Host Range and Some Properties of Desmodium Yellow Mottle Virus

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ABSTRACT

A virus causing yellow mottling and occurring naturally in *Desmodium* spp. infects many leguminous hosts by mechanical inoculation, with no infection occurring in inoculated plants in families other than Leguminoseae. Great Northern bean plants became systemically infected with dilutions up to 10^{-7}, but not 10^{-8}. The virus was infectious when heated 10 min at 70 C, but not at 75 C. Virus stored at 20 C was infectious for 38 days, but not after 44 days. No transmission of the virus was obtained with three species of beetles and the green peach aphid. Electron micrographs of purified virus preparations revealed "full" and "empty" spherical particles ca. 30 nm in diam. Gel-diffusion tests resulted in spur formation when the virus was reacted against antiserum of turnip yellow mosaic virus. The virus has been named "Desmodium yellow mottle virus".
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Additional key words: wild cucumber mosaic virus, cocoa yellow mosaic virus, insect transmission.

Several viruses have been found to naturally infect *Desmodium* species. Dale (5) reported a mosaic disease of cowpea, *Vigna unguiculata*, as infecting *Desmodium frutescens* in Trinidad. Van Velson & Crowley (19) found that centrosema mosaic virus occurred in *D. distortum*. Hutton & Grylls (8) showed that the "little leaf" virus of legumes naturally infects D. *uncinatum*. Walters & Dodd (20) found a strain of cowpea chlorotic mottle virus naturally infecting *D. laevigatum*. Recently, Edwardson et al. (6) isolated a virus designated as Desmodium mosaic virus from *D. canum*. Other viruses have been reported to be mechanically transmitted to *Desmodium* species. Murphy & Pierce (15) found that the common mosaic of garden pea, *Pisum sativum*, could infect *Desmodium canadense*, and Adsuar (1) reported that a mosaic disease of cowpea in Puerto Rico was transmitted to *D. distortum*.

A new virus disease was found in *D. laevigatum* and *D. paniculatum* growing in several areas in Arkansas (21). The virus is serologically related to turnip yellow mosaic virus (TYMV), and has been named "Desmodium yellow mottle virus" (DYMV).

Markham & Smith (12) first described TYMV in England. The virus has been reported in Portugal (2) and Denmark (10), but has not been reported in countries other than those in Europe. Other than *Reseda odorata* (3) and *Nicotiana tabacum* var. *Xanthi* (17), TYMV has been reported to infect mainly members of the Cruciferae (14). Wild cucumber mosaic virus (7) and cocoa yellow mosaic virus are serologically related to TYMV (4). The relationship is unusual in that WCMV antiserum reacted with both CYMV and TYMV, but there was no reaction between TYMV and CYMV antiserum or CYMV and TYMV antiserum.

MATERIALS AND METHODS.—Because *Desmodium* spp. are not a very good source of virus, the inoculum for DYMV for all tests was prepared from *Phaseolus vulgaris* L. 'Great Northern' plants that had been infected for 10-14 days. Great Northern bean was used as the assay plant for all tests.

For host range studies, at least six plants of each species or cultivar were inoculated with crude sap in 0.01 M phosphate buffer, pH 7.2. Regardless of the type or lack of symptoms, assays were made from inoculated plants to Great Northern bean to determine the presence of the virus.

Crude sap diluted with distilled water was used for dilution end point studies. For thermal inactivation and longevity in vitro tests, the sap was diluted 1:10 with distilled water.

The bean leaf beetle, *Ceratoma trifurcata* Forst.; the spotted cucumber beetle, *Diabrotica undecimpunctata* Mann.; the weevil, *Apion roseae* (Kiss.); and the green peach aphid, *Myzus persicae* (Sulz.) were used in transmission tests with DYMV. Following a 24-hr acquisition feeding on infected Great Northern bean plants, individual bean leaf beetles and spotted cucumber beetles were transferred daily to healthy young Great Northern bean plants for 3 days. *Apion roseae* was tested in a similar manner, with five beetles placed on each test plant. Green peach aphids were allowed a 30- to 60-sec acquisition feeding on DYMV-infected tissue, transferred in groups of 10 to leaves of healthy bean plants for about 1 hr, and then killed by fumigation.

For purification, Steere's extraction method (18) was modified by the homogenizing of the infected leaf tissue in a blender with 1 volume of 0.2 M phosphate buffer, pH 7, with an equal amount of a mixture of 1:1 *n*-butanol and chloroform. After centrifugation for 15 min at about 2,500 *g*, the aqueous phase was removed, allowed to sit overnight, and clarified at 10,000 *g*. Three differential centrifugations were made for 1 hr at 80,000 *g* and 10 min at 3,000 *g*. Pellets were suspended in 0.01 M phosphate buffer, pH 7.2.

Serological studies were made with the Ouchterlony gel-diffusion test (16).

Purified virus was stained with 2% phosphotungstic acid, pH 6.8. Micrographs were

taken with a Siemens Elmiskop 1A electron microscope.

RESULTS.—*Symptomatology.*—*Desmodium* plants infected with DYMV under natural conditions had a yellow mottle with some leaf deformity. Desmodium yellow mottle virus has been found occurring in nature with cowpea chlorotic mottle virus (H. J. Walters, *unpublished data*), and symptoms are more severe than when DYMV occurs by itself. A mixture of the two viruses causes severe malformation of the leaves, some stunting, and a deep yellow mottle.

Host range.—Numerous hosts were infected with DYMV by mechanical inoculation. In general, four types of host-susceptible responses were observed: (i) yellow mottle; (ii) green to mild chlorotic mottle; (iii) local lesion; and (iv) symptomless.

Yellow mottle occurred in *Desmodium laevigatum* (Nutt.) DC.; *D. canum* Schinz & Thellung; *D. cuneatum* Hook. & Arn.; *D. hassleri* (Schindl.) Burkart; *D. cilane* (Muyl. ex Willd.) DC.; *D. marylandicum* (L.) DC.; *D. paniculatum* (L.) DC.; *Lespedeza stipulacea* Macim. 'Summit' and 'Climax'; *L. striata* (Thunb.) H. & A. 'Kobe' and 'F. C. 31057-5'; *Trifolium incarnatum* L. 'Dixie Auburn' and 'Frontier'; *T. repens* L. 'Rega'; *T. histum* All.; *Medicago orbicularis* (L.) Bartalini; *Crotalaria juncea* L.; *C. longithyrsa* E. G. Baker; *C. intermedia* Kotshy; *C. mucronata* Desv.; and *C. lupulina* H.B.K.

Green-to-mild chlorotic mottle developed in *Phaseolus vulgaris* L. 'Great Northern', 'Navy', 'Black', and 'Blue Lake'; *P. aureus* Roxb.; *P. lathyroides* L.; *Vigna sinensis* (Torner) Savi 'Black', 'Clay', 'Early Ramshorn', 'Georgia 21', 'Iron', and 'Monarch'; *V. sesquipedalis* (L.) Fruwirth; *Canavalia ensiformis* (L.) DC.; *Lespedeza sericea* Miq.; *Strophostyles helvola* (L.) Ell.; *Lupinus albus* L.; *L. angustifolius* L.; *Rhynochosia phaseoloides* DC.; *R. minima* (L.) DC.; *Galactia elliottii* Nutt.; *Sesbania exaltata* (Raf.) Cory; and *Dolichos biflorus* L.

Virus was recovered from the following symptomless hosts that were inoculated and observed from 4 to 6 weeks: *Phaseolus limenses* Macf. 'Henderson', *Pisum sativum* L. 'Thomas Laxton', and *Lathyrus odoratus* L.

Local lesions, although not suitable for assay studies, developed on the following cultivars of beans: Black Valentine, Bountiful, Pinto, Tendercrop, and Wade. Local lesions developed on several *Desmodium* spp. and other hosts, and these subsequently became systemically infected. Distinct local lesions suitable for assay studies developed on inoculated leaves of a species of broad-leafed *Desmodium* (Fig. 1).

Desmodium yellow mottle virus was not recovered from the following inoculated plant species: *Phaseolus vulgaris* L. 'Red Kidney' and 'Small White'; *P. atropurpureus* DC.; *Trifolium pratense* L. 'Kenland', 'Orbit', and 'Tensas'; *Medicago sativa* L. 'Vernal'; *Lotus corniculatus* L.; *Mucuna duringianum* (Bort) Merr.; *Glycine max* (L.) Merr. 'Hill' and 'Lee'; *Lens culinaris* Medik.; *Dolichos falcatus* Klein.; *D. lablab* L.; *Aster laevis* L., *Calendula officinalis* L.; *Tithonia rotundifolia* (Mill.) Blake; *Zinnia elegans*

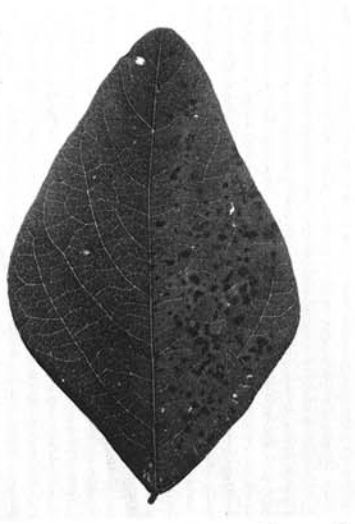


Fig. 1. Local lesions caused by Desmodium yellow mottle virus on a broad-leafed species of *Desmodium*. Left half of leaf was not inoculated.

Jacq.; *Chenopodium album* L.; *C. hybridum* L.; *C. quinoa* Willd.; *Brassica oleracea* L. 'Viridis'; *B. rapa* L.; *B. juncea* (L.) Coss; *B. chinensis* L.; *B. pekinensis* (Lour) Rupr.; *Matthiola incava* (L.) R. Br. var. *annua* (L.) Voss; *Datura stramonium* L.; *Nicotiana glutinosa* L.; *N. rustica* L.; *N. tabacum* L. 'Havana 38' and 'F_2Cl'; *Petunia hybrida* Vilm.; *Ipomoea leptophylla* Torr.; *Antirrhinum majus* L.; *Dianthus barbatus* L.; *Gomphrena globosa* L.; *Ricinus communis* L.; *Sesamum indicum* L.; *Centrosema virginianum* (L.) Benth.; and *Cyamopsis tetragonolobus* (L.) Taub.

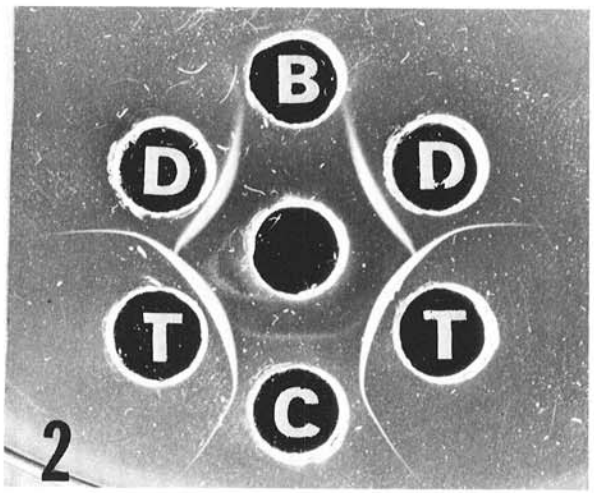
Physical properties.—Plants inoculated with dilutions up to 10^{-7} became systemically infected. No infection occurred at the dilution of 10^{-8}. The virus was infectious when heated 10 min at 70 C, but not at 75 C. Virus stored at 20 C was infectious for 38 days, but not after 44 days. Infective tissue, diced and dried over $CaCl_2$ at 5 C, was infectious after 2 years.

Transmission.—No transmission was obtained when 40 bean leaf beetles and 35 spotted cucumber beetles were fed on infected bean plants for 24 hr, then transferred daily to test plants, one insect/plant. Similar results were obtained with 100 *Apion roseae*, using five weevils/plant. One hundred-fifty green peach aphids failed to transmit DYMV.

Serology.—Ouchterlony, gel-diffusion tests using either viruliferous sap or purified virus resulted in spur formation when DYMV and TYMV were tested with TYMV antiserium (Fig. 2).

Electron microscopy.—The virus particles were spherical, with approximate diameters of 30 nm. The micrographs showed virus particles which were apparently normal, and particles which appeared to be empty shells (Fig. 3).

DISCUSSION.—The physical properties of DYMV are similar to those of TYMV. The two viruses are inactivated between 70 and 75 C. Both viruses have a rather high dilution end point. Markham & Smith



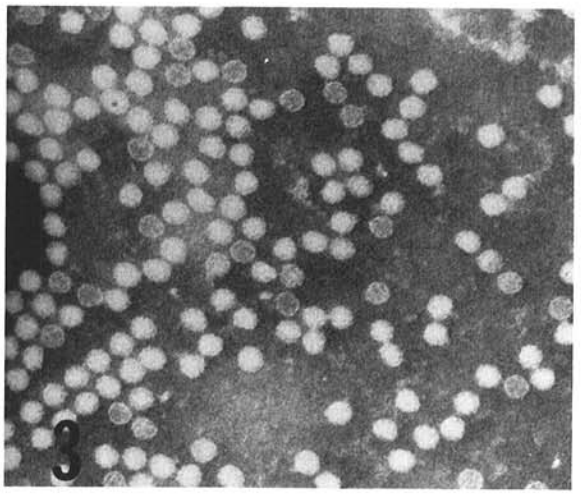


Fig. 2-3. 2) Agar gel diffusion test with Desmodium yellow mottle virus and turnip yellow mosaic virus. The center well contains antiserum to turnip yellow mosaic virus. The outer wells contain (B) sap of healthy Great Northern bean, (D) purified Desmodium yellow mosaic virus, (T) purified turnip yellow mosaic virus, and (C) sap of healthy Chinese cabbage. **3)** Electron micrograph of purified Desmodium yellow mottle virus stained with phosphotungstic acid showing both "full" and "empty" virus particles.

(13) reported the dilution end point of TYMV to be 10^{-6}, whereas that of DYMV was 10^{-8}.

Agar diffusion tests showed that DYMV and TYMV are closely related but not serologically identical.

The particles of DYMV have similar shape, size, and external morphology as those of TYMV. Markham (11) reported two types of protein associated with TYMV, the virus protein and T-protein which is hollow and contains no nucleic acid. The electron micrographs of Huxley & Zubay (9) revealed "empty" particles and particles containing nucleic acid in negatively stained preparations of TYMV. The two types of particles associated with DYMV appear to be similar to those reported by Markham (11) and Huxley & Zubay (9) for TYMV.

The host range of DYMV differs from that of TYMV. No hosts outside of the family Leguminosae are known to be susceptible to DYMV. Other than *Reseda odorata* and *Nicotiana tabacum*, only the members of Cruciferae are susceptible to TYMV.

Wild cucumber mosaic virus and cocoa yellow mosaic virus have been shown to be serologically related to TYMV (4). Of this group of related viruses, only TYMV has been shown to be transmitted by insects. Markham & Smith (13) showed that various types of insects with biting mouth parts would transmit TYMV. No transmission of DYMV was obtained with the spotted cucumber beetle, bean leaf beetle, the weevil *Apion roseae*, and the green peach aphid.

The economic importance of DYMV is not known. The virus will infect a number of important legumes, and may have deleterious effects on these crops.

LITERATURE CITED

1. ADSUAR, J. 1964. A mosaic disease of cowpea (Vigna sinensis Savi) in Puerto Rico. J. Agr. Univ. Puerto Rico 48:264.
2. BORGES, M. DE L. V. 1947. O virus do mosiaco amarelo do Nabo. Agron. Lusitana 9:253-264.
3. BROADBENT, L., & G. D. HEATHCOTE. 1958. Properties and host range of turnip crinkle, rosette, and yellow mosaic viruses. Ann. Appl. Biol. 46:535-592.
4. BRUNT, A. A., R. H. KENTON, A. J. GIBBS, & H. L. NIXON. 1965. Further studies on cocoa yellow mosaic virus. J. Gen. Microbiol. 38:81-90.
5. DALE, W. T. 1949. Observations on a virus disease of cow pea in Trinidad. Ann. Appl. Biol. 36:327-332.
6. EDWARDSON, J. R., D. E. PURCIFULL, F. W. ZETTLER, R. G. CHRISTIE, & S. R. CHRISTIE. 1970. A virus isolated from Desmodium canum: Characterization and electron microscopy. Plant Dis. Reptr. 54:161-164.
7. FREITAG, J. H. 1941. Comparison of the transmission of four cucurbit viruses by cucumber beetles and aphids. Phytopathology 31:8 (Abstr.).
8. HUTTON, E. M., & N. E. GRYLLS. 1956. Legume "little leaf", a virus disease of subtropical pasture species. Aust. J. Agr. Res. 7:85-97.
9. HUXLEY, H. E., & G. ZUBAY. 1960. The structure of the protein shell of turnip yellow mosaic virus. J. Mol. Biol. 2:189-196.
10. KRISTENSEN, H. R. 1957. Gulmosaic hos Kalroer i Danmark. Ugeskrift Landmaend, p. 49-60.
11. MARKHAM, R. 1951. Physiochemical studies of turnip yellow mosaic virus. Discussion Faraday Soc. 11:221-227.
12. MARKHAM, R., & K. M. SMITH. 1946. A new crystalline virus. Nature 157:300.
13. MARKHAM, R., & K. M. SMITH. 1949. Studies on the virus of turnip yellow mosaic. Parasitology 39:330-342.

14. MATTHEWS, R. E. F., & R. K. RALPH. 1966. Turnip yellow mosaic virus. Advances Virus Res. 12:273-328.
15. MURPHY, D. M., & W. H. PIERCE. 1937. Common mosaic of the garden pea, Pisum sativum. Phytopathology 27:710-721.
16. OUCHTERLONY, O. 1962. Diffusion-in-gel methods for immunological analysis. III. Progress Allergy 6:30-154.
17. SANDER, E., & G. SCHRAMM. 1963. Die Bedeutung der Proteinhulle fur die Wirtsspezifitat vor Pflanzenviren. Z. Naturforschung 18b:199-202.
18. STEERE, R. L. 1956. Purification and properties of tobacco ringspot virus. Phytopathology 46:60-69.
19. VAN VELSON, R. J., & N. C. CROWLEY. 1961. Centrosema mosaic: A plant virus disease transmitted by both aphids and plant bugs. Nature 189:858.
20. WALTERS, H. J., & N. L. DODD. 1969. Identification and beetle transmission of an isolate of cowpea chlorotic mottle virus from Desmodium. Phytopathology 59:1055 (Abstr.).
21. WALTERS, H. J., & H. A. SCOTT. 1968. A virus isolate from Desmodium related to turnip yellow mosaic. Phytopathology 58:1071 (Abstr.).