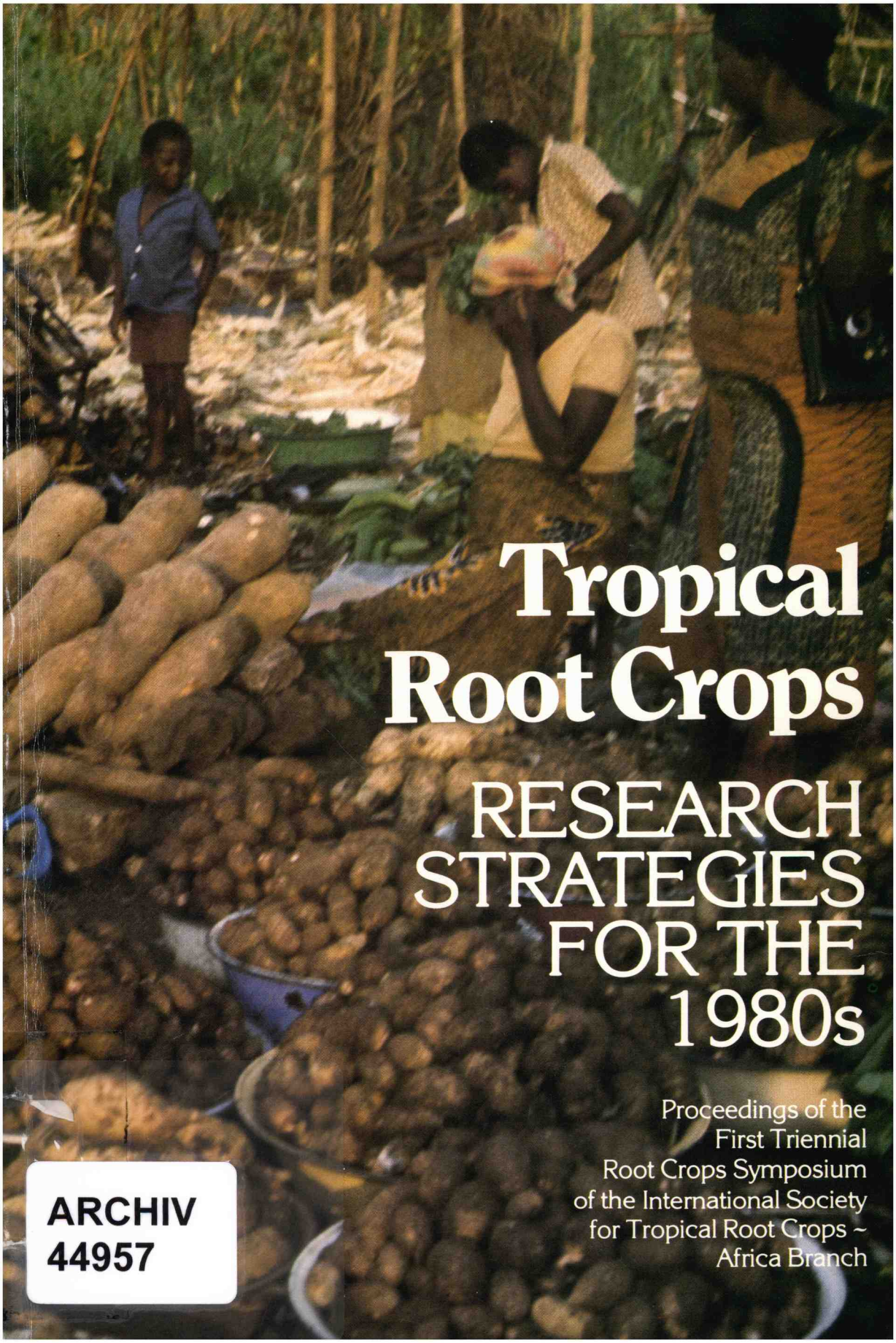

Tropical Root Crops

RESEARCH STRATEGIES FOR THE 1980s

Proceedings of the
First Triennial
Root Crops Symposium
of the International Society
for Tropical Root Crops ~
Africa Branch

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TROPICAL ROOT CROPS: RESEARCH STRATEGIES FOR THE 1980S

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EDITORS: E.R. TERRY, K.A. ODURO, AND F. CAVENESS

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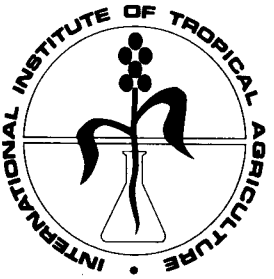

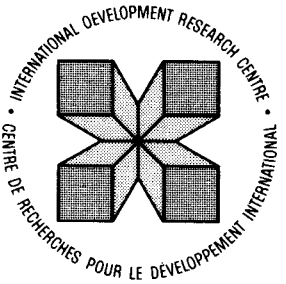

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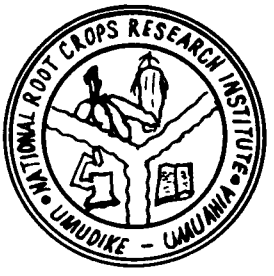

CONTENTS

PROPERTIES OF A SEVERE STRAIN OF CASSAVA LATENT VIRUS ISOLATED FROM FIELD-GROWN TOBACCO IN NIGERIA

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I isolated and tested the agent causing severe stunting, leaf curl, leaf epinasty, and chlorosis mottle in field-grown tobacco in Nsukka, Nigeria. The agent was shown to be a virus, isolates of which were able to infect *Datura stramonium* and several *Nicotiana* species. The virus exhibited thermal inactivation at 55–60°C, dilution endpoint of less than 10^{-1}, longevity in vitro of fewer than 24 hours, and a spherical morphology. It was transmitted by *Bemesia tabaci*. Based on these properties, I consider the virus to be a severe strain of cassava latent virus (CLV), although results of studies on agar to demonstrate the possible serological relationship between the Nsukka virus and CLV were inconclusive.

L'agent responsable du rabougrissement sévère, de la frisolée, de l'épinastie et de la marbrure chlorotique du tabac cultivé à Nsukka, Nigeria a pu être isolé et étudié. C'est un virus et plusieurs isolats ont été injectés à *Datura stramonium* et à d'autres espèces *Nicotiana*. Le virus, de morphologie sphérique, est inactif à 55–60 °C ainsi que lorsque dilué à moins de 10^{-1}, et il ne vit pas plus de 24 heures en laboratoire. Il est transmis par *Bemesia tabaci* et attendu les caractères décrits plus haut, il est possible qu'il appartienne à une variété du virus latent du manioc (VLM), bien que des recherches sur l'agar n'aient démontré aucune relation sérologique entre VLM et Nsukka.

Cassava latent virus (CLV) is relatively new, having been described in Kenya in 1978 by Bock et al. (1978). CLV is sap-transmissible and is inactive at temperatures higher than about 55°C. Its dilution endpoint is about 10^{-3}; longevity in vitro about 3 days; it infects Euphorbiaceae and Solanaceae in which it causes leaf curl, chlorotic lesions, and chlorotic vein banding (Bock et al. 1978).

There is new interest in CLV because it has consistently been isolated from cassava showing symptoms of cassava mosaic disease (CMD) (Bock et al. 1978; Igwegbe, unpublished results; Thottappilly, personal communication, and Cobaruko, unpublished thesis). CMD is a serious disease of cassava wherever the crop is grown. Although CLV has been transmitted experimentally from cassava to tobacco, the virus has never been observed in nature on tobacco.

In a 1979 survey of viruses infecting tobacco at the University of Nigeria tobacco plot at Nsukka, Nigeria, severely stunted plants with symptoms of leaf curl, leaf epinasty, and chlorotic mottle were found. Studies done to determine the causal agent of this disease showed that it was caused by a strain of CLV.

MATERIALS AND METHODS

Of 10 virus isolates (6 from field-grown tobacco and 4 from CMD-infected cassava kept in the greenhouse) obtained in Nsukka, one designated CLV-NN2 was used in my study. CLV-NN2 isolate was from field-grown tobacco. After several serial local lesion passages in *Nicotiana occidentalis*, CLV-NN2 was maintained in *N. tabacum*, NC 95 or a local cultivar. Unless otherwise indicated, these two varieties served as virus sources in this study.

I obtained the inoculum for a host-range study by grinding, in a mortar and pestle, infected tobacco leaves, with 0.05 M potassium phosphate buffer, pH 8.0 (1 : 2, V/V). The brei was passed through two layers of cheesecloth before the inoculum was applied to carborundum-dusted leaves of test plants. The inoculum was washed from the leaves with tap water immediately after application. Inoculated plants and appropriate uninoculated controls were moved to an insect-controlled greenhouse for observation.

Four or more young, rapidly growing, plants of each species were inoculated. If no symptoms resulted on reported hosts after 4–6 weeks, inoculations were made from uninoculated leaves to local or NC 95 variety of *N. tabacum*.

CLV-NN2 was partially purified from NC 95 tobacco leaves harvested about 4 weeks after inoculation. Twenty grams of freshly harvested infected leaves were ground in an ice-cold mortar

and pestle in the presence of 40 ml of 0.1 M potassium phosphate buffer (pH 7.0) containing 1% 2-mercaptoethanol. The homogenate was filtered through two layers of cheesecloth, and then the filtrate was clarified by use of n-butanol–chloroform mixture (1 : 1, V/V), which was used at the rate of 1 ml/10 ml of extract. The mixture was stirred intermittently for 2 hours at 4°C before the emulsion was centrifuged at 3000 rpm for 30 minutes. To precipitate the virus, I dissolved polyethylene-glycol and NaCl in the aqueous phase to final concentrations of 7.5% and 0.4 M, respectively. The mixture was incubated with intermittent stirring for about $2\frac{1}{2}$ hours at 4°C, and the precipitate was collected by centrifugation at 10000 rpm for 20 minutes at 4°C. The precipitate was resuspended in 5 ml of 0.05 M potassium phosphate buffer (pH 8.0) and then clarified by low-speed centrifugation. This final supernatant is referred to as partially purified virus preparation. To test the pathogenicity of the partially purified virus preparation, I inoculated four or more young, actively growing NC 95 tobacco and cassava seedlings. The control plants received similar extract obtained from uninoculated tobacco.

In a study of the particle morphology of CLV-NN2, partially purified virus preparation was negatively stained with 2% neutralized potassium phosphotungstate and then viewed under an electron microscope.

All serological tests were carried out with partially purified virus preparation in Ouchterlony double diffusion plates of 0.8% agarose and 0.1% sodium azide dissolved in saline; 6-mm-diameter wells were spaced 4 mm apart. CLV antiserum employed in this test had a titre of 1 : 256 and was a gift from K.R. Bock of Nairobi, Kenya.

I investigated properties of the crude sap in vitro with expressed juice obtained by titrating infected leaves of a local cultivar of *N. tabacum* in two volumes of 0.05 M potassium phosphate buffer, pH 8.0. Tissues were taken 3–4 weeks after inoculation. All infectivity assays were done on a local cultivar of *N. tabacum*. I determined thermal inactivation point (TIP) by heating 5 ml of the extract for 10 minutes at 10°C intervals from 40 to 80°C. At the end of heat treatment, tubes were immediately cooled and infectivity was determined (five plants/treatment). I determined dilution endpoint (DEP) and again determined infectivity using five plants/dilution. Longevity in vitro (LIV) was determined by incubation of the extract at 21°C. The extract was assayed on five tobacco plants 0–6 days after preparation.

Virus-free adult whiteflies reared on *Crotalaria* spp. were starved for 1 hour and then allowed to feed on infected tobacco for intervals of 30–60 minutes and transferred to test plants for inoculation feeding of 4 days. In another test, viruliferous adult whiteflies collected from CMD-infected cassava kept in the greenhouse were transferred to test plants and allowed to feed undisturbed for 4 days. Whiteflies in both tests were used at the rate of 10/plant and were removed at the end of each inoculation feeding period. Test plants in both tests were tobacco and cassava seedlings in the five- to six-leaf stage. In each test appropriate controls were included.

RESULTS

My results were similar to those of Bock et al. (1978) in showing that the host range of CLV-NN2 was confined to Solanaceous plants (*Nicotiana* spp. and *Datura stramonium*). *Nicotiana* spp. found susceptible that were not investigated by Bock et al. (1978) are *N. tabacum*, Burley 21, Samsun-NN, Havana 425, NC 95, and *N. occidentalis*. With the exception of the last, all the plants developed systemic symptoms. However, local lesions on *N. occidentalis* were erratic and often too few for quantitative assay.

On the other susceptible species, the sequence of symptoms was: 8–10 days after inoculation, crinkles appeared in the leaf base (lower one-half of leaf lamina) of the second, third, or fourth leaf above the youngest inoculated leaf. Within 24 hours, the crinkles became more conspicuous, and chlorotic lesions appeared in the crinkled area. These lesions were usually associated with veins and soon coalesced to form chlorotic vein banding. Within 36–48 hours from the time the first symptoms appeared, the mid-vein portion in the crinkled area became necrotic, especially in *N. tabacum* NC 95, and the local variety. A few hours later, infected plants developed leaf curl followed immediately by severe leaf epinasty. The point of leaf curl usually coincided with the necrotic portion along mid-vein. Temporary cessation of growth and the presence of epinastic leaves gave infected plants a flattened-top appearance. Within 7–10 days after the first reaction, the plant resumed growth.

New leaves lacked epinasty but had a characteristic chlorotic mottle. With time, infected plants became severely stunted compared with uninoculated controls. Sequence of symptom development in *D. stramonium* was similar to that described for tobacco except that in *Datura* the first symptom noted was petiole curl or leaf curl or both. CLV-NN2 in several tests failed to produce necrotic lesions on *D. stramonium*, a finding that supported results of Bock et al. (1978).

Among *Nicotiana* spp., *N. tabacum* NC 95 and the local variety were the most sensitive to inoculation with CLV-NN2, whereas *N. glutinosa* and *N. tabacum* Havana 425 were infected with difficulty. CLV-NN2 in several tests failed to produce symptoms on *D. ferox*, *Solanum nigrum*, and *N. rustica*, which are hosts of CLV.

CLV-NN2 did not incite symptoms in cassava or in any of the following: *Ageratum* spp., *Lycopersicon esculentum* (Marglobe and Roma), *Calapagonium mucoides*, *Crotalaria* spp., *Physalis floridana*, *Capsicum annuum* (California Wonder), *Capsicum frutescens* (Tabasco), *Ipomoea purpurea*, *Gossypium hirsutum*, *Chenopodium amaranticolor*, *Solanum melongena*, *Cucumeropsis* spp., *Urena lobata*, *C. quinoa*, *Ricinis communis*, *Cucumis sativus* (Supermarket and National Pickling), *Vinca rosae*, *Sesamum indicum*, *Datura metel*, *Glycine max* (Lincoln), *Jatropha multifida*, *Pisum sativum* (Bonneville), *Vigna unguiculata* (Blackeye No. 5), and *Hibiscus esculentus*. Except for cassava, none of the above species were assayed for latent virus infection.

Partially purified preparations of CLV-NN2 contained paired particles and some single particles. The paired particles had a total size of 30 × 20 nm, and single particles measured about 18–20 nm in diameter. All tobacco plants inoculated with partially purified virus showed typical symptoms of CLV-NN2 within 2 weeks. None of the tobaccos inoculated with extract obtained from healthy tobacco showed symptoms. None of the inoculated cassava became infected even after three cutbacks within 3 months. Furthermore, I repeatedly failed to recover CLV-NN2 from the inoculated cassava plants.

Partially purified virus preparation of CLV-NN2 did not react with antiserum from Bock in the agar test. The TIP of CLV-NN2 was between 50 and 60°C, the DEP was less than 10^{-1}; and LIV was less than 24 hours.

Virus-free whiteflies failed to transmit CLV-NN2 from infected tobacco to either tobacco or cassava. In contrast, viruliferous whiteflies collected from CMD-infected cassava transmitted CLV-NN2 to tobacco but not to cassava.

DISCUSSION

Host range, symptomatology, and physical properties of CLV-NN2 indicate that the virus is a severe strain of Bock's CLV. Although the host range, symptomatology, and physical properties of CLV-NN2 and CLV are quite close, obvious differences exist. Unlike CLV, my virus failed to infect *S. nigrum*, *N. rustica*, and *D. ferox*; infected *N. glutinosa* and *D. stramonium* only when young infected leaves and buffer were present in a ratio of 1 : 1; caused severe leaf epinasty and chlorotic mottle in susceptible hosts; failed to induce necrotic lesions on *D. stramonium*; and gave slightly lower TIP, LIV, and DEP values than have been recorded for CLV.Because strains of a virus may differ in host range, symptomatology, and physical properties (Bozarth et al. 1977; Fischer and Lockhart 1977; Fribourg 1977), these differences are not inconsistent with the conclusion that CLV and CLV-NN2 are related.

The failure to demonstrate a serological relationship between CLV and CLV-NN2 suggests that the reactants have not occurred in optimal concentrations (Waterworth et al. 1973) or that CLV has serotypes, as is the case with some plant viruses (Harrison 1964; Harrison and Woods 1966). Definitive conclusions regarding a possible serological relationship between CLV and CLV-NN2 must await the results of additional studies, especially the development of improved purification procedures and production of high-titre antiserum.

My failure to return CLV-NN2 to cassava and produce typical CMD symptoms is similar to the experience of Bock et al. (1978). Because the same virus preparation that failed to produce symptoms on cassava caused typical CLV-NN2 symptoms on inoculated tobacco, my inability to infect cassava is unexplained. Even more surprising was my inability to recover CLV-NN2 from inoculated cassava seedlings. These results raise some doubts as to whether CLV is truly latent in cassava. Furthermore, they do not support the view that CLV is the causal agent of CMD.

My attempts to return CLV-NN2 to cassava were hindered in two ways: whiteflies transferred to infected tobacco died within 60 minutes; even when they were allowed to feed for only a very short time, they died soon afterward. Until a more reliable method of returning CLV to cassava is developed, its role, if any, in the etiology of CMD will remain uncertain; however, I suspect the involvement of a complex in the etiology of CMD.

The origin of CLV-NN2 in the field-grown tobacco at Nsukka is not known but is possibly CMD-infected cassava. In the area in which the virus was found, tobacco seedbeds were prepared in close proximity to infected cassava plots. Because this virus has not been isolated from any other hosts in nature, it is quite likely that the tobacco became infected in the seedbeds.

My results show that CLV-NN2 is no longer a mere laboratory curiosity.