

Morphology, Host Range, and Serological Relationships of Pepper Mottle Virus

D. E. Purcifull, T. A. Zitter, and Ernest Hiebert

First and third authors, Department of Plant Pathology, University of Florida, Gainesville 32611; and second author, Agricultural Research and Education Center, University of Florida, P.O. Drawer A, Belle Glade 33430.

Supported in part by National Science Foundation Grant GB-32093. We thank A. A. Brunt for pepper veinal mottle virus and its antiserum, M. R. Nelson for the Arizona pepper virus antigens, and S. R. Christie and W. E. Crawford for technical assistance.

Florida Agricultural Experiment Station Journal Series Paper No. 5583.

Accepted for publication 5 December 1974.

ABSTRACT

Filamentous particles with a predominant length of about 737 nm were found in extracts from plants infected with a Florida isolate of pepper mottle virus (PeMV). PeMV induced symptoms in six species of the family Solanaceae, but not in three other solanaceous species or in 19 species representing 10 other families. On the bases of immunodiffusion tests with antisera to PeMV, this virus is distinct from, but related to, three potato Y group viruses which infect pepper: pepper veinal mottle, potato Y, and

tobacco etch viruses. Evidence was obtained that PeMV is serologically identical to a pepper virus from Arizona previously reported by Nelson and Wheeler, and also that the lamellar inclusions (pinwheels and related structures) induced by these two viruses are serologically identical. The morphological and serological properties of PeMV provide further evidence that it is a member of the PVY group.

Phytopathology 65:559-562

Zitter (14) recently isolated an atypical potato virus Y (PVY) from pepper in Florida. This isolate has subsequently been included in several studies under the tentative name of pepper mottle virus (PeMV) (5, 8, 12, 15). Previous work has indicated that PeMV induces pinwheel inclusions in its hosts (5, 8), and on this basis it has been included as a member of the PVY group (5). Preliminary studies have indicated that PeMV is serologically distinguishable from tobacco etch virus (TEV) and PVY (12, 14), both of which commonly occur in pepper in Florida. Two other PVY-group viruses that have been reported to occur naturally in pepper elsewhere are pepper veinal mottle virus in Ghana (3) and Arizona pepper virus (10).

This report presents evidence that PeMV is serologically identical to the Arizona pepper virus, but that PeMV is distinct from PVY, TEV, and pepper veinal mottle viruses. Morphological evidence is also presented to further support the classification of PeMV as a member of the PVY group.

MATERIALS AND METHODS.—The PeMV culture used in this study was isolated from naturally infected pepper (14) and maintained by mechanical transfer in tobacco (*Nicotiana tabacum* L. 'Turkish NN') or in a *Nicotiana* hybrid (4). Both types of PeMV-infected *Nicotiana* were used as sources of virus for purification.

In the host-range trials, crude sap from infected plants was rubbed on Carborundum-dusted leaves of test plants, which were observed for symptoms for at least 1 month after inoculation. In some cases, extracts from leaves of test plants were checked in an electron microscope for presence of rod-shaped particles.

For serological comparison with PeMV, the following viruses were used: Bidens mottle virus (PV-165), TEV (PV-69), and lettuce mosaic virus (PV-63) (American Type Culture Collection accession numbers listed in parentheses); a PVY isolate from North Carolina (9); Arizona pepper virus (10); pepper veinal mottle virus (3); and a Florida isolate of turnip mosaic virus.

Leaf extracts were mounted in potassium phosphotungstate as previously described (11) to study particle and inclusion morphology. Particle lengths were determined by comparison to a diffraction grating, 2,156 lines/mm (54,864 lines/inch).

PeMV was purified by the following procedures. Infected leaf tissue was homogenized in 0.5 M potassium phosphate buffer, pH 7.5, containing 1% sodium sulfite (g tissue/1-2 ml buffer). The homogenate was clarified by addition of *n*-butanol (8%, v/v) or with chloroform (10%, v/v). After slow-speed centrifugation, the virus was precipitated from the supernatant by addition of polyethylene glycol 6000 (8%, w/v) with stirring for at

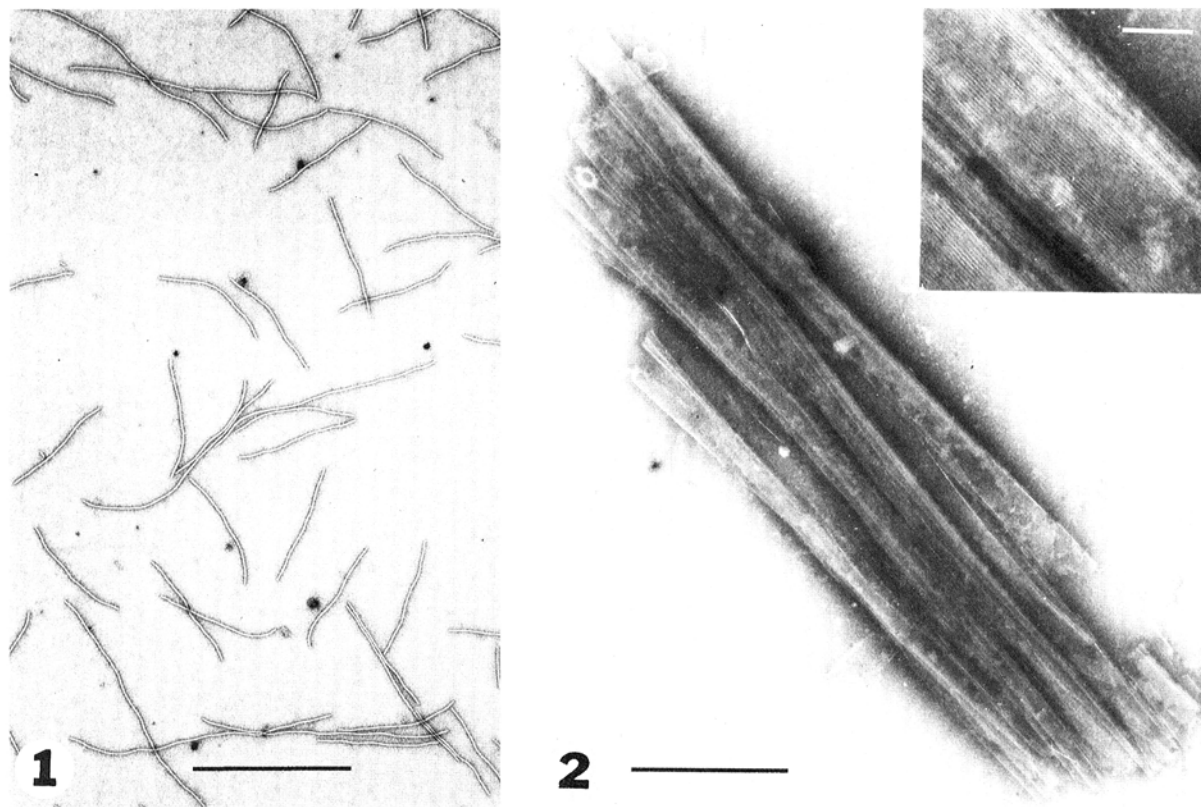


Fig. 1-2. 1) Electron micrograph of purified preparation of pepper mottle virus (PeMV) negatively stained with potassium phosphotungstate (Bar = 1 μ m). 2) Electron micrograph of PeMV-induced lamellar inclusions in crude extract from leaves of a pepper plant inoculated with partially purified PeMV (Bar = 1 μ m). Note striated nature of inclusions as depicted in inset (Bar in inset = 100 nm).

least 1 hour. The virus was pelleted by low-speed centrifugation, resuspended in 0.02 M phosphate, pH 7.5, or in 0.02 M borate, pH 8.0, and given one cycle of differential centrifugation. The virus preparations were then further purified by rate-zonal sucrose density-gradient centrifugation and/or by equilibrium density-gradient centrifugation in CsCl. The purified preparations were emulsified 1:1 with Freund's incomplete adjuvant and injected intramuscularly into two rabbits. One animal received a first injection of 3 mg, with a second injection of 0.5 mg seven weeks later. The other rabbit was injected initially with 2 mg followed 3 weeks later by an injection of 1.5 mg. Both animals were bled periodically for several weeks following the second injection.

An antiserum specific for PeMV-induced lamellar inclusions (12) was used for serological detection and comparison of inclusion proteins.

Immunodiffusion tests were conducted in agar gels containing sodium dodecyl sulfate (SDS) (6) using crude extracts prepared in SDS (12). The extracts were used fresh or were lyophilized in the presence of SDS and reconstituted with water prior to use (Purcifull and Christie, *unpublished*). Gel patterns consisted of six peripheral wells (7 mm in diameter) at a distance of 5 mm from a central well (also 7 mm in diameter). Results were recorded after the plates were incubated for 24-48 hours at 24 C.

RESULTS.—Host range.—PeMV induced symptoms only on certain solanaceous species. The following species developed a systemic mottle: *Capsicum annuum* L., *Lycopersicon esculentum* Mill., *Nicotiana* hybrid, *N. tabacum* 'Turkish NN' and 'Havana 425', *Physalis floridiana* Rybd., and *Solanum* sp. (nightshade). Tabasco pepper (*C. frutescens* L.) developed local lesions on inoculated leaves and systemic symptoms consisting of a mottle in older plants or necrosis in younger plants (14).

The following test plants showed no symptoms and rod-shaped particles were not detected by electron microscopic examination of negatively stained extracts from leaves above the point of inoculation: *Cucurbita pepo* L. 'Early Prolific Straightneck', *Datura stramonium* L., *Gomphrena globosa* L., *N. tabacum* L. 'V20', *Pisum sativum* L. 'Little Marvel', *Solanum tuberosum* L. 'Russet Burbank', *Zea mays* L. 'Golden Cross Bantam', *Zinnia elegans* Jacq.

No symptoms were observed on any of the following inoculated species, but neither recovery attempts nor electron microscopic examinations were made: *Beta vulgaris* L., *Bidens pilosa* L., *Brassica pekinensis* (Lour.) Rupr., *Carthamus tinctorius* L., *Cassia tora* L., *Chenopodium amaranticolor* Coste et Reyn., *Chenopodium quinoa* Willd., *Cichorium endivia* L., *Datura metel* L., *Helianthus annuus* L., *Lepidium sativum* L., *Momordica charantia* L., *Phytolacca americana* L., *Portulaca oleracea* L., and *Verbena hybrida* Voss.

Electron microscopy.—Flexuous, rod-shaped particles were consistently found in crude extracts from tobacco leaves infected with PeMV. Of 129 particles measured, 102 particles were between 729-745 nm long with the modal class at 737 nm. Partially purified preparations of PeMV also contained flexuous, rod-shaped particles (Fig. 1) and were infectious when mechanically

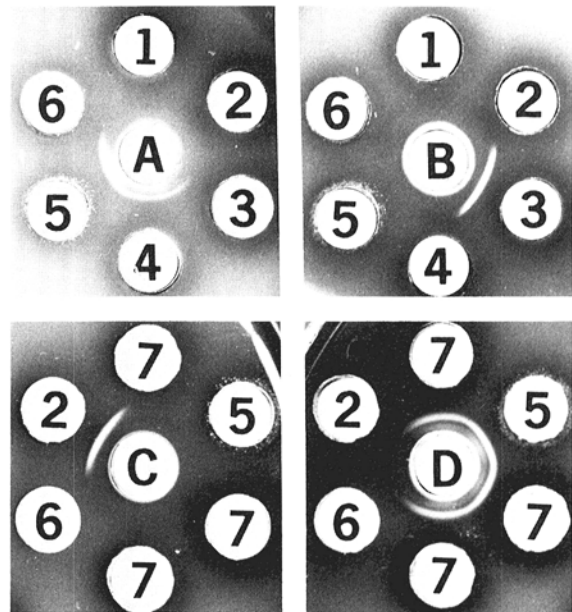


Fig. 3. Immunodiffusion tests with pepper mottle virus (PeMV) and other potato Y (PVY) group viruses which naturally infect pepper. The center wells were charged with: (A) PeMV antiserum, (B) PVY antiserum, (C) Brunt's pepper vein mottle virus antiserum, and (D) PeMV-inclusion antiserum. The peripheral wells were charged with sodium dodecyl sulfate-treated extracts from: (1) Tobacco etch virus-infected pepper, (2) pepper vein mottle virus-infected pepper, (3) PVY-infected pepper, (4) PeMV-infected pepper, (5) Arizona pepper virus-infected pepper, (6) healthy pepper, and (7) pepper infected by inoculation with purified PeMV.

inoculated to pepper or tobacco. Rod-shaped particles also were found in leaf extracts from plants inoculated with purified virus.

Striated, lamellar inclusions were readily detected in crude plant extracts, including extracts from plants infected by inoculation with purified PeMV (Fig. 2).

Serology.—The virus preparations were immunogenic and the PeMV antisera gave strong reactions with PeMV in gels containing SDS, but they did not react with sap from healthy plants (Fig. 3-A). PeMV antiserum from one rabbit was specific for PeMV, and it did not react with antigens of the following viruses which infect pepper: TEV, PVY, pepper vein mottle (Fig. 3-A). This PeMV antiserum also did not react with lettuce mosaic, bidens mottle, and turnip mosaic viruses. The PeMV antiserum from the other rabbit reacted weakly with PVY and pepper vein mottle virus, but the homologous reactions spurred over the heterologous precipitin lines. Two PVY antisera either reacted weakly with PeMV or failed to react with it (e.g., Fig. 3-B). One of three TEV antisera also reacted weakly with PeMV, but PeMV antigens failed to react with antisera to pepper vein mottle (Fig. 3-C), bidens mottle, turnip mosaic, and lettuce mosaic viruses. All antisera tested gave positive reactions with homologous antigens.

Antigens in sap from plants infected with the Arizona pepper virus gave reactions of identity to PeMV when tested against antisera either to PeMV (Fig. 3-A) or to

PeMV-inclusion protein (Fig. 3-D). The PeMV inclusion antiserum used in this study did not react with extracts from plants infected with pepper veinal mottle virus, TEV or PVY. Extracts from plants which had been infected by partially purified PeMV reacted with both the PeMV-inclusion antiserum (Fig. 3-D) and PeMV antiserum.

DISCUSSION.—On the bases of its particle morphology, serological relationships, aphid transmissibility (Zitter, *unpublished*), and ability to induce pinwheel inclusions in its host cells (5), PeMV should be classified in the PVY group (1, 2, 5) or potyvirus group (7). In addition to PeMV, Edwardson (5) listed four viruses of the PVY group that are known to infect pepper: Arizona pepper virus, pepper veinal mottle virus, PVY, and TEV. PeMV was found to be serologically distinct from, although related to, the latter three viruses.

Cross reactivity of the degraded proteins of different PVY group viruses can be expected on the basis of previous work (e.g., 13). The Arizona pepper virus, however, gave reactions of identity with PeMV, and it is concluded that these two viruses are closely related strains. The types of inclusion bodies induced by Arizona pepper virus and PeMV are also strikingly similar (5). They appear to differ somewhat in host range, however, in that the Arizona isolate infected *Datura stramonium* L. (10) whereas the Florida isolate did not infect this species.

The antiserum specific for PeMV-inclusions reacted only with extracts from plants infected with PeMV from Florida and the Arizona isolate of PeMV, but not with extracts from plants infected by TEV, PVY, or pepper veinal mottle virus. Partially purified preparations of PeMV, which were devoid of inclusions, induced morphologically and serologically characteristic lamellar inclusions when inoculated to susceptible hosts. These observations support the previous suggestions that the lamellar inclusions are virus-coded and that serological properties of the inclusions are useful for diagnosis and classification (9, 12).

LITERATURE CITED

- BRANDES, J. 1964. Identifizierung von gestreckten pflanzenpathogenen viren auf morphologischer Grundlage. Mitt. Biol. Bundesanst. Land Forstwirtsch. (Berlin-Dahlem) 110:1-130.
- BRANDES, J., and R. BERCKS. 1965. Gross morphology and serology as a basis for classification of elongated plant viruses. Adv. Virus Res. 11:1-24.
- BRUNT, A. A., and R. H. KENTEN. 1971. Pepper veinal mottle virus—a new member of the potato Y group from peppers (*Capsicum annum* L. and *C. frutescens* L.) in Ghana. Ann. Appl. Biol. 69: 235-243.
- CHRISTIE, S. R. 1969. Nicotiana hybrid developed as a host for plant viruses. Plant Dis. Rep. 53:939-941.
- EDWARDSON, J. R. 1974. Some properties of the potato Y-group. Fla. Agric. Exp. Stn. Monogr. 4. 398 p.
- GOODING, G. V., JR., and W. W. BING. 1970. Serological identification of potato virus Y and tobacco etch virus using immunodiffusion plates containing sodium dodecyl sulfate. Phytopathology 60:1293 (Abstr.).
- HARRISON, B. D., J. T. FINCH, A. J. GIBBS, M. HOLLINGS, R. J. SHEPHERD, V. VALENTA, and C. WETTER. 1971. Sixteen groups of plant viruses. Virology 45:356-363.
- HIEBERT, E., and J. G. MC DONALD. 1973. Characterization of some proteins associated with viruses in the potato Y group. Virology 56:349-361.
- HIEBERT, E., D. E. PURCIFULL, R. G. CHRISTIE, and S. R. CHRISTIE. 1971. Partial purification of inclusions induced by tobacco etch virus and potato virus Y. Virology 43:638-646.
- NELSON, M. R., and R. E. WHEELER. 1972. A new virus disease of pepper in Arizona. Plant Dis. Rep. 56:731-735.
- PURCIFULL, D. E., J. R. EDWARDSON, and S. R. CHRISTIE. 1970. A morphological comparison of inclusions induced by tobacco etch and potato Y viruses. Phytopathology 60:779-782.
- PURCIFULL, D. E., E. HIEBERT, and J. G. MCDONALD. 1973. Immunochemical specificity of cytoplasmic inclusions induced by viruses in the potato Y group. Virology 55:275-279.
- SHEPARD, J. F., G. A. SECOR, and D. E. PURCIFULL. 1974. Immunochemical cross-reactivity between the dissociated capsid proteins of PVY group plant viruses. Virology 58:464-475.
- ZITTER, T. A. 1972. Naturally occurring pepper virus strains in south Florida. Plant Dis. Rep. 56:586-590.
- ZITTER, T. A., and A. A. COOK. 1973. Inheritance of tolerance to a pepper virus in Florida. Phytopathology 63:1211-1212.