

Cucumber Mosaic Virus and Desmodium Yellow Mottle Virus Infections in Wild Groundnut (*Apios americana*)

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ABSTRACT

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Cucumber mosaic virus (CMV) and Desmodium yellow mottle virus (DYMV) were the causal agents of two previously unreported diseases in wild groundnut (*Apios americana*) grown in experimental plots in Baton Rouge, LA. CMV was isolated from 12 and DYMV from three of 20 wild groundnut plants showing mosaic or mottle symptoms. CMV caused a severe foliar mosaic, whereas DYMV induced a mild yellow mottle. The presence of these two viruses in naturally infected plants was verified by electron microscopy, serology, double-stranded RNA analysis, and host reaction. CMV and DYMV incited symptoms in artificially inoculated wild groundnut plants similar to those observed on naturally infected plants. The DYMV isolate from wild groundnut was serologically related but not identical to the type strain.

Wild groundnut (*Apios americana* Medik.), a native legume of eastern North America, is being evaluated as a food crop in Louisiana (1). This nitrogen-fixing plant produces seed and tubers, the latter containing about 16% protein and 47% carbohydrates (7). The aims of the wild groundnut research program at the Louisiana Agricultural Experiment Station include screening a large collection of accessions of this species for desirable agronomic and horticultural characteristics and for resistance to diseases and abiotic agents.

Wild groundnut is propagated primarily by tubers, because they perpetuate the desirable genetic traits of individual accessions. However, this method of propagation is also an efficient way of spreading pathogens, particularly viruses. Consequently, before the Louisiana wild groundnut germ plasm is offered to other domestic and foreign institutions, it would be desirable to determine whether it harbors pathogens.

During 1988, a number of plants of the Louisiana State University collection

were found to have mild to severe symptoms of disease, similar to those induced by viruses. Preliminary studies revealed that two virus isolates, one related to cucumber mosaic virus (CMV) and the other to Desmodium yellow mottle virus (DYMV), were the causal agents. Because there have been no previous reports of viral infections in *A. americana*, this study was undertaken to identify and partially characterize these wild groundnut virus isolates.

MATERIALS AND METHODS

Virus isolates. Leaf specimens showing mosaic or mottle symptoms were collected from 20 wild groundnut plants grown in an experimental field in Baton Rouge, LA. Each sample was ground in 0.02 M sodium phosphate buffer, pH 7.0, and extracts were rubbed on Carborundum-dusted leaves of the following plant species: *A. americana*; *Chenopodium quinoa* Willd.; *Cucumis sativus* L. 'Marketer'; *Cucurbita pepo* L. 'Seneca Zucchini'; *Nicotiana tabacum* L. 'Havana 423'; *Phaseolus vulgaris* L. 'Black Turtle Soup,' 'Great Northern,' and 'Pinto'; *Pisum sativum* L. 'Bonneville' and 'Ranger'; and *Vigna unguiculata* (L.) Walp. subsp. *unguiculata* 'Blackeye No. 5' and 'TVu 612.'

Based on the reactions of these diagnostic species, two distinct virus isolates from *A. americana*, designated CMV-A and DYMV-A, were selected for in-depth

studies. Cultures of the legume strain of CMV (CMV-L) and two tymoviruses (the type strain of DYMV [DYMV-T] and turnip yellow mosaic virus [TYMV]) were used for comparative studies. DYMV-T was obtained from H. A. Scott (University of Arkansas), and CMV-L and TYMV were part of our collection.

Electron microscopy. Extracts from plants infected with the two wild groundnut virus isolates were negatively stained with 2% uranyl acetate (pH 6.8) and viewed with a Jeol 100 CX transmission electron microscope.

Double-stranded RNA extraction and analysis. Double-stranded RNA (dsRNA) was extracted from 7.0 g of Blackeye No. 5 cowpea infected with the two wild groundnut virus isolates, DYMV-T, and CMV-L and from *Brassica campestris* L. plants infected with TYMV. Extracts from healthy cowpea and *B. campestris* were used as controls. DsRNA was extracted using the CF-11 cellulose column chromatography method described by Morris and Dodds (4), with minor modifications (6). The dsRNAs were analyzed in 6% polyacrylamide gels at 100 V for 2.5–3.5 hr. Molecular weight markers were a mixture of dsRNAs of tobacco mosaic virus (4.3 and 0.4×10^6) and tobacco necrosis virus (2.3 , 0.8 , and 0.6×10^6).

Serology. Double-diffusion tests were employed to determine the reactions of wild groundnut virus isolates to antisera to CMV-C (titer 1:256) and to DYMV-T (titer 1:256) (both antisera provided by H. A. Scott). Dilutions (1:2) of extracts from infected and healthy plants in sodium phosphate buffer (0.01 M, pH 7.0) were placed in wells of plates containing 1% agarose in the same buffer. Purified virus preparations (1.0 mg/ml) were also included. Undiluted antisera were added to the center wells of the plates, which were incubated for 48 hr.

Virus purification. Both wild groundnut isolates were purified from leaves of

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infected Blackeye No. 5 cowpea plants. DYMV-A was partially purified following the method described by Walters and Scott (8) and further purified by linear sucrose density gradient centrifugation (10–40%). The preparation obtained in this way was suspended in 0.01 M sodium phosphate buffer, pH 7.2. CMV-A was purified using a modification of Scott's method (5). After differential centrifugation, virus pellets were resuspended in 5 mM sodium borate buffer and 0.5 mM ethylenediaminetetraacetic acid at pH 9.0.

Electrophoresis of virion protein subunits. To obtain virion protein subunits for electrophoretic analyses, protein subunits from purified virus preparations (DYMV-A, CMV-A, DYMV-T, and CMV-L) were dissociated following the method described by Laemmli (3).

Samples were loaded in a sodium dodecyl sulfate (SDS) polyacrylamide gel, and electrophoresis was performed in a vertical gel apparatus (Mini-gel, Idea Scientific, Corvallis, OR) at 100 V for 3 hr. The stacking gel was 4% polyacrylamide and the separating gel 12% (1.5 mm thick). Molecular weight markers were lactalbumin (14.2×10^3), trypsin inhibitor (20.1×10^3), trypsinogen (24.0×10^3), and carbonic anhydrase (29.0×10^3).

RESULTS

Virus isolates and host reaction. Symptoms on field-grown wild groundnut were severe foliar mosaic or mild chlorotic mottle and stunting. In the greenhouse, CMV-A and DYMV-A caused symptoms in diagnostic plant

species similar to those incited by CMV-L and DYMV-T, respectively (Table 1); however, DYMV-A, unlike DYMV-T, did not infect *P. vulgaris* 'Great Northern.' Symptoms in artificially inoculated wild groundnut plants grown from seeds were similar to those observed under field conditions. CMV-A and CMV-L incited prominent mosaic and leaf distortion (Fig. 1A), and DYMV-A and DYMV-T caused mild and diffuse chlorotic mottle (Fig. 1B).

Electron microscopy. Electron microscopy of purified preparations and leaf dips of each virus isolate revealed isometric particles about 28 nm in diameter.

DsRNAs. When dsRNA was extracted from cowpea plants infected with CMV-A and analyzed in 6% polyacrylamide gels, four dsRNAs (2.0, 1.9, 1.3, and 0.5×10^6) were obtained. These dsRNAs were similar to those obtained with CMV-L (Fig. 2, lanes G and H). The first two dsRNAs of CMV in Figure 2 migrated as a single band; nevertheless, longer electrophoretic runs (3.5 hr) resolved both bands.

The dsRNA from plants infected with DYMV-A was identical to that of DYMV-T (Fig. 2, lanes E and F). A major dsRNA (4.1×10^6) was obtained, together with minor bands. TYMV had a major dsRNA of about 3.9×10^6 and several bands of lower molecular weight.

Virus purification and serology. After purification, yields of about 300 and 100 mg per kilogram of tissue were obtained for DYMV-A and CMV-A, respectively. Extracts from infected plants and purified preparations of CMV-A reacted with antiserum to CMV-C (Fig. 3A), as did 11 other isolates from wild groundnut. Similarly prepared extracts and purified preparations of DYMV-A reacted with antiserum to DYMV-T. However, a spur was evident when the precipitin line of DYMV-T was adjacent to that formed by DYMV-A, indicating antigenic difference (Fig. 3B). Two other wild groundnut isolates also gave a positive reaction with this antiserum. TYMV, another tymovirus used for comparison, also reacted with antiserum to DYMV-T.

Protein electrophoresis. Capsid protein subunits with molecular mass of about 24 and 25 kDa were detected in SDS polyacrylamide gel for DYMV-A and CMV-A, respectively, and comigrated with the coat protein of DYMV-T and CMV-L, respectively.

DISCUSSION

The results clearly established that two viruses, CMV and DYMV, were causal agents of diseases observed in *A. americana*. Although this preliminary survey of viral infection in *A. americana* was limited to an experimental field in Baton Rouge, many of the wild groundnut accessions in the field were derived from

Table 1. Reactions^a of diagnostic plant species inoculated with the legume strain of cucumber mosaic virus (CMV-L), the type strain of Desmodium yellow mottle virus (DYMV-T), and two virus isolates from *Apios americana* (DYMV-A and CMV-A)

Species	DYMV-T	DYMV-A	CMV-A	CMV-L
<i>Apios americana</i>	MSM	MSM	SSM	SSM
<i>Chenopodium quinoa</i>	—	—	CLL	CLL
<i>Cucumis sativus</i> 'Marketer'	—	—	SM	SM
<i>Cucurbita pepo</i> 'Seneca Zucchini'	—	—	SSM	SSM
<i>Nicotiana tabacum</i> 'Havana 423'	—	—	SM	SM
<i>Phaseolus vulgaris</i>				
'Black Turtle Soup'	CLL, MSM	CLL, MSM	SM	SM
'Great Northern'	MSM	—	SM	SM
'Pinto'	NLL	NLL	SM	SM
<i>Pisum sativum</i>				
'Bonneville'	SM	SM	SM, SS	SM, SS
'Ranger'	SM	SM	SM, SS	SM, SS
<i>Vigna unguiculata</i>				
'Blackeye No. 5'	MSM	MSM	CLL, SM	CLL, SM
'TVu 612'	NLL	NLL	CLL, SM	CLL, SM

^a CLL = chlorotic local lesions; NLL = necrotic local lesions; SM = systemic mottle or mosaic; SSM = severe mosaic; MSM = mild mosaic or mottle; SS = stem streak; — = no symptoms and no virus recovery.



Fig. 1. Wild groundnut (*Apios americana*) leaves infected with two virus isolates from wild groundnut. (A) Mosaic and leaf distortion induced by cucumber mosaic virus. (B) Mottle induced by Desmodium yellow mottle virus.

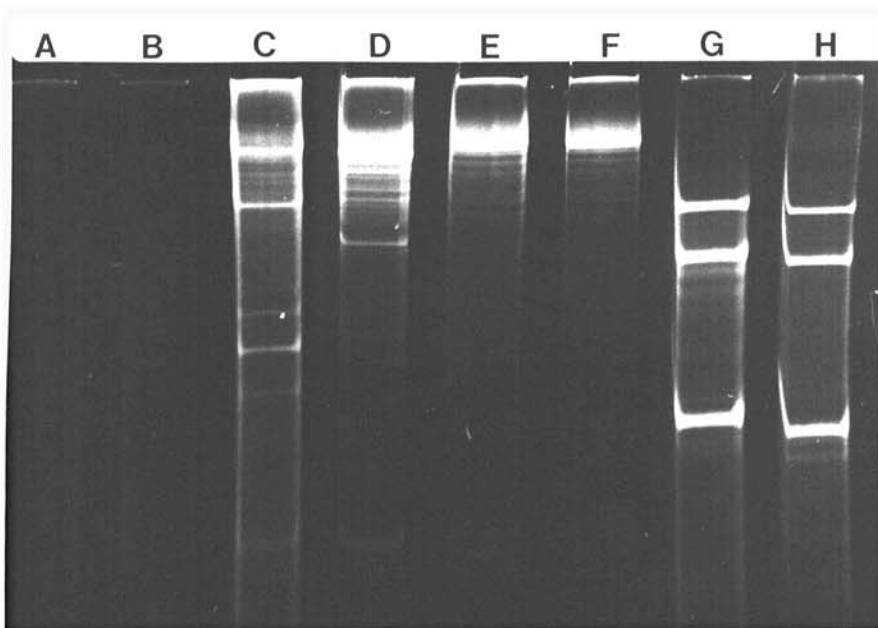


Fig. 2. Polyacrylamide gel electrophoresis (6%) of double-stranded RNAs (dsRNAs) extracted from plants infected with Desmodium yellow mottle virus (DYMV), cucumber mosaic virus (CMV), and turnip yellow mosaic virus (TYMV). Lane A, healthy *Brassica campestris*. Lane B, healthy cowpea (cv. Blackeye No. 5). Lane C, molecular weight markers (dsRNAs from tobacco mosaic virus and tobacco necrosis virus). Lane D, TYMV. Lane E, a wild groundnut isolate of DYMV (DYMV-A). Lane F, the type strain of DYMV (DYMV-T). Lane G, a wild groundnut isolate of CMV (CMV-A). Lane H, the legume strain of CMV (CMV-L). Gels were run at 100 V for 2.5 hr and were stained in ethidium bromide (10 µg/ml).

vegetatively propagated material collected in several regions of North America. Thus, viruses recovered from infected plants may have been of local origin or may have been introduced with infected tubers.

CMV was the most common virus infecting the crop in the experimental field, and it caused symptoms more severe than those incited by DYMV. CMV infects many crops in Louisiana and elsewhere and is spread by a large number of aphid species (2). Although its host range includes more than 800 plant species, to our knowledge CMV has not been reported previously in the genus *Apios*.

DYMV was described by Walters and Scott in Arkansas in 1971 (8). Like other tymoviruses, DYMV is easily transmitted by mechanical means and in nature may be spread by beetles; however, no specific vector is known at present. The isolate of DYMV recovered from wild groundnut (DYMV-A) differs serologically from the type strain (DYMV-T). The formation of a spur in double-diffusion tests indicates that some antigens present in DYMV-T are lacking in DYMV-A. Also, unlike DYMV-T, DYMV-A is unable to infect Great Northern beans. Thus, DYMV-A

is a new serotype of DYMV. This is the first report of the natural occurrence of DYMV in a genus other than *Desmodium*.

Additional surveys in Louisiana and elsewhere may reveal that other viruses infect *A. americana*. No virus could be transmitted mechanically from five of the 20 field-collected samples showing mosaic and mottle symptoms. These plant samples could have been infected with a nonmechanically transmitted virus(es). Although the effect of CMV and DYMV on wild groundnut production is not yet known, every effort should be made to propagate only accessions found to be free of viral infections.

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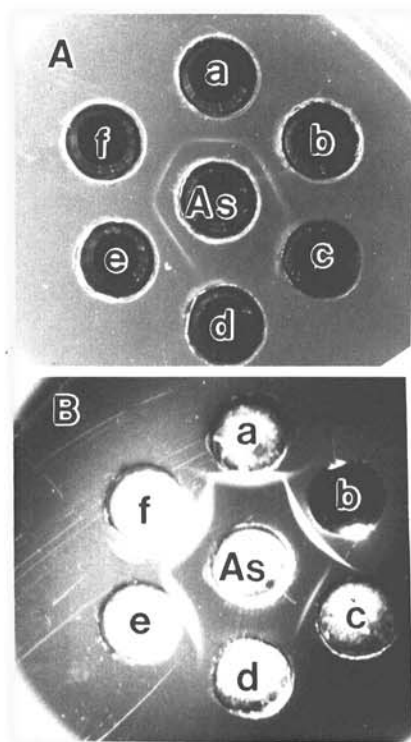


Fig. 3. Ouchterlony double-diffusion tests in 1% agarose in 0.01 M sodium phosphate buffer, pH 7.0. (A) The center well contained antiserum to cucumber mosaic virus (CMV) isolate C (CMV-C). Peripheral wells contained sap extracts of a wild groundnut isolate of CMV (CMV-A) (a and f); the legume strain of CMV (CMV-L) (b); healthy cowpea (c and d); and a purified preparation (1.0 mg/ml) of CMV-A (e). (B) The center well contained antiserum to the type strain of Desmodium yellow mottle virus (DYMV) (DYMV-T). Peripheral wells contained a sap extract of DYMV-T (a); a purified preparation (1.0 mg/ml) of a wild groundnut isolate of DYMV (DYMV-A) (f); turnip yellow mosaic virus (b); healthy cowpea (cv. Blackeye No. 5) (c); and sap extracts of DYMV-A (e and d).

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