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IDENTIFICATION OF AN APHID-TRANSMITTED COWPEA MOSAIC VIRUS[1, 2]

Identificatie van een door bladluizen overgebracht Vigna-*mozaïekvirus*

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A virus disease of cowpea widespread in North Italy has been found to be caused by a virus which has the following properties: a. rod-shaped particles about 750 mμ in length; b. serological affinity with bean common mosaic virus; c. aphid-transmission; d. seed-transmission in cowpea at a percentage of 0.3–1.59; e. fairly wide host range covering 19 species representing 13 genera and 6 families; f. dilution end-point 1:4000; g. thermal inactivation point 60–62 °C; h. longevity in vitro 5 days. The virus is tentatively called "cowpea aphid-borne mosaic virus".

In North Italy a mosaic virus disease of cowpea, *Vigna unguiculata* (L.) Walp. (= *Vigna sinensis* (L.) Endl.), first recorded and described by VIDANO (VIDANO, 1959; RUI, 1960), is widespread and sometimes very damaging.

From cowpea plants with symptoms on the whole similar to those described by VIDANO and coming from the same localities (Mantova and Cremona provinces), we obtained 6 isolates of a specific virus the main properties of which are described below. In order to avoid nomenclatural confusion among the several viruses that have, as far as we know, the cowpea as the main natural host, we propose tentatively to call our virus "cowpea aphid-borne mosaic virus" (CAMV).

HOST RANGE

CAMV infects, by mechanical inoculation or by aphids, 19 species of the families Amarantaceae, Chenopodiaceae, Cucurbitaceae, Labiatae, Leguminosae and Solanaceae.

The following plants reacted with systemic infection: *Chenopodium foetidum* Schrad., *Cucumis sativus* L. cv. 'Gele tros', *Cucurbita pepo* L. var. *verrucosa*, *Glycine max* (L.) Merr. var. *lutea* and *vilnensis*, *Petunia hybrida* hort., *Phaseolus lunatus* L., *Physalis alkekengi* L., *Ph. floridana* Rydb. (latent), *Pisum sativum* L. (latent), *Trigonella foenum-graecum* L., *Vigna unguiculata* (L.) Walp. cvs. 'Black', 'Nostrana', 'Midget' and 'Iron Clay' (Fig. 1).

The plants which gave local symptoms only were: *Chenopodium album* L., *Ch. amaranticolor* Coste et Reyn., *Ch. quinoa* Willd., *Ch. vulvaria* L., *Gomphrena globosa* L., *Nicotiana tabacum* L. cv. 'White Burley', *Ocimum basilicum* L. and *Phaseolus vulgaris* L. cvs. 'Canadian Wonder', 'Prince' and 'Saxa' (French bean does not react during the winter).

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No symptoms appeared, and no CAMV could be detected, in the following plants: *Amarantus ascendens* Lois., *Brassica chinensis* L., *Capsicum annuum* L., *Datura stramonium* L., *Desmodium canadense* DC., *Lamium bifidum* Cyr., *Lupinus albus* L., *Lycopersicum esculentum* Mill., *Nicotiana glauca* R. Grah., *N. glutinosa* L., *Phaseolus aureus* L., *Trifolium repens* L., *Vicia faba* L.

The hosts which proved most useful as test plants or for the multiplication of CAMV were: *Ocimum basilicum* (Fig. 2), *Chenopodium amaranticolor* (Fig. 3), *Ch. album*, *Ch. quinoa*, French bean (Fig. 4), *Gomphrena globosa* (Fig. 3), petunia, cowpea, soybean and *Trigonella foenum-graecum*.

METHODS OF TRANSMISSION

CAMV can normally be transmitted mechanically; carborundum powder and phosphate buffer (0.01 M, pH 6.9) were generally used.

CAMV is seed-borne in cowpea; this was ascertained in the following trials carried out in insect-proof glasshouses. The first trial was made with commercial seed of the cv. 'Nostrana' harvested in 1962 in localities where CAMV was present. In this trial, in which 985 plants were grown from this seed, which was then about two years old, we isolated CAMV from three plants (0.3%). In a second trial, using the same type of cowpea seed but harvested in 1964 and tested when about one year old, 2578 plants were grown and 41 (1.59%) were found to be infected with CAMV. Seed transmission was confirmed again in a third trial, which was carried out with 'Iron Clay' cowpea seed one to two months old, harvested from plants mechanically inoculated on their primary leaves: out of 4445 plants grown 19 (0.42%) were found to be infected with CAMV. From these results it is clear that, although CAMV is seed-borne in cowpea, seed transmission occurs very erratically. Further observations are needed to discover the factors favouring seed transmission. In all our tests CAMV could be isolated only from plants showing symptoms.

The CAMV described in this paper has been shown by VIDANO & CONTI (1965) to be transmitted by the aphids *Myzus persicae* Sulz., *Aphis fabae* Scop., *A. medicaginis* Koch, *A. gossypii* Glov. and *Macrosiphum euphorbiae* Thomas; the virus proved to be non-persistent (stylet-borne).

PROPERTIES OF THE VIRUS IN PLANT SAP

The *in vitro* properties were generally determined by using sap from young systemically infected petunia plants; comparisons were also made with sap extracted from cowpea and *Trigonella foenum-graecum*.

Chenopodium amaranticolor, *Ch. album*, *Ch. quinoa*, *Ocimum basilicum* and *Gomphrena globosa* were used as local lesion hosts. The procedure suggested by BOS *et al.* (1960) was followed.

Thermal inactivation point: CAMV was inactivated after 10 minutes heating at 60–62°C; the virus was usually still infectious after 10 minutes at 57°C, but rarely after the same period at 60°C.

Dilution end point: the virus in the sap was still infective at a dilution of 1:4000 but never at 1:6000.

Longevity in vitro: the virus in the crude sap stored at 21°C (± 3°) was still infective after 120 hours but not after 125.

ELECTRON MICROSCOPY AND SEROLOGY

Electron microscope trials carried out by Dr. J. BRANDES, Braunschweig, using the dip method, showed that CAMV consists of elongated particles with normal length of about 750 mμ, morphologically indistinguishable from those of Dr. QUANTZ's cowpea mosaic virus (BRANDES, 1964).

Preliminary serological tests performed by Prof. R. BERCKS, Braunschweig, showed the existence of a serological relationship between CAMV and bean common mosaic virus. Other tests, carried out with antisera against cowpea mosaic virus ('Severe' and 'Yellow' strains) kindly supplied by Dr. H. AGRAWAL, Wageningen, have made quite sure that our isolates of CAMV were not contaminated by these viruses.

DISCUSSION

The identity, and consequently the nomenclature, of the several viruses reported in naturally infected cowpea is rather confusing, as has been pointed out by several authors and recently by AGRAWAL (1964), BOS (1964) and KUHN (1964).

A number of viruses inducing mosaic symptoms in cowpea – especially those described by MCLEAN (1941), SNYDER (1942), YU (1946), CAPOOR & VARMA (1956), HINO (1960), NARIANI & KANDASWAMY (1961), and others – possess features in common with CAMV, the most important of which are aphid transmission, seed transmission and symptomatology on cowpea. Nevertheless it is not possible to identify CAMV with any of them for the following reasons: 1. nothing is known about the morphology of these viruses; 2. they are said to infect certain leguminous plants only.

CAMV cannot be assigned to any of the seven groups tentatively proposed by ANDERSON (1955b) in order to classify *Vigna* and *Crotalaria* viruses.

CAMV seems also to be clearly different from the following viruses causing field diseases of cowpea and which are clearly definable:

- cowpea mosaic viruses transmitted by leaf beetles (cf. CHANT, 1962; AGRAWAL, 1964)
- cowpea chlorotic mottle (KUHN, 1964)
- cowpea strain of bean southern mosaic virus (cf. KUHN, 1963)
- cowpea strain of tobacco mosaic virus (LISTER & THRESH, 1955)
- cowpea strain of cucumber mosaic virus (cf. ANDERSON, 1955a)
- blackeye cowpea mosaic strain of bean yellow mosaic virus (cf. CORBETT, 1957; ANDERSON, 1959).

The virus most similar to CAMV seems to be one studied by Dr. L. QUANTZ and briefly described by BRANDES (1964).

Comparisons, either experimental or on the basis of literature data, with the viruses having particles with a normal length of about 750 mμ ("Y-group" according to BRANDES, 1964), failed to reveal identity of CAMV with any of them. Particular attention was paid to differences between CAMV and the leguminous viruses of the "Y-group", such as bean yellow mosaic virus (cf. HAGEDORN & WALKER, 1954; DEVERGNE, 1964; etc.), soybean mosaic virus (cf. QUANTZ, 1961; GALVEZ, 1963), and pea mosaic virus (cf. HAGEDORN & WALKER, 1954; DEVERGNE, 1964; etc.). As already mentioned, serological relationships exist between CAMV and bean common mosaic virus; further research is therefore in progress in order to clear up this important point.

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SAMENVATTING

Van een in Noord-Italië veel voorkomende en soms zeer schadelijke mozaïekziekte van *Vigna unguiculata* (fig. 1) werd aangetoond dat deze door een virus wordt veroorzaakt. Het virus heeft draadvormige deeltjes van ongeveer 750 mμ lengte en vertoont serologische verwantschap met het rolmozaïekvirus van boon. Het wordt verspreid door bladluizen en kon hiermee of met sap kunstmatig worden overgebracht op 19 plantesoorten uit 13 geslachten en 6 families (fig. 2–4). In *Vigna* gaat het virus voor 0,3–1,59% over met zaad. Het verdunningseindpunt van het virus in uitgeperst sap bedroeg 1:4000, het thermale inactiveringspunt 60–62°C en de houdbaarheid in vitro vijf dagen. Aan het virus werd voorlopig de naam "Cowpea aphid-borne mosaic virus" gegeven.

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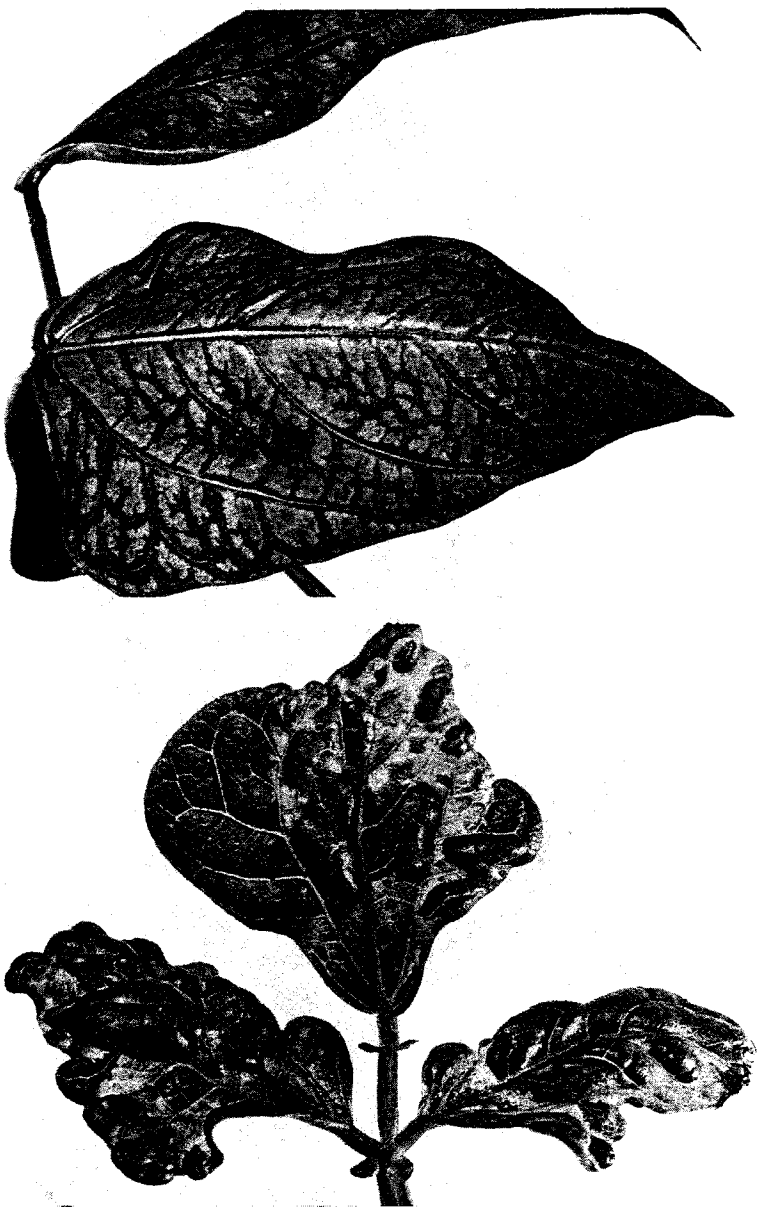

FIG. 1. Symptoms induced in cowpea by cowpea aphid-borne mosaic virus (CAMV). Top: first systemic symptoms ten days after inoculation. Bottom: typical mosaic and blistering four months after inoculation.

Symptomen in Vigna, *teweeggebracht door het door bladluizen overgebrachte* Vigna-*mozaïekvirus. Boven: eerste systemische verschijnselen tien dagen na inoculatie. Onder: Karakteristiek mozaïek en misvorming vier maanden na inoculatie.*

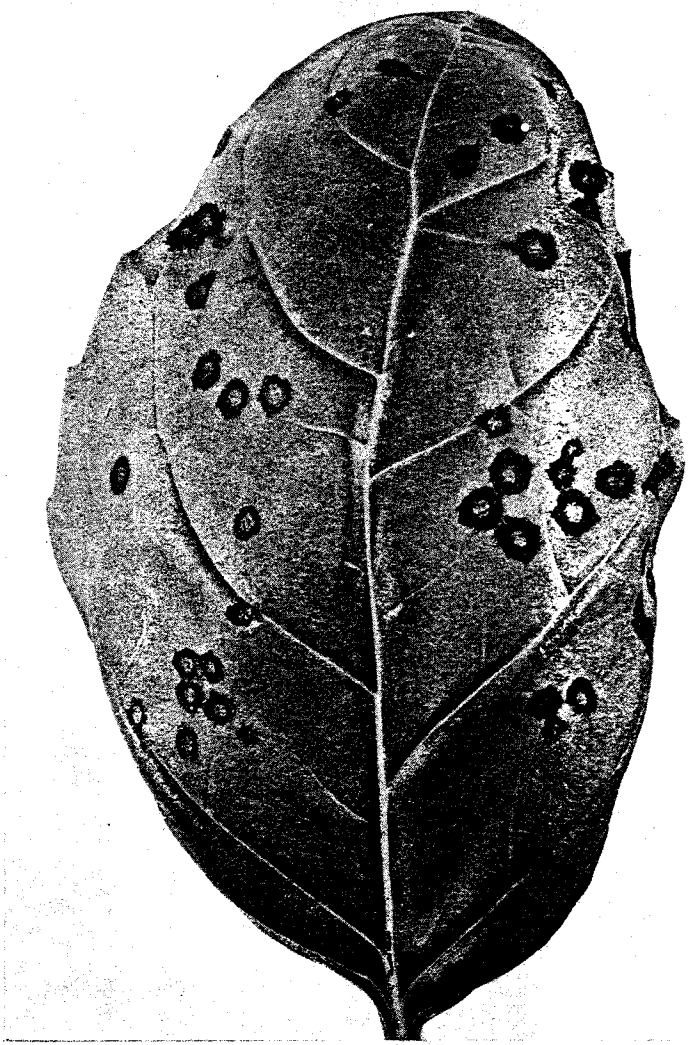

FIG. 2. Local symptoms due to CAMV on *Ocimum basilicum*, 13 days after inoculation.
Lokale symptomen in Ocimum basilicum, *13 dagen na inoculatie.*

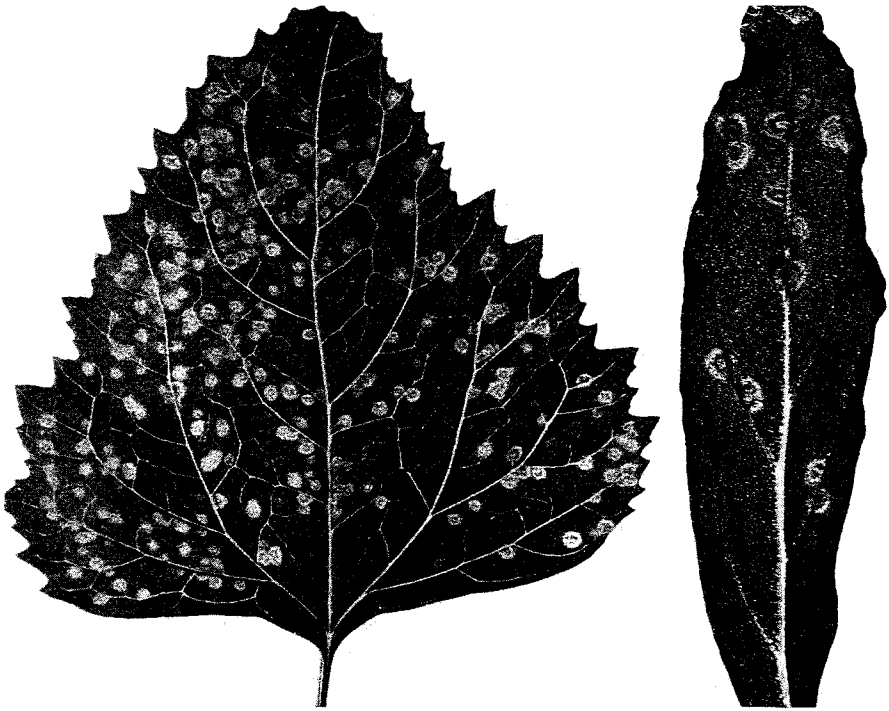

FIG. 3. Local symptoms due to CAMV on *Chenopodium amaranticolor* (left) and on *Gomphrena globosa* (right), 13 days after inoculation.

Lokale symptomen in Chenopodium amaranticolor *(links) en* Gomphrena globosa *(rechts), 13 dagen na inoculatie.*

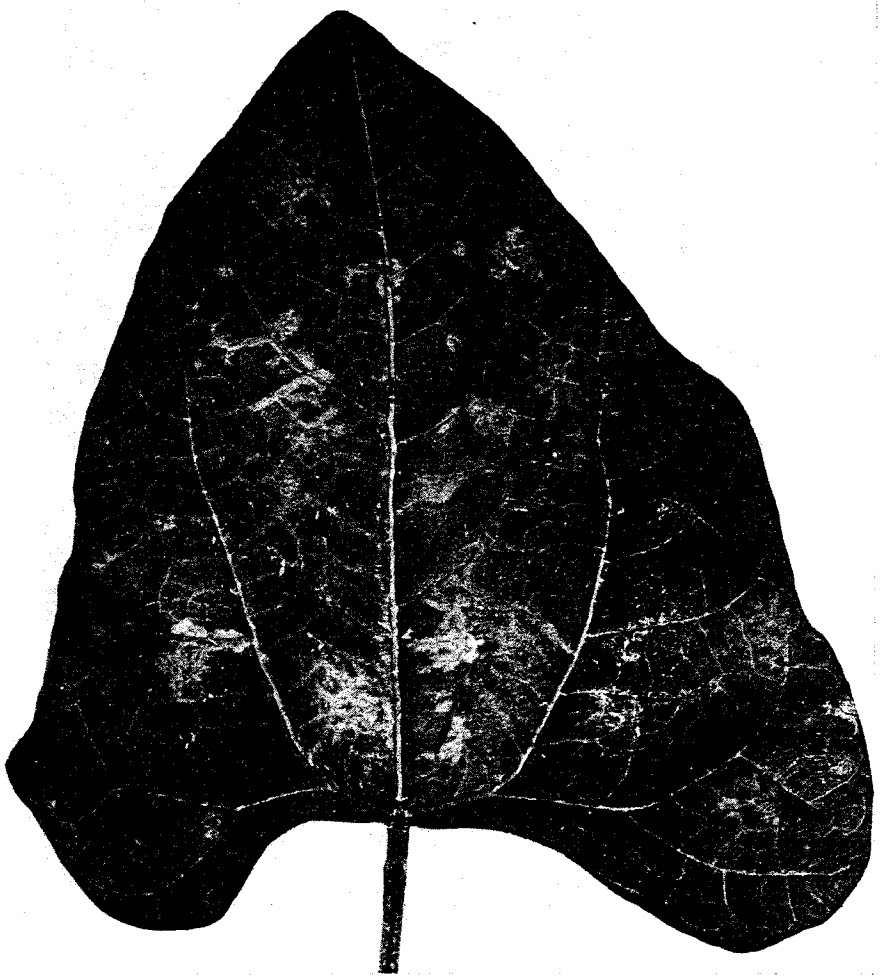

FIG. 4. Local symptoms due to CAMV on French bean, cv. 'Prince', 18 days after inoculation.
Lokale symptomen in boon, cv. 'Prince', 18 dagen na inoculatie.