

Association of Cassia Mild Mosaic Virus with Dieback of *Cassia macranthera* in Central Brazil

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ABSTRACT

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A slightly flexuous rod-shaped virus measuring 650×15 nm was isolated from leaves of *Cassia macranthera* which showed chlorotic spots, vein chlorosis, and mild mosaic. The virus was identified as cassia mild mosaic virus (CaMMV) based on symptoms, host range, physical properties in vitro, serology, and reproduction of the symptoms in *C. macranthera* seedlings mechanically inoculated with CaMMV. Field observations of apparently healthy and CaMMV-infected *C. macranthera* trees indicate the association of CaMMV infection and dieback.

Cassia macranthera is a common tropical tree in southeast Brazil, including the states of Minas Gerais, Rio de Janeiro, Espirito Santo, and São Paulo. This leguminous plant with dense

foliage and beautiful long-lasting yellow flowers was one of the shade trees used for arborization in Brasilia, the capital of Brazil, and satellite cities. According to the Department of Parks and Gardens of NOVACAP (the organization responsible for arborization), 1,846 *C. macranthera* trees were planted in parks and along

avenues in Brasilia (1,260 trees), Sobradinho (365), Taguatinga (172), Planaltina (35), Gama (9), and Ceilandia (5) in the early 1970s. In the past few years, however, most of the trees died of unknown cause and were removed, and no more than 300 trees can now be found in these cities.

In July 1977, a *C. macranthera* tree at the Brasilia zoo showed chlorotic spots and vein chlorosis in young leaves and mild mosaic in old leaves. Electron microscopic examination of leaf dips prepared from these leaves revealed slightly flexuous rods measuring 650×15 nm (Fig. 1A). Aggregates of elongated particles resembling the rods in the leaf dips were present in the cytoplasm of parenchymal cells of these leaves (Fig. 1B).

Sap prepared from the leaves was mechanically inoculated (2) to plants commonly used as virus indicators. Among these plants, *Gomphrena globosa* showed necrotic local lesions (Fig. 2) in 7–10 days, and *Cassia obtusifolia* showed chlorotic spots and vein chlorosis in 2 wk and mosaic a few days later. Rod-shaped particles were observed in the leaf dips made from the leaves of *G. globosa* and

C. obtusifolia with symptoms. A single lesion virus isolate was obtained from *G. globosa* and multiplied in *C. obtusifolia*. Five seedlings of *C. macranthera* that were mechanically inoculated with the isolate developed chlorotic spots and vein chlorosis (Fig. 3) in 10–14 days. These results confirmed that the rod-shaped virus was responsible for the symptoms in the leaves of the *C. macranthera* tree at

the zoo.

This virus also infected *C. occidentalis* and *C. coluteoides* and induced the symptoms similar to those in *C. macranthera*. When mechanically inoculated with this virus, the following plants were not infected: *Apium graveolens*, *Arachis hypogaea*, *Bidens pilosa*, *Brassica alba*, *B. alba* var. *perviridis* 'Tendergreen,' *B. chinensis*, *B. oleracea* var. *capitata*, *Capsicum annuum*, *Chenopodium amaranticolor*, *C. murale*, *C. quinoa*, *Chrysanthemum* sp., *Cichorium endivia*, *Citrullus lanatus*, *Cucumis melo*, *C. metuliferus* PI 292190, *C. sativus*, *Cucurbita pepo* 'Zucchini,' *Datura stramonium*, *Emilia sonchifolia*, *Euphorbia pruniflora*, *Glycine max*, *Helianthus annuus*, *Lactuca sativa*, *Luffa acutangula*, *Lupinus albus*, *L. angustifolius*, *Lycopersicon esculentum*, *Malva parviflora*, *Manihot esculenta*, *Medicago sativa*, *Nicandra physaloides*, *Nicotiana debneyi*, *N. glutinosa*, *N. sylvestris*, *N. tabacum*, *Petunia* sp., *Phaseolus vulgaris*, *Physalis floridana*, *Pisum sativum*, *Portulaca oleracea*, *Raphanus sativus*, *Solanum melongena*, *S. tuberosum*, *Sonchus oleraceus*, *Sorghum bicolor*, *Tetragonia expansa*, *Vigna unguiculata*, *Zea mays*, and *Zinnia elegans*. The dilution end point and thermal inactivation point were 10^{-5} to 10^{-6} and 55–60 C, respectively.

Based on particle morphology, symptomatology in *G. globosa* and *C. obtusifolia*, host range, and physical properties in vitro, the virus was tentatively identified as cassia mild mosaic virus (CaMMV), a carlavirus previously isolated from a native cerrado

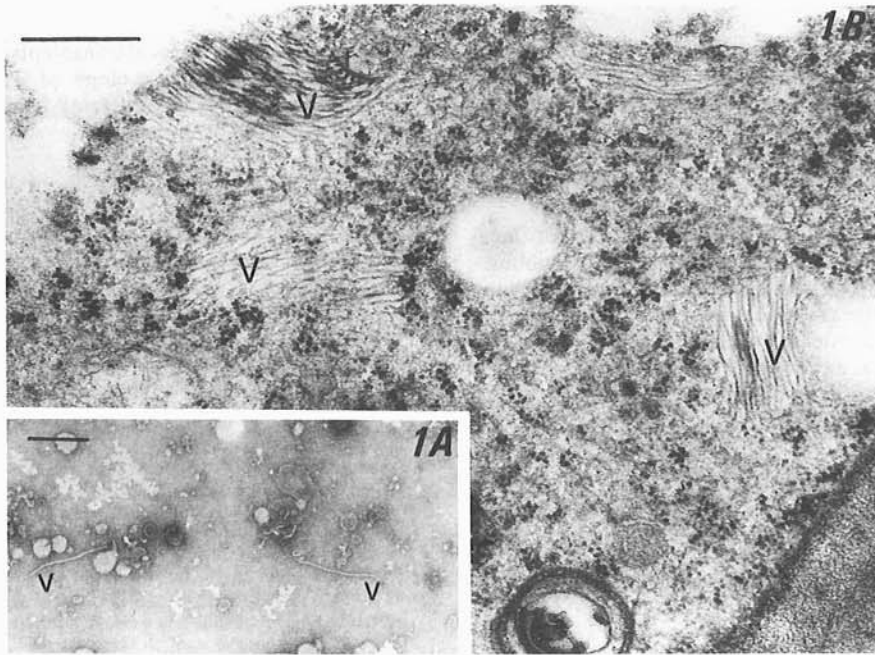
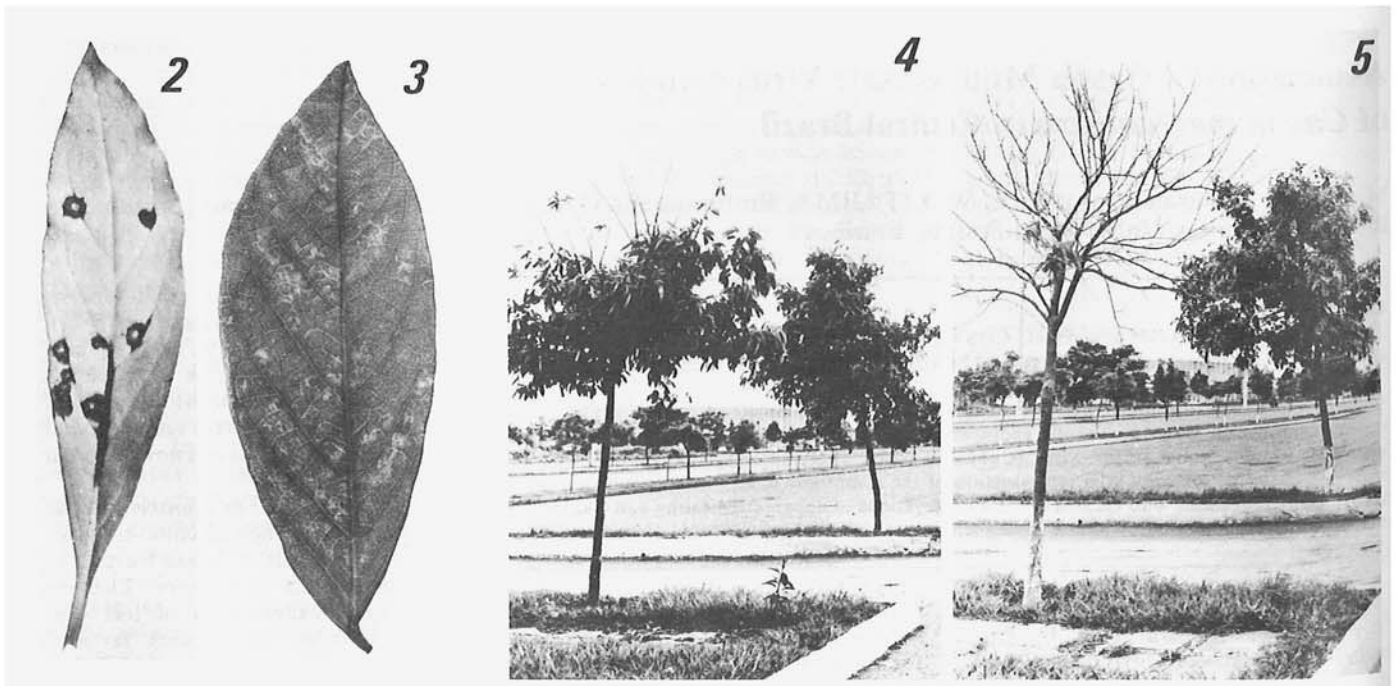


Fig. 1. Electron micrographs showing slightly flexuous rod-shaped particles (v) in leaf dip (1A) and in cytoplasm of a parenchymal cell (1B) of *Cassia macranthera* with chlorotic spots and vein chlorosis. Scale bars represent 500 nm.



Figs. 2 and 3. Symptoms induced by the virus isolated from *C. macranthera*: (2) Necrotic local lesions on a leaf of *Gomphrena globosa*. (3) Systemic chlorotic spots and vein chlorosis on a leaf of *Cassia macranthera*.

Figs. 4 and 5. Development of dieback in two *Cassia macranthera* trees in Brasilia photographed (4) in November 1977 and (5) 1 yr later. Both trees were naturally infected with cassia mild mosaic virus, but only the plant on the left had the dieback symptoms when these pictures were taken. The plant on the right began to show twig blight (initial symptom of dieback) in July 1979.

plant, *Cassia sylvestris* (2). Identification was further confirmed by development of chlorotic spots and vein chlorosis in *C. macranthera* seedlings inoculated with the CaMMV isolate from *C. sylvestris* and by serologic identity between CaMMV and the virus from *C. macranthera* when they were tested against an antiserum to CaMMV (1) in a sodium dodecyl sulfate-immunodiffusion test.

A field survey made in October 1977 in Brasilia, Sobradinho, and Planaltina indicated that most of the *C. macranthera* trees (84 of 101) had symptoms typical of CaMMV infection, and 54 of the 84 infected trees also showed some degree of dieback, but none of 17 apparently healthy trees showed any dieback (Table 1). This indicated that the dieback of *C. macranthera* trees is associated with CaMMV infection. Under experimental conditions, CaMMV has induced dieback of the annual shrub *C. obtusifolia* (2). It is also possible that CaMMV infection predisposes the trees to further infection by other agents.

Development of dieback in *C. macranthera* seems to be slow and gradual. In November 1977, one tree in Brasilia showed early signs of dieback with blight of top twigs (Fig. 4). One year later, the blight affected almost the entire tree (Fig. 5), but it was not completely dead when this article was written in December 1979. To provide direct

Table 1. Association of cassia mild mosaic virus (CaMMV) infection with dieback of *Cassia macranthera* trees in central Brazil^a

City	Plants with CaMMV symptoms ^b		Plants without CaMMV symptoms and dieback	Total
	With dieback	Without dieback		
Brasilia	11	1	0	12
Sobradinho	33	13	9	55
Planaltina	10	16	8	34
Total	54	30	17	101

^a Field survey conducted in October 1977.

^b CaMMV symptoms included chlorotic spots, vein chlorosis, and mild mosaic on leaves.

evidence that CaMMV causes dieback in *C. macranthera*, seedlings would have to be mechanically inoculated and development of dieback observed for several years.

Occurrence of CaMMV in *C. macranthera* seems not to be restricted to central Brazil. Two trees at Itaipava in Rio de Janeiro (about 1,400 km from Brasilia) and one at Sete Lagoas in Minas Gerais (650 km from Brasilia) show typical chlorotic spots, vein chlorosis, and mild mosaic symptoms in the leaves.

C. macranthera and five other *Cassia* spp. (*C. fistula*, *C. multijuga*, *C. javanica*, *C. siamea*, and *C. grandis*) have been used as shade trees in the NOVACAP arborization program. Of 6,136 *C. siamea* trees planted, 1,176 had died of unknown cause by 1975. Although evidence was not conclusive, several factors including insect damage, soil

fertility, and root infection by *Fusarium* spp. were implicated (3). Development of dieback symptoms in *C. siamea* closely resembles that in *C. macranthera*. The possible involvement of CaMMV in the dieback of *C. siamea* is being investigated.

ACKNOWLEDGMENTS

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