Yellow Mosaic Disease of *Crotalaria spectabilis* in Nigeria Caused by a Potexvirus

E. C. K. IGWEGBE, Department of Crop Science, University of Nigeria, Nsukka, Anambra State

ABSTRACT

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A slightly flexuous, rod-shaped virus measuring about 500 nm was isolated from leaves of *Crotalaria spectabilis* showing stunting, veinclearing, and yellow mosaic. Host range of the virus apparently was confined to legumes. The virus was readily transmitted by sap inoculation and by the aphid *Myzus persicae*. The virus in crude sap of infected *Calopogonium mucunoides* had a thermal inactivation point of between 55 and 60 C, a dilution end-point between 10^{-3} and 10^{-4}, and longevity in vitro between 24 and 48 hr (20–22 C). The virus appears similar to centrosema mosaic virus reported from Papua and New Guinea. This is the first report of a potexvirus from Nigeria and possibly Africa.

In November 1979, I observed several *Crotalaria spectabilis* Roth plants, a common weed found in scattered areas of Anambra State, Nigeria, that appeared to be infected by a virus. Affected plants showed mild veinclearing, stunting, and yellow mosaic or chlorotic mottle. Leaves on axillary shoots of infected plants sometimes showed general chlorosis. Because these symptoms were similar to those caused by centrosema mosaic virus (CaMV) described by van Velsen and Crowley (6,7), I attempted to identify the causal agent and to determine whether it was a threat to other important crops, especially legumes.

MATERIALS AND METHODS

The virus was isolated from field-infected *C. spectabilis* at Nsukka, Nigeria, and maintained and assayed in *Calopogonium mucunoides* Desv. by mechanical transmission. Leaves showing symptoms were triturated in 0.05 M phosphate buffer, pH 8.0, and the expressed sap was rubbed onto Carborundum-dusted leaves with a cheesecloth pad. Back-inoculations to detect symptomless infected plants were not made.

I used crude sap from systemically infected leaves in 0.05 M phosphate buffer (pH 8.0) to determine dilution end-point, thermal inactivation point, and longevity in vitro by methods described earlier by Ross (5).

For electron microscopy, leaf dip preparations from infected plants were negatively stained on Formvar-coated copper grids using 2% neutralized potassium phosphotungstate.

Aphid transmission tests were done with nonviruliferous, apterous green peach aphid (*Myzus persicae* (Sulz.)) adults reared on eggplant (*Solanum melongena* L.). Aphids were starved for 2 hr and allowed an acquisition access of 5–10 min on infected, detached leaves of *Calopogonium mucunoides*. Four to six aphids were then transferred to each of five healthy *Calopogonium mucunoides* plants (two- to three-leaf stage). Inoculation access was terminated at 24 hr by aphicidal sprays. Because whiteflies were almost invariably associated with infected *C. spectabilis* plants in the field, we investigated whitefly transmissibility of the virus. Apparently healthy tobacco whiteflies, *Bemisia tabaci* (Gennadius), were reared on *Nicotiana tabacum* L. 'Xanthi' and starved for 2 hr. About 50 insects were then transferred to infected *Calopogonium mucunoides* plants and allowed to feed overnight. Thereafter, insects were transferred, about 10 insects per plant, to each of five healthy leaves of *C. mucunoides*. Insects were removed after inoculation access of 24 hr.

For seed transmission tests, *C. spectabilis* plants were mechanically inoculated at the two- to three-leaf stage and allowed to set fruit in the greenhouse. Harvested seeds were air-dried, sown in sterilized soil in the greenhouse, and observed regularly for virus symptoms for over 2 mo.

RESULTS

The *Crotalaria* virus infected seven plant species after mechanical inoculations. In *C. spectabilis*, initial symptoms were systemic, mild net-vein yellowing followed by stunting, yellow mosaic, or chlorotic mottle. Occasionally, older leaves developed green vein banding. *Calopogonium mucunoides* developed occasional diffuse chlorotic spots or areas on the youngest inoculated leaf 3–4 days after inoculation, followed by mild chlorotic mottle and mild veinclearing of new leaves. Infected plants later showed slight curl and deformation of young leaves, green veinbanding, and pronounced hairiness of older leaves. In *Canavalia ensiformis* DC., the diagnostic symptom was systemic chlorotic mottle of leaflet margins of young and old leaves. Infected plants were stunted, and older leaves sometimes showed interveinal chlorosis and were cupped downwards.

Phaseolus vulgaris L. 'Bountiful' developed diffuse chlorotic spots on primary inoculated leaves, but these spots were usually too few for quantitative assay. No systemic symptoms developed. In *P. lunatus* L. 'Henderson Bush,' systemic mosaic mottle symptoms developed. *Vigna unguiculata* (L.) Walp. 'California Blackeye' developed systemic, mild net-vein chlorosis that developed into a mottle with time. Plants of *Glycine max* Merr. 'Williams' and 'TGM 280-3' developed a systemic, mild net-vein chlorosis that became mosaic with time.

The following plants did not show symptoms after mechanical inoculation: *Abelmonchus esculentum*; *Amaranthus hybridus*; *Arachis hypogaea*; *Brassica oleraceae*; *Cajanus cajan*; *Capsicum annuum* 'California Wonder' and 'Nsukka Yellow'; *C. frutescens* 'Tabasco'; *Celosia argentea*; *Centrosema pubescens*; *Chenopodium amaranticolor*; *C. quinoa*; *Crotalaria juncea*; *Cucumeropsis edulis*; *Cucumis sativus* 'Supermarket' and 'Improved Long Green'; *Cyamopsis tetragonoloba*; *Gomphrena globosa*; *Lycopersicon esculentum* 'Roma' and 'Marglobe'; *Nicotiana clevelandii*; *N. glutinosa*; *N. tabacum* 'N.C. 95' and 'Havana 425'; *Phaseolus lathyroides*; *P. mungo*; *P. vulgaris* 'Black Turtle-1,' 'Black Turtle-,' 'Contender,' and 'Tendergreen'; *Physalis floridana*; *Pisum sativum* 'Alaska,' 'Bonneville,' and 'Ranger'; *Sesamum indicum*; *Sesbania exaltata*; *Sida linifolia*; *Solanum aethiopicum*; *S. melongena*; *S. nigrum*; *Telfairia occidentalis*; *Vigna unguiculata* 'Dinner' and 'New Era'; and *Vinca rosea*.

Virus obtained from crude sap from *Calopogonium mucunoides* plants had a thermal inactivation point between 55 and 60 C, dilution end-point between 10^{-3} and 10^{-4}, and longevity in vitro between 24 and 48 hr.

Leaf dip preparations from infected *C. mucunoides* plants contained slightly flexuous, rod-shaped particles (Fig. 1).

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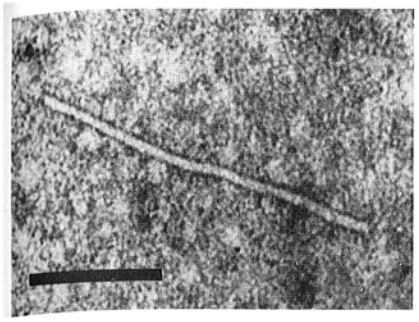

Fig. 1. Electron micrograph showing a slightly flexuous, rod-shaped particle in leaf dip of *Calopogonium mucunoides* inoculated with the virus isolated from *Crotalaria spectabilis* plant showing veinclearing and yellow mosaic. Bar = 200 μm.

Of 103 particles examined, 70% were between 432 and 576 nm in length; 32% were 504 nm. Normal length of the particles was assumed to be about 500 nm.

The *Crotalaria* virus was efficiently transmitted by *M. persicae*. In a representative test, aphids transmitted the virus from *Calopogonium mucunoides* to four out of four healthy plants of the same species and five out of five healthy *C. spectabilis* plants. Typical symptoms usually appeared 10–14 days after inoculation. *Bemisia tabaci* failed to transmit the virus in three separate tests.

The virus was not transmitted through the 500 seeds of *C. spectabilis* tested. Seed transmission in *Calopogonium mucunoides* was not tested because very few seeds were produced by infected plants.

DISCUSSION

A mosaic disease of *C. spectabilis* found in Nsukka, Anambra State, Nigeria, was caused by a rod-shaped virus about 500 nm long. Therefore, this virus is not one of the following viruses reported to naturally infect *C. spectabilis*: a potyvirus commonly infecting *Calopogonium mucunoides* in Nigeria (E. C. K. Igwegbe, *unpublished data*), crotalaria mosaic virus (3), or cowpea mosaic virus (1).

The size range of the virus suggests that it is a member of potexvirus group (4). Based on host range, particle size, and physical properties, the virus may be identical with CaMV as described by Crowley and Francki (2) and van Velsen and Crowley (6,7). However, the *Crotalaria* virus and CaMV differ somewhat in host range. The former virus infected *Phaseolus vulgaris* 'Bountiful,' *P. lunatus*, and *Canavalia ensiformis*, which are not hosts of CaMV, but did not infect *Centrosema pubescens* and *Crotalaria juncea*, which are hosts of CaMV. Failure to infect *Centrosema pubescens* suggests that the *Crotalaria* virus is not identical with CaMV. We were unable to determine possible serologic relationship between the *Crotalaria* virus and CaMV because antiserum to CaMV was not available (R. Francki, *personal communication*).

Our data suggest that aphids may spread the virus in nature. Although symptoms incited by the virus in most hosts except *Calopogonium mucunoides* were relatively mild, the ease with which aphids transmitted the virus indicates that it is potentially dangerous. The severe symptoms incited by the virus on *Calopogonium mucunoides* are of great interest because this plant is presently under investigation as a feed and forage crop in Nigeria (C. P. E. Omaliko, *personal communication*).

This is apparently the first report of a potexvirus isolated from legumes in Nigeria and of a potexvirus infecting plants in Africa.

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