From the Virology Laboratory, Adiopodumé Research Station, Office de la Recherche Scientifique et Technique, Outre-Mer, Abidjan, Ivory Coast

Centrosema pubescens,
a natural host of groundnut crinkle virus in Ivory Coast

By

J. Dubern
Groundnut crinkle disease has been observed in the southern region of the Ivory Coast (Duberne and Dollet 1979). A study of groundnut crinkle virus (GCV) enabled to determine some of its physico-chemical and serological properties, and to establish that it was a new filamentous virus, belonging to the carlaviruse group (Duberne and Dollet 1980). Centrosema pubescens was easily infected with GCV by mechanical transmission and, in spite of failure of natural transmission (Duberne and Dollet 1980), this plant which was one of the most common weeds in contaminated fields, was supposed to be naturally-infected by GCV.

The present paper reports the results obtained and describes Centrosema pubescens as a new natural host plant of GCV.

Materials and Methods

Samples of Centrosema pubescens, consisting of whole plants with roots, were collected from fields contaminated by GCV and kept on ice.

To test the possible presence of GCV in the samples, Arachis hypogaea L. cv. Te3 was inoculated with crude sap extract from symptomatic leaves. This groundnut variety develops very typical symptoms when inoculated with GCV. Test plants were kept in optimal conditions for GCV multiplication, that is in a glasshouse where the temperature is 30—35 °C and humidity reaches 90%. For inoculations, samples were ground in pH 7.3, 0.05 M potas-
Centrosema pubescens, a natural host of groundnut crinkle virus

Sodium phosphate buffer (4 ml/g of tissue) containing 0.01 M sodium diethyldithiocarbamate and 12.5 mg/ml magnesium bentonite (Frankel-Conrat et al. 1961). The extract obtained was rubbed manually on Carborundum-dusted leaves.

Aphid transmission experiments were done in the laboratory using Aphis craccivora, A. citricola reared on groundnut plants, and A. gossypii reared on Eupatorium sp. Adults and late instar apterous aphids were starved for 1—3 h, then allowed an acquisition access time of 24 h on diseased samples, and an inoculation access period of 24 h, 48 h or 72 h, on healthy groundnut seedlings. They were then killed by applying an insecticide (systoate: dimethyldithiophosphorylacetic monomethyl amide). Ten aphids per plant were used in each experiment. Aphids used in the tests were virus free; this was established by placing control lots of insects on healthy groundnut seedlings serving as negative controls.

The standard procedures described by Bos, Hagedorn and Quanta (1960) were used with crude sap extracts from young leaves of groundnut plants infected at least five weeks by inoculation of Centrosema pubescens extracts, as the source of the virus. For all the studies, extracts were prepared as inocula for mechanical transmission. The dilution end point was determined by serial dilutions in 0.05 M potassium phosphate buffer, at pH 7.3. The thermal inactivation point was tested by immersing 2 ml quantities of extracts, contained in 5 ml tubes, in a water bath at various temperatures for 10 min, and placing the tubes in an ice bath and inoculating immediately. To test aging in vitro, the infectivity of extracts kept at 25°C was checked daily. Resistance to air-drying was determined by testing daily the infectivity from Arachis hypogaea leaves dried in boxes dehydrated by Silica gel.

Serological tests were made, by using the microprecipitin technique, under paraffin oil in Petri dishes (van Slogteren 1954). The antisera used were gifted by Dr. Wetter; no antiserum against GCV was produced in the laboratory.

Results

Mechanical transmission

Samples of C. pubescens collected in GCV-contaminated fields were symptomless plants and plants showing mosaic and distortion. Only some symptomless plant extracts produced crinkling and stippling on inoculated groundnut seedlings, symptoms that are typical of groundnut crinkle virus (Dubern and Dollet 1979). Symptoms were developed 4—6 weeks after inoculation. Centrosema pubescens, inoculated mechanically with these symptomless samples or with the first infected groundnut seedlings, developed necrotic local lesions on inoculated cotyledonary leaves 8 days after inoculation; a very light mottling appeared 3—5 weeks after inoculation on the new-emitted leaves, so light that plants seemed nearly symptomless. Necrotic local lesions were developed on the cotyledonary leaves of Soja max, Vigna unguiculata, Dolichos jacquinii, Phaseolus lathyroides, Desmodium polycarpum, Cassia occidentalis and C. obtusifolius. Mottling was developed 3—6 weeks after inoculation by Soja max, Vigna unguiculata, Canavalia ensiformis, Psophocarpus tetragonolobus, Cassia occidentalis and C. obtusifolius. Only these species in this family were infected. Other plant species belonging to Compositae, Chenopodiaceae, Aizoaceae, Scrophulariaceae, Malvaceae, Solanaceae and Passifloraceae were inoculated; none was infected (back inoculation to groundnut seedlings gave negative transmission).
Aphid transmission assay

All the attempts to transmit the disease by this way failed.

In vitro properties

Dilution end point was about $10^{-3}$; no infection was obtained with sap diluted to $10^{-4}$. Thermal inactivation point was about 65°C; infectivity was abolished after 10 min at 70°C. Dried groundnut leaves lost slowly infectivity, but they kept still infectivity two months after air-drying. Infectivity of groundnut extracts was kept three days at 4°C but no longer.

Serology

Clarified sap extracts of diseased groundnut plants, inoculated with collected symptomless samples, reacted with antisera to passiflora latent virus (up to a dilution of 528, homologous titre 1024), potato virus S (up to a dilution of 1024, homologous titre 1024), and carnation latent virus (up to a dilution of 16,384, homologous titre 16,384). These antiserum were previously absorbed with clarified sap extract of healthy groundnut plants.

Discussion

From symptomless *Centrosema pubescens* samples collected in GCV contaminated fields, it was possible to recover GCV. Groundnut seedlings, inoculated mechanically, developed typical crinkling and stippling. Host range obtained with the sample extracts was identical with GCV host range. Aphid transmission attempts failed, that is to compare with failure of GCV aphid transmission. In vitro properties were the same and clarified extracts reacted with four virus of the carlaviruses group. So it may be considered that *Centrosema pubescens* is a natural host plant of groundnut crinkle virus.

The author is much indebted to Dr. Remaudières (Institut Pasteur) for identifying the aphids, M. J.-L. Renard (I.R.H.O.) for providing Te3 cultivar seeds, Dr. C. Wetter for providing antisera and, M. Aho Kouakou for technical assistance.

Summary

*Centrosema pubescens* was found as a natural host plant of groundnut crinkle virus in Ivory Coast. The virus from *C. pubescens* was characterized by mechanical transmission, host range, in vitro properties, lack of aphid transmission and, serological relationship with groundnut crinkle virus.

Résumé

*Centrosema pubescens*, hôte naturel du virus de la frisolée de l’Arachide

Le virus de la frisolée de l’Arachide infecte naturellement *Centrosema pubescens*. Cette observation a été effectuée en champ dans le sud de la Côte
**Centrosema pubescens**, a natural host of groundnut crinkle virus

Centrosema pubescens, a natural host of groundnut crinkle virus of Ivory Coast. The virus isolated from C. pubescens in Ivory Coast has been characterized by its mechanical transmission, its range of hosts, the absence of transmission by aphids, its physico-chemical properties and its serological relationships with the virus of the blighted groundnut.

**Zusammenfassung**

**Centrosema pubescens**, ein natürlicher Wirt des Erdnuß-Kräuselvirus an der Elfenbeinküste


**Literature**


Dubern, J., and M. Dollet, 1979: Groundnut crinkle, a new virus disease observed in Ivory Coast. Phytopath. Z. 95, 279—283.


Author's address: Dr. J. Dubern, Centre de Bel Air, ORSTOM, B.P. 1386 Dakar (République du Sénégal).