

Properties of a Virus Causing Severe Mosaic of *Cucumeropsis edulis* in Nigeria

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ABSTRACT

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A virus causing leaf mosaic, blisters, puckering, malformation, and flower abortion on *Cucumeropsis edulis* in Nigeria induced chlorotic local lesions without systemic spread in *Chenopodium amaranticolor* and *C. quinoa* and incited systemic symptoms in cantaloup, cucumber, pumpkin, squash, and watermelon. *Luffa acutangula*, *Nicotiana benthamiana*, *Pisum sativum* 'Alaska,' and *Phaseolus vulgaris* 'Bountiful' were immune to the virus. The virus had a thermal inactivation point between 45 and 50 C, longevity in vitro between 4 and 5 days, dilution end point between 10^{-3} and 10^{-4} , and flexuous-rod morphology and was readily transmitted by the green peach aphid, *Myzus persicae*. The virus failed to react with antisera to muskmelon necrotic spot virus, watermelon mosaic virus-1, watermelon mosaic virus-2, or a Moroccan isolate of watermelon mosaic virus.

Egusi (*Colocynthis citrullus* L.) is an important, widely cultivated cucurbitaceous vegetable in West Africa, including Nigeria, where the kernel is used for soup (8). The kernel is also rich in oil (5,8). In 1966, an estimated 73,000 tons of egusi were grown in Nigeria (4).

In parts of Nigeria, especially around Nsukka, ahu (*Cucumeropsis edulis* L.), a close relative of egusi, is cultivated instead of egusi. Egusi is a trailing plant, whereas ahu is a climber (*C. Oyolu, personal communication*).

In 1978, I observed viruslike symptoms on *C. edulis* plants on several farms in and around Nsukka. Diseased plants were severely stunted with leaves showing green mosaic, severe puckering, and blisters. Diseased plants also showed severe flower abortion and produced no fruit or a few distorted small fruits that were usually seedless. This paper describes studies to determine the causal agent of the disease.

MATERIALS AND METHODS

Inoculum was leaf tissue from naturally infected *Cucumeropsis* plants collected from several locations and ground in a mortar containing 0.05 M potassium phosphate buffer, pH 8.0, and filtered through double layers of cheesecloth. Test plants grown in steam-sterilized soil in the greenhouse were dusted with Carborundum and rubbed with a cheesecloth pad dipped in the

inoculum. Plants were rinsed with tap water immediately after inoculation. Inoculated plants were kept in the greenhouse and observed for at least 4 wk. Each of 10 isolates of the *Cucumeropsis* virus was given three serial local-lesion passages in *Chenopodium quinoa* L. The isolates were maintained and assayed by sap inoculations in *Cucumis sativus* L. 'Supermarket' or in *C. edulis*. Back-inoculations were made about 4 wk after inoculation to Supermarket cucumber to detect symptomless hosts.

In vitro properties were determined using a crude leaf extract of Supermarket cucumber (2-3 wk after inoculation) in 0.05 M potassium phosphate buffer, pH 8.0 (11). Five Supermarket cucumber seedlings in the two- to three-leaf stage were inoculated per treatment, and experiments were repeated three times.

Aphid transmission of the *Cucumeropsis* virus was determined using virus-free apterous adult green peach aphids (*Myzus persicae* Sulzer). Insects reared on healthy eggplants (*Solanum melongena* L.) were brushed into a petri dish, starved for 60-90 min, and given an acquisition access period of 1-5 min on infected Supermarket cucumber or *Cucurbita pepo* L. 'Small Sugar' plants. Insects were then transferred to Supermarket cucumbers in the two- to three-leaf stage and allowed to feed for 24 hr. Five insects were used per test plant, and plants were sprayed with an aphicide to terminate inoculation feeding.

Immunodiffusion tests were done in plates containing 0.5% sodium dodecyl sulfate, 1.0% sodium azide, and 0.6% agarose dissolved in distilled water (6). Crude antigens were obtained from infected leaves of Supermarket cucumber,

Cucurbita maxima Duch. 'Emerald,' or Small Sugar pumpkin according to the methods described by Purcifull and Hiebert (10).

For electron microscopy, leaves from experimentally infected Supermarket cucumber were ground or chopped on a glass microscope slide containing 2% phosphotungstic acid (PTA), pH 6.8, and a drop of the extract was placed on Formvar-coated grids. Leaf-dip preparations were made in 1% PTA, pH 6.8.

RESULTS

The *Cucumeropsis* virus was readily sap-transmissible from naturally infected *Cucumeropsis* plants to several plants belonging to the Chenopodiaceae and Cucurbitaceae. The single-lesion isolates used in this study produced symptoms in *C. edulis* identical to those in naturally infected plants, indicating that a single virus was involved.

The virus induced chlorotic local lesions and chlorotic ring spots on inoculated leaves of *Chenopodium quinoa* and *C. amaranticolor*, respectively, but the reactions were too erratic for infectivity assays. The virus systemically infected the following cucurbits: *C. edulis*; *Cucumis sativus* 'Marketer,' 'Supermarket,' 'Mini-Cute,' 'Straight Eight,' 'National Pickling,' 'Improved Long Green,' and 'Chicago Pickling'; *Cucumis melo* L. 'Delicious 51'; *Citrullus vulgaris* Schrad. 'Kleckleys Sweet'; *Cucurbita pepo* L. 'Small Sugar'; and *Cucurbita maxima* 'Emerald.' The virus induced mosaic and leaf puckering in Mini-Cute cucumber and severe stunting and mild mottle in Delicious 51 cantaloup. Symptoms induced in all other cucurbits tested included severe stunting, severe leaf deformation, faint light to dark green or yellow mosaic, leaf puckering, and small, distorted fruits (Figs. 1 and 2). In addition, the virus induced leaf necrosis in Kleckley's Sweet watermelon and chlorotic spots in Emerald squash.

Plants immune to the virus were Amaranthaceae: *Gomphrena globosa* L.; Apocynaceae: *Vinca rosea* L.; Chenopodiaceae: *Spinacia oleracea* L.; Cucurbitaceae: *Cucumis melo*, an unidentified variety from the Ivory Coast, *Luffa acutangula* Roxb., *Telfairia occidentalis* L., *Colocynthis citrullus*, and *Cucurbita pepo* 'Local'; Euphorbiaceae: *Ricinus communis* L.; Legum-

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inosae: *Phaseolus vulgaris* L. 'Black Turtle-1,' 'Black Turtle-2,' 'Bountiful,' 'Top Crop,' and 'Pinto,' *P. mungo* L., *P. lunatus* L. 'Henderson Bush,' *P. lathyroides* L., *Pisum sativum* L. 'Ranger,' 'Alaska,' and 'Bonneville,' *Vigna unguiculata* (L.) Walp. 'Blackeye,' *Crotalaria* sp., *Crotalaria juncea* L., *Calopogonium mucunoides* L., *Canavalia ensiformis* DC., *Glycine max* (L.) Merr. 'Jupiter' and TGM '280-3'; Malvaceae: *Abelmoschus esculentus* (L.) Moench; Pedaliaceae: *Sesamum indicum* L.; Solanaceae: *Nicotiana benthamiana* Domin., *N. tabacum* L. 'Havana 425,' 'Burley 21,' 'NC 95' and 'Xanthi-nc,' *N. glutinosa*, *Lycopersicon esculentum* Mill. 'Roma,' 'Bonny Best' and 'Ife No. 1,' *Solanum melongena* 'Local' and 'New York,' *S. nigrum*, *Physalis floridana* Rydb., and *Capsicum annum* L. 'California Wonder' and 'Nsukka Yellow.'

The *Cucumeropsis* virus was transmitted efficiently by *M. persicae*. In one representative test, the virus was transmitted from Small Sugar pumpkin inoculated 1 wk earlier to five of five healthy Supermarket cucumber seedlings. Typical symptoms appeared within 1 wk of inoculation.

Crude sap from leaves of infected Supermarket cucumber, Small Sugar pumpkin, or *Cucurbita maxima* failed to react with antisera to muskmelon necrotic spot virus, watermelon mosaic virus-1 (WMV-1), 2 (WMV-2), or a Moroccan isolate of WMV (WMV-Mo). In a comparable test, each of these viruses

reacted with its homologous antiserum. There was no reaction between these antisera and extracts from healthy cucurbits.

Electron microscopy of leaf dips or diluted plant extracts revealed flexuous rod-shaped particles with a modal length of about 740 nm.

Sap from infected Supermarket cucumber remained infectious when diluted 10^{-3} but not at 10^{-4} , stored at 20–22 C for 4 but not 5 days, and heated at 45 C for 10 min but not at 50 C.

DISCUSSION

The only virus reported to date on cucurbitaceous plants in Nigeria is telfairia mosaic virus (TeMV), which is not aphid transmissible (7). Because the *Cucumeropsis* virus was aphid transmitted and did not infect telfairia (*T. occidentalis*), it is not TeMV.

Host range, symptomatology, particle morphology, and aphid transmissibility indicate that the virus is WMV. Two serotypes of WMV are recognized (1,9,12): WMV-1, with a host range restricted to cucurbits, and WMV-2, which infects both cucurbits and plants of other families. Recently, the existence of a third type of WMV was suggested by Purcifull and Hiebert (10), based on results of reactions of four differential hosts and serological tests involving WMV-1 FL (Florida isolate of WMV-1), WMV-2 FL, and WMV-2 Mo (Moroccan isolate of WMV-2). These three WMV isolates induced symptoms on Small Sugar pumpkin, the only differential host infected by WMV-2 Mo; however, WMV-1 FL also infected *Luffa acutangula* but not Alaska pea and *Nicotiana benthamiana*. Conversely, WMV-2 FL infected the two latter plant

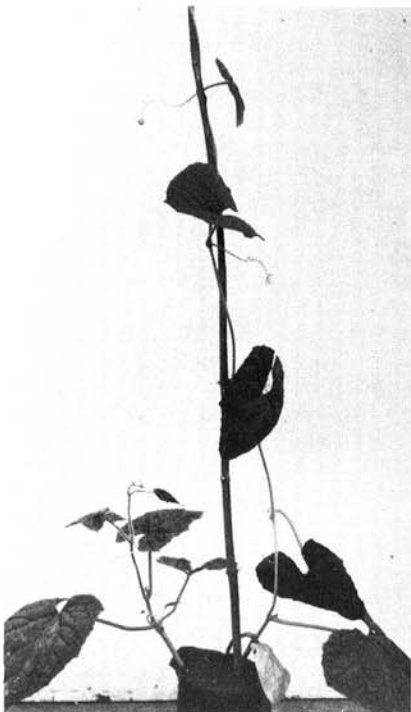


Fig. 1. Growth reduction in a *Cucumeropsis edulis* plant 3 wk after inoculation with a Nigerian isolate of a watermelon mosaic-like virus; healthy plant on right.

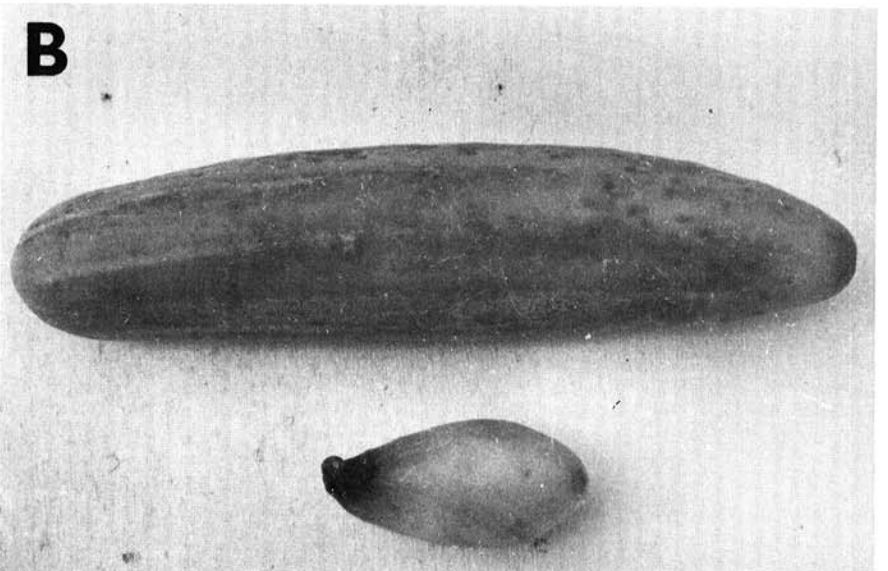
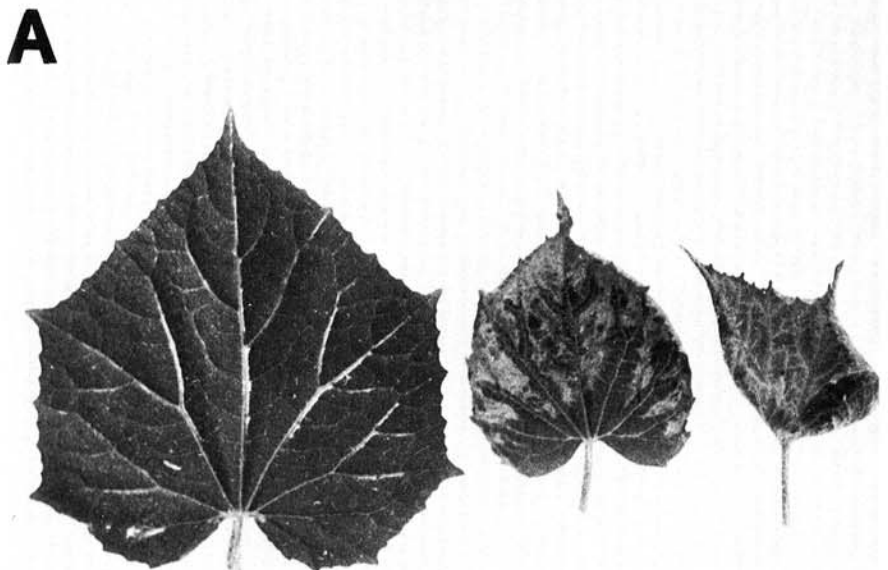


Fig. 2. Symptoms induced by a Nigerian isolate of a watermelon mosaic-like virus in Supermarket cucumber. (A) Wide green veinbanding and leaf distortion (two leaves on right) and a healthy inoculated leaf (left). (B) Small deformed fruit (below) from an infected plant and a normal fruit (above) from a healthy plant.

species but not the former.

The Nigerian virus differs from WMV-1 in host range and serological properties. The host range and physical properties of the virus indicate that it may be a strain of WMV-2; however, it did not react with antiserum to WMV-2 FL or WMV-Mo. Purcifull and Hiebert (10) and Baum et al (2) reported that WMV-2 Mo did not react with antiserum to WMV-2 FL. The failure of the Nigerian virus to react with antiserum of WMV-Mo is of interest because both infected Small Sugar pumpkin but neither infected *L. acutangula*, Alaska pea, or *N. benthamiana* nor reacted with WMV-2 FL antiserum. Our results and those of others (2,3,10) emphasize the need for an international cooperative research effort to resolve the problem of variability of WMV strains.

Because the Nigerian virus caused severe flower abortion in field-grown *C. edulis* and infected plants produced only a few small distorted seedless fruits, it is of major economic importance to

growers. Because the virus is readily transmitted by aphids and the disease is widespread, breeding for resistance appears to be the most practical control measure. We suggest that cucurbitaceous plants that are immune to the virus be included in any breeding program to control the disease. This apparently is the first report of isolation of WMV-like virus from cucurbits in Nigeria.

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