting of plants and mosaic on leaves, on *Dioscorea cayenensis* and *D. alata* growing in many localities of the Ivory Coast. Recently, the most common disease was shown to be caused by a virus, which was named yam mosaic virus (YMV) (Thouvenel & Fauquet, 1977). This paper describes the transmission, host range, purification and some properties of YMV.

MATERIALS AND METHODS

Unless otherwise stated, methods of growing test plants, studying *in vitro* properties, electron microscopy, and serology were as described previously (Thouvenel, Dollet & Fauquet, 1976).

The original inoculum consisted of naturally infected leaves collected from an infected *Dioscorea cayenensis* plant growing at Adiopodoume near Abidjan. For mechanical transmission, fresh leaves of yam were ground in 0·1 M potassium phosphate buffer, pH 7·1 (10 ml/g of tissue) containing 0·02 M cysteine hydrochloride, 2·5 mg/ml bentonite and 5 mg/ml activated charcoal. Young seedlings of *Dioscorea* spp. were used as test plants. Isoelectric-focusing of virus particles was done as follows: an LKB column was loaded with 110 ml ampholyte (to give a gradient of pH 3·5–10) and about 1 mg of purified virus, and a potential difference of 300 V applied for 72 h to establish the pH gradient. The pH and optical density of the column contents were recorded with the flow cell of a Tacussel ISIS 20 000 pH-meter and the 3 mm flow cell of a LKB Uvicord absorptiometer.

The molecular weight of the capsid polypeptide was determined by electrophoresis in 7·5, 10

and 12·5% polyacrylamide-SDS gels, using the procedure of Weber & Osborn (1969). Phosphoglycerate kinase (mol. wt 47 000), alcohol dehydrogenase (35 000), carbonic anhydrase (31 000), trypsin inhibitor from soybean (21 000) and lysozyme (14 300) were used as standards.

RESULTS

Host range and symptomatology

Dioscorea cayenensis was easily infected by mechanical inoculation with sap from diseased *D. cayenensis*, developing in 4 to 6 wk systemic symptoms that are similar to those observed in the field. The symptoms were apt to differ considerably in different leaves of one plant: mosaic, vein-banding, green spotting or flecking, curling and mottling (Plate, figs 1, 2, 3, 4, 5). Similar symptoms appeared in inoculated *D. liebrechtsiana*, *D. praehensilis* and *D. preussii* plants; but *D. bulbifera*, *D. composita* and *D. floribunda* were not infected by mechanical inoculation, no symptoms occurred and no virus detected by back inoculation to *D. cayenensis*.

Similar isolates were likewise transmitted from naturally infected *D. alata* to *D. cayenensis*.

Nicotiana benthamiana was the only host found in families other than the Dioscoreaceae; symptoms are vein-clearing, followed by systemic mottle.

No virus was detected by inoculating sap from inoculated and non-inoculated leaves of the following species to *D. cayenensis*:

Aizoaceae: *Tetragonia expansa*; Amaranthaceae: *Gomphrena globosa*; Apocynaceae: *Vinca rosea*; Chenopodiaceae: *Beta vulgaris*, *Chenopodium amaranticolor*, *Chenopodium quinoa*; Cruciferae: *Brassica oleracea*; Cucurbitaceae: *Cucumis sativus*, *Cucurbita pepo*; Malvaceae: *Hibiscus esculentus*; Leguminosae: *Crotalaria striata*, *Phaseolus vulgaris*, *Pisum sativum*, *Vigna unguiculata*; Solanaceae: *Capsicum annuum*, *Capsicum frutescens*, *Datura stramonium*, *Nicotiana clevelandii*, *N. glutinosa*, *N. megalosiphon*, *N. rustica*, *N. suaveolens*, *N. tabacum* 'Samsun', *N. tabacum* 'Xanthi-nc', *Petunia hybrida*, *Physalis floridana*.

Tuber and seed transmission

Fifty tubers produced by diseased *D. cayenensis* plants, planted in a sterilised soil in an insect-free glasshouse, all gave diseased yam plants.

Symptoms of YMV were detected in none of *c.* 2700 plants grown from seed collected from naturally infected *D. cayenensis*.

Properties in sap

Crude sap from diseased *D. cayenensis* leaves was not infective, so properties of YMV were studied using leaf extracts obtained as described in Methods, with phosphate buffer containing cysteine hydrochloride, bentonite and activated charcoal (10 ml buffer/1 g leaf = dilution 10^{-1}). Yam seedlings inoculated with leaf extracts diluted in water up to 10^{-2} but not 10^{-3} became systemically infected. The extracts were infective after heating for 10 min at 55 °C but not at 60 °C. Sap stored at 25 °C remained infective for 12 but not 24 h, and when stored at 4 °C it lost its infectivity after 40 days. Extracts frozen for 150 days were still infective.

Purification

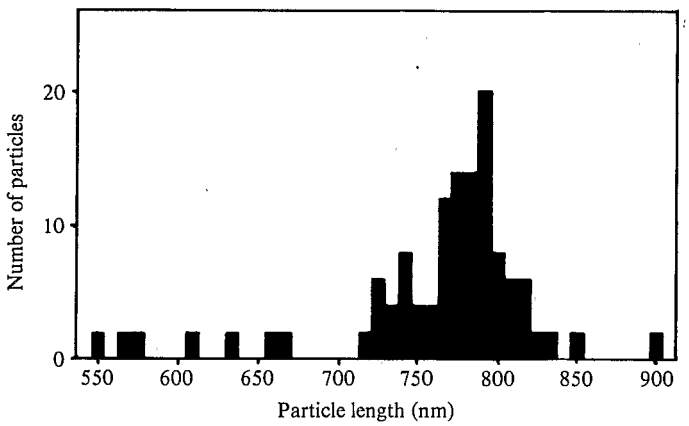
The presence of mucilaginous substances in yam leaves made the purification of the virus difficult. Good results were obtained by the following method. Fresh leaves of diseased *D. cayenensis* were ground (1 g/4 to 5 ml) with 0·2 M potassium phosphate buffer (pH 8) containing 0·4% mercaptoethanol for 3 min in a Waring Blender, cold chloroform (2 ml/1 g leaf) was added and the blender run again for 1 min. The extract was centrifuged for at least 10 min at 6000 *g*. The pellets were discarded and ammonium sulphate was added to the supernatant fluid (20 g/100

ml). The mixture was left 30 min at 4 °C, centrifuged for 10 min at 10 000 *g* and the supernatant fluid discarded. The pellets were resuspended in water (1/10 of the initial volume). After removal of the insoluble material by centrifugation at 8000 *g*, the virus was sedimented by ultracentrifugation at 78 000 *g* for 135 min. It was then resuspended overnight in 0·05 M borate buffer (pH 8) (2% of the initial volume), and the suspension clarified by centrifugation for 10 min at 10 000 *g*. The virus was then sedimented through a sucrose density gradient column (10–40% sucrose in 0·05 M potassium borate buffer, pH 8) for 3 h at 27 000 rev/min in a Beckman SW 27 rotor. At the end of the centrifugation, a single opalescent band was apparent in the middle of the column. The band was removed, diluted three-fold in 0·05 M potassium borate buffer, pH 8, and concentrated by sedimentation at 78 000 *g* for 150 min.

Taking $A_{260}^{0·1}$; 1 cm = 3·0, an extinction coefficient close to that found for morphologically similar viruses, virus yields were estimated to be 15–25 mg/kg leaf.

Properties of purified preparations

Ultraviolet absorption. The u.v. absorption spectrum of purified virus preparations was characteristic of that of a nucleoprotein and showed a maximum at 262 and a minimum at 247 nm. The $A_{262/247}$ ratio was 1·13 ± 0·01, and the $A_{260/280}$ ratio was 1·20 ± 0·01, indicating a nucleic acid content of *c.* 6% (Layne, 1954).



Text-fig. 1. Length distribution of YMV particles from a purified virus suspension observed by electron microscopy.

Particle morphology. Purified virus suspensions were negatively stained with 1% uranyl acetate, at pH 7. Particles of YMV appeared in the electron microscope as flexuous filaments of about 13 nm diameter (Plate, fig. 7). Of 130 particles measured, 60 were 785 ± 15 nm long (Text-fig. 1).

Behaviour in isoelectric focusing. When submitted to electro-focusing, the virus particles migrated to form a single band at pH 4·3 ± 0·3. Some of the particles were aggregated, but the virus remained infective after isoelectric focusing.

Molecular weight of coat protein. Preparations of YMV protein gave a major component with estimated mol. wt 34 000, and two minor bands with mol. wts 32 000 and 29 000.

Serological properties. Serological studies were performed using the microprecipitin technique under paraffin oil. As crude chloroform clarified extracts of healthy *D. cayenensis* leaves produce abundant precipitates caused by the presence of mucilaginous substances, purified suspensions of YMV with OD_{260} of 0·1 or 0·03 were used for the tests. The antiserum produced to YMV had a homologous titre of 1/2048. A slight reaction up to an antiserum dilution of 1/4 was produced with normal plant proteins prepared with healthy leaves of *D. cayenensis* following

the purification method. A positive reaction was obtained between this antiserum and the following purified viruses at $OD_{260} = 0{\cdot}1$: groundnut eyespot (Dubern & Dollet, 1978) up to an antiserum dilution of 1/32, and pepper veinal mottle up to a dilution of 1/32. A reaction occurred between YMV and antisera to the following viruses (homologous titres in parentheses): guinea-grass mosaic strain A (1/1024) up to an antiserum dilution of 1/128, groundnut eyespot (1/2048) up to a dilution of 1/64, and passionfruit ringspot (1/4096) up to a dilution of 1/16.

Transmission by aphids

YMV was transmitted from diseased *D. cayenensis* to healthy D. cayenensis and *D. preussii* seedlings, in the non-persistent manner, by all four aphid species tested. A period of 5 min was sufficient for the acquisition of the virus, and a time of less than 15 min for the inoculation access period. Using 10 aphids per test plant, it was transmitted by *Aphis gossypii* to 38 of 92 plants, by *Toxoptera citricidus* (21/30), by *Aphis craccivora* (13/24) and by *Rhopalosiphum maidis* (1/20). It was not transmitted by *Aphis gossypii* from *D. cayenensis* to *D. bulbifera* (0/20), *D. composita* (0/24) or *D. floribunda* (0/24) seedlings.

Attempt at heat therapy

The effect of heat treatment was tested by exposing tuber fragments (weight ranging from 50 to 150 g) from diseased *D. cayenensis* for 15 to 60 min at 45 to 60 °C, in hot-water bath. The treatment greatly decreased the sprouting ability of the tubers, but all the tuber fragments that grow produced diseased plants.

DISCUSSION

The mosaic of *D. cayenensis* in the Ivory Coast is caused by a virus with flexuous particles 785 nm long, and mechanically transmissible to some species of *Dioscorea* and to *Nicotiana benthamiana*. The virus is transmitted by aphids in the non-persistent manner and is serologically related to the potyviruses like pepper veinal mottle, guinea-grass mosaic and groundnut eyespot viruses. These properties place YMV in the potyvirus group.

Some of the viruses reported from yams in other countries have similar particle morphology. Of these, dioscorea green-banding virus (DGBV), found in *D. floribunda* in Puerto Rico (Ruppel, Delpin & Martin, 1966; Lawson, Hearon, Smith & Kahn, 1973), is mechanically transmissible to *Crotalaria striata* and *Nicotiana glutinosa*, whereas YMV is not. Moreover, YMV did not infect *D. composita* and *D. floribunda* which are natural hosts of DGBV. Other viruses with filamentous particles are recorded in the Caribbean area on *D. alata*, *D. trifida*, *D. cayenensis*, *D. esculenta* and *D. rotundata* (Mohamed & Mantell, 1976; Migliori & Cadilhac, 1976) but there is little information on these viruses. There is also a report from Nigeria on a disease of *D. rotundata* associated with a mechanically and aphid transmissible virus that has properties in leaf extracts similar to those of YMV (Terry, 1976), but no further information about it is available.

We therefore consider the Ivory Coast isolates to be of a previously uncharacterised virus, which we name yam mosaic virus, and which has the cryptogram */*:*/(6):E/E:S/Ve/Ap, potyvirus group.

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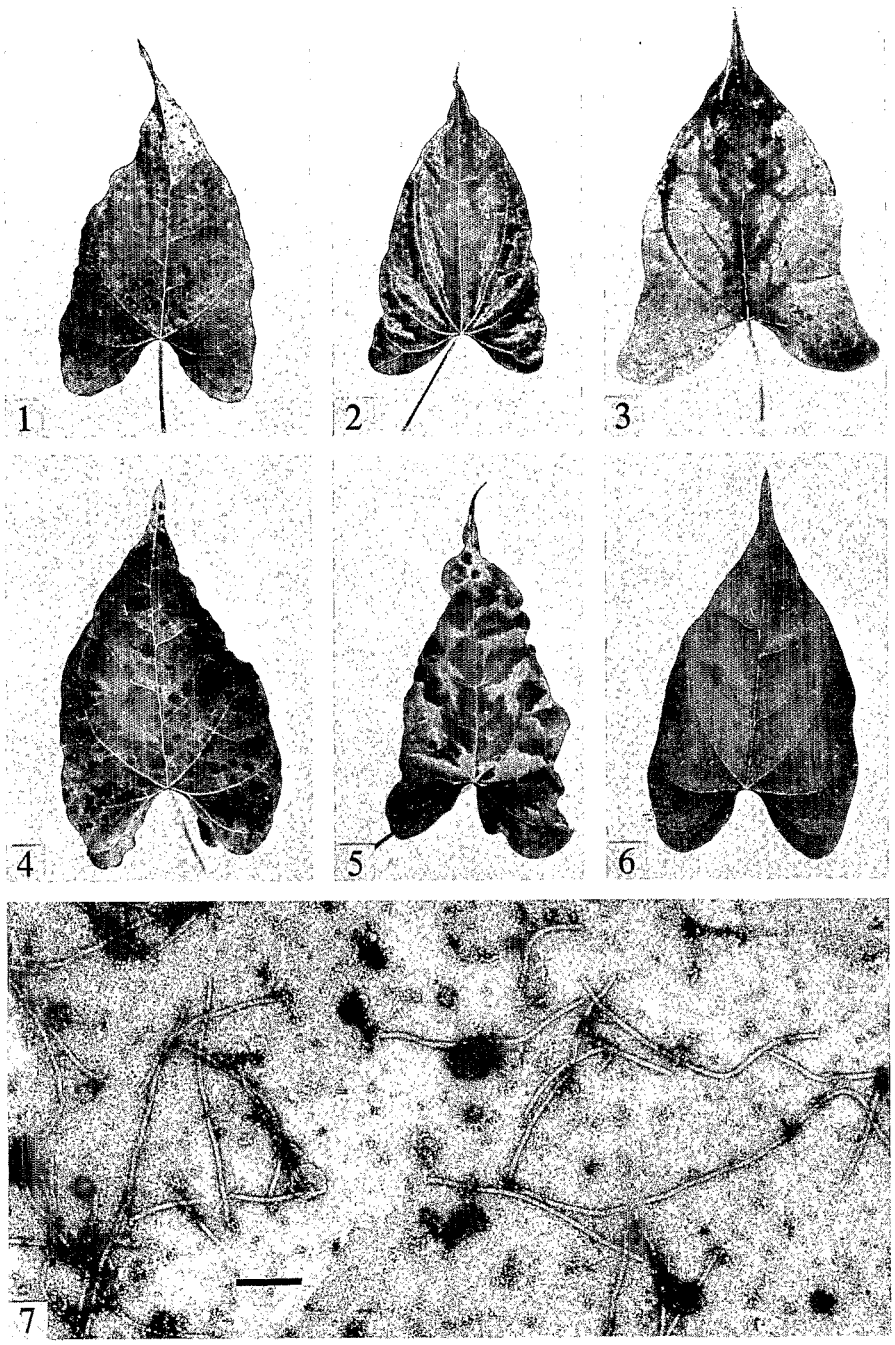
REFERENCES

COURSEY, D. G. (1967). *Yams*. London: Longmans, 230 pp.

DUBERN, J. & DOLLET, M. (1978). Observation of a new virus disease in the Ivory Coast: groundnut eyespot disease. *Oléagineux* **33**, 175–177.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HARRISON, B. D. & ROBERTS, I. M. (1973). Association of virus-like particles with brown spot of yam (*D. alata*). *Tropical Agriculture, Trinidad* **50**, 335–340.

LAWSON, R. H., HEARON, S. S., SMITH, F. F. & KAHN, R. P. (1973). Electron microscopy and separation of viruses in *D. floribunda*. *Phytopathology* **63**, 1435.

LAYNE, E. (1954). Spectrophotometric and turbidimetric methods for measuring proteins. In *Methods in Enzymology*. [*Ed. S. P. Colowick & N. O. Kaplan*], **3**, 447–454. New York: Academic Press.

MIÈGE, J. (1957). Influence de quelques caractères des tubercules semences sur la levée et le rendement des ignames cultivées. *Journal d'Agriculture Tropicale et de Botanique Appliquée* **4**, 315–342.

MIGLIORI, A. & CADILHAC, B. (1976). Contribution à l'étude de la maladie à virus de l'igname *Dioscorea trifida* en Guadeloupe. *Annales de Phytopathologie* **8**, 73–78.

MOHAMED, N. A. & MANTELL, S. H. (1976). Incidence of virus symptoms in yam (*Dioscorea* sp.) foliage in the Commonwealth Caribbean. *Tropical Agriculture, Trinidad* **53**, 255–261.

RUPPEL, E. R., DELPHIN, H. & MARTIN, F. W. (1966). Preliminary studies on a virus disease of sapogenin producing *Dioscorea* species in Puerto Rico. *Journal of Agriculture of the University of Puerto Rico* **50**, 151–157.

TERRY, E. R. (1976). Incidence, symptomatology, and transmission of a yam virus in Nigeria. In *Proceedings of the 4th Symposium of the International Society for Tropical Root Crops. Cali, Columbia, August 1976* (Ed. J. Cock, R. McIntyre & M. Graham).

THOUVENEL, J.-C., DOLLET, M. & FAUQUET, C. (1976). Some properties of peanut clump, a newly discovered virus. *Annals of Applied Biology* **84**, 311–320.

THOUVENEL, J.-C. & FAUQUET, C. (1977). Une mosaïque de l'igname (*Dioscorea cayenensis*) causée par un virus filamenteux en Côte d'Ivoire. *Comptes rendus hebdomadaires des séances de l'Académie des sciences* **284**, 1947–1949.

WEBER, K. & OSBORN, M. (1969). The reliability of molecular weight determination by SDS-polyacrylamide gel electrophoresis. *Journal of Biological Chemistry* **244**, 4406–4412.

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EXPLANATION OF PLATE

Figs 1, 2, 3, 4, 5. Different types of symptoms in leaves of infected *Dioscorea cayenensis*.

Fig. 6. Healthy leaf of *D. cayenensis*.

Fig. 7. Electron micrograph of purified YMV, stained with 1% uranyl acetate, at pH 7. Micrograph by P. Pfeiffer. Bar represents 200 nm.