Plantago Mottle Virus, a New Member of the Tymovirus Group

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ABSTRACT

Plantago mottle virus (PlMV) is a new member of the b-subgroup of the tymoviruses. Virus particles are isometric and average 26.7 nm in diam. Particles occasionally form pseudo-crystals within leaf cytoplasm. PlMV has an extinction coefficient of 8.51 at 260 nm. The nucleic acid of the virus has a molar base composition of guanine 17%, adenine 21%, cytosine 34%, and uracil 28%. Serologically, it is related to turnip yellow mosaic, ononis yellow mottle, andean potato latent, dulcamara mottle, and belladonna mottle viruses.

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Additional key word: *Pisum sativum*.

Tymovirus is one of 16 groups of plant viruses proposed by Harrison et al. (5), and turnip yellow mosaic (TYMV) represents the type member. The two subgroups into which it was divided contained seven viruses, and to these a few more have been added in recent years (1, 12). This paper presents evidence that a virus originally isolated from *Plantago major* L. (4), and herein known as plantago mottle (PlMV), is a new member of this group.

MATERIALS AND METHODS.—Plantago mottle virus was maintained in *Plantago major* or *Pisum sativum* L. 'Ranger'.

For host range studies, infected pea leaves were ground in 0.01 M phosphate buffer, pH 7 and mechanically inoculated onto Carborundum-dusted leaves of test plants grown in the greenhouse. To determine the effect of temperature on symptom expression, a few tests were conducted in growth chambers. Recovery tests were made from all the host plants tested, and infection was confirmed by reinoculation to pea, electron microscopy, or serology.

The physical properties of PlMV were determined with sap extracted from infected pea leaves diluted with 0.1 M phosphate buffer, pH 7. Sap was sealed in capillary tubes and heated in a water bath for 10 min to determine the heat inactivation temperature. *Plantago major* or pea were mechanically inoculated for bioassays.

For electron microscopy, crude plant extracts as well as purified preparations were negative-stained with neutral 2% (w/v) potassium phosphotungstate, and viewed on a Jeolco 100 B electron microscope at × 30,000. In addition, leaf tissue or pellets containing purified virus were processed for 1 hr each in cold Karnovsky's fixative (6), cold 0.1 M cacodylate buffer, pH 7, and 1% osmium tetroxide buffered with s-collidine at pH 7. Dehydration was carried out in an acetone series and propylene oxide, after which the pieces were embedded in a low viscosity epoxy resin (10). Sections less than 100 nm thick were cut on a Porter-Blum MT-2 microtome with a diamond knife, mounted on copper grids, and stained with 2% uranyl acetate for 1 hr and lead citrate for 2 min (11).

The virus was purified by gradually adding infected pea leaves to two volumes (v/w) of 0.05 M phosphate buffer, pH 7 containing 0.1% mercaptoacetic acid in a Waring Blendor. The blender speed was increased until the leaves were homogenized and the resulting pulp was filtered through cheesecloth. The extract was clarified by adding 8% (v/v) butanol (washed with 15% sodium bisulfite and water) and refrigerated overnight at 5 C. After centrifugation (10,000 *g*, 10 min, 5 C), 242 g solid ammonium sulfate were added to each liter of the butanol phase supernatant. The resulting precipitate was extracted by centrifugation (10,000 *g*, 10 min, 5 C) and was dialyzed against 0.05 M phosphate buffer, pH 7, for 24 hr at 5 C before two series of differential centrifugations (12,000 *g*, 10 min, 5 C and 66,000 *g*, 120 min, 5 C). Further purification, when necessary, was accomplished by sucrose density gradients of 10 to 40% centrifuged in a Spinco No. SW 25.1 rotor (50,000 *g*, 180 min, 5 C), overnight dialysis of the individual opalescent bands, and final high-speed centrifugation (93,000 *g*, 90 min, 5 C).

The preparations were analyzed on a Beckman DB-G spectrophotometer. A sample of purified virus was freeze dried, weighed, and resuspended in distilled water. The optical density at 260 nm of several dilutions of this solution was plotted. The extinction coefficient was calculated from a line of best fit.

Base ratios were determined by hydrolyzing the virus in 1N HCl followed by paper chromatography (Whatman No. 1 paper) with an isopropanol: conc HCl: H_2O (170:44:36, v/v) solvent system (7).

Antiserum to PlMV was prepared in mice (courtesy of J. K. Uyemoto) by giving three intramuscular injections of 0.4 ml PlMV. The first contained 1.2 mg virus; the second injection, 8 days later, contained 47 mg; and the third, after 38 days, contained 1.2 mg. An additional injection of 0.3 ml ascites tumor virus was made immediately after the third injection. The animal was sacrificed 8 days after the final injections and the ascites fluid was collected.

Serological tests (9) were conducted in agar gel diffusion plates using 0.75% Ionagar gel dissolved in saline with 0.1% sodium azide added as a preservative. Either eight or four wells, 5-mm diam, surrounded a central well. The outside wells were 4-5 mm from the center well and 1-3 mm from each other. All reactions were repeated at least three times.

The following investigators kindly supplied materials for the comparisons: J. H. Hill, Iowa virus related to belladonna mottle (BMVi) and antiserum; R. Koenig, antisera to andean potato latent (APLV), belladonna mottle (BMV), dulcamara mottle (DMV), and ononis

yellow mottle viruses (OYMV); H. A. Scott, desmodium yellow mottle virus (DYMV); J. K. Uyemoto, wild cucumber mosaic virus antiserum (WCMV); and H. J. Walters, antiserum to desmodium yellow mottle virus. Antiserum to turnip yellow mosaic virus had been previously prepared in California (Granett, *unpublished*).

RESULTS.—*Host range and physical properties.*—Of over 45 species tested belonging to 20 families, only eight species were susceptible to PlMV. Symptoms usually appeared 5 to 14 days after mechanical inoculation. In *Antirrhinum majus* L. symptoms consisted of expanding necrotic ringspots without systemic invasion. Mild systemic mottle was observed in *Glycine max* (L.) Merr., *Petunia hybrida* Vilm., *Plantago major*, and *Tetragonium expansa* Thunb. In *Nicotiana clevelandi* A. Gray, PlMV induced a severe systemic mottle. On

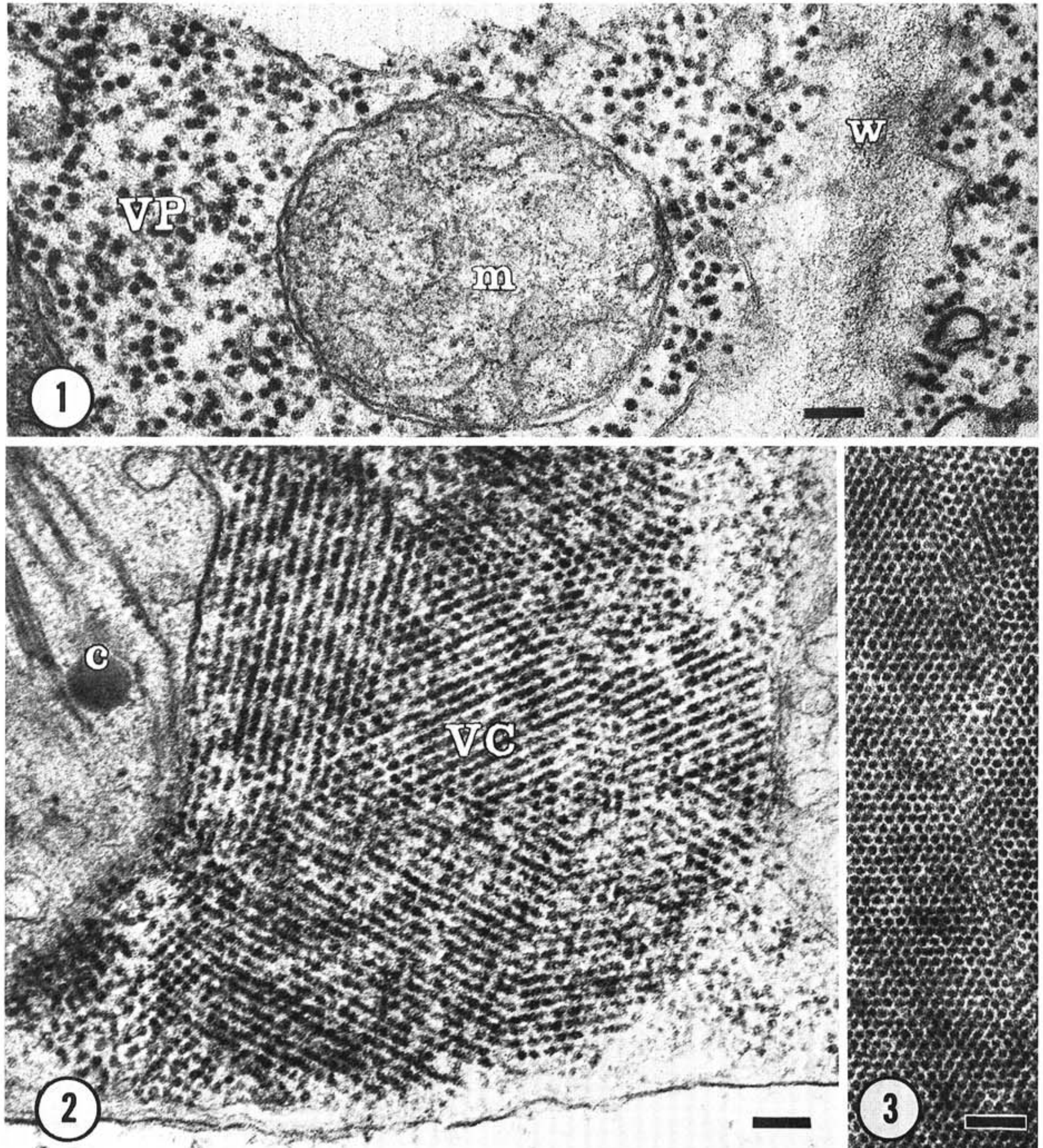

Fig. 1-3. Electron microscopy of plantago mottle virus in thin sections. Marker represents 100 nm. **1)** Cytoplasm of pea leaf containing scattered virus particles (VP). **2)** Virus cluster (VC) in pea leaf cytoplasm. **3)** High-speed centrifuge pellet of virus. c = chloroplast; m = mitochondria; VC = virus cluster; VP = virus particles; w = cell wall.

martynia [*Proboscidea jussieu* (Mill.) Thell.], the virus incited tiny primary necrotic lesions and systemic mottle. Ranger pea produced systemic mottle with a gradual disappearance of symptoms in new growth. Some cultivars were resistant to infection. In 1972, PlMV was isolated from a foreign introduction pea plant growing in an open field (R. Provvidenti, *personal communication*).

No infection was obtained in the following species: *Brassica pekinensis* (Lour.) Rupr.; *B. oleracea* var. *botrytis* L., *B. oleracea* var. *capitata* L.; *B. oleracea* var. *gemmifera* DC.; *B. oleracea* var. *italica* Plenck; *Capsicum frutesens* L.; *Chenopodium amaranticolor* Coste & Reyn.; *C. quinoa* Willd.; *Cucumis sativis* L.; *Cucurbita moschata* Dcne.; *C. pepo* L., *Datura stramonium* L.; *Gomphrena globosa* L.; *Hordeum vulgare* L.; *Lactuca sativa* L.; *Lupinus albus* L.; *Lycopersicon esculentum* Mill.; *Melilotus italicus* Lam.; *Nicotiana multivalvis* Lindl.; *N. sylvestris* Speg. & Comes; *N. tabacum* L. 'Havanna 423' and 'Turkish'; *Phaseolus vulgarus* L.; *P. lunatus* L.; *Pisum sativum* 'Bonneville'; *Plantago lanceolata* L.; *Spinacia oleracea* L.; *Trifolium pratense* L.; *T. repens* L.; *Triticum aestivum* L.; *Vicia faba* L.; *Vigna sinensis* (Torner) Savi; *Vinca rosea* L.; *Zea mays* L.; and *Zinnia elegans* Jacq.

PlMV in sap had a dilution end point between 10^{-7} and 10^{-8} and was inactivated at about 65 C.

Temperature and light effect.—Temperature had an important effect on the infectivity and symptom expression of most hosts infected by PlMV. Higher temperatures (30 C) generally delayed or suppressed symptoms whereas lower temperatures (20 C) increased infection. Inoculated pea plants incubated at a constant 25 C were 100% infected whereas 67% became infected in the greenhouse with fluctuating temperatures. Inoculated *P. major* plants incubated at 25 C and 21,520 lx (2,000 ft-c) produced large reddish brown local lesions, but those receiving only 2,152 lx (200 ft-c) produced only mild mottle symptoms.

Purified virus.—Yields of approximately 0.6 mg of virus per g infected leaf tissue were obtained. The extinction coefficient of purified virus, before density gradient separations, was 8.51 at 260 nm and the ultraviolet spectrum was a typical nucleoprotein curve with a 260/280 ratio of 1.9. When 2 ml of a solution containing 5 mg PlMV was centrifuged through a sucrose density gradient, four separate bands resulted. The top two bands, A and B, were each 1- to 2-mm wide and were located 10 and 15 mm from the meniscus. The third band, C, was 18 mm from the meniscus and was 5- to 6-mm wide while the fourth band, D, was 25 mm from the meniscus and 1- to 2-mm in width. Bands B and D were quite faint. In one experiment the bands from three density-gradient columns were extracted and pooled, and the solutions were dialyzed overnight against distilled water at 5 C before centrifugation (66,000 *g*, 150 min, 5 C). All pellets were resuspended in 1 ml 0.01 M phosphate buffer, pH 7; and samples were removed for bioassay and electron microscopy. Bands A and B were non-infectious and contained abundant numbers of stain-penetrated ("empty") isometric particles. Fractions C and D were infectious and contained both penetrated and nonpenetrated particles. Preparations of 5 mg of TYMV, centrifuged through sucrose density gradients, separated into four bands corresponding in depth and width to those produced with PlMV preparations.

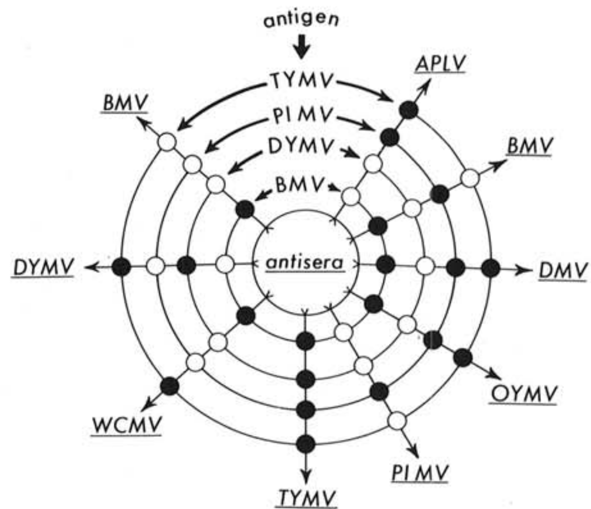

Fig. 4. Serological relationship between tymoviruses. Four viruses were tested against nine antisera. Positive reactions are indicated by filled circles (•); open circles (o) represent no reaction.

The molar composition of the nucleic acid bases of PlMV was guanine 17%, adenine 21%, cytosine 34%, and uracil 28%.

Electron microscopy.—Negative-stained infected leaf extracts and purified preparations revealed both penetrated and nonpenetrated isometric particles. As noted above, however, fewer nonpenetrated particles were found in the top density-gradient fractions, bands A and B.

Thin sections of infected pea and *P. major* leaves revealed isometric particles in the cytoplasm (Fig. 1). Pseudo-crystals were found rarely (Fig. 2). Virus pellets of purified preparations which were also embedded and sectioned showed clusters (Fig. 3) similar to those in the leaf cells.

Sections of virus pellets or clusters seen in the cytoplasm produced individual measurements averaging 22.7 nm diam, while negative-stained virus preparations measured 26.7 nm in diam.

Serology.—The serological reactions between four viruses and nine antisera, as tested by gel diffusion, are summarized in Fig. 4. PlMV is related to APLV, BMV, DMV, OYMV, and TYMV. No serological relationship to BMVi, DYMV, or WCMV was detected. When PlMV and one of the other three viruses reacted with the same antiserum, continuous precipitant bands were never formed, but spurs were noted. Although PlMV and BMVi reacted with TYMV antiserum, their respective antisera did not react with TYMV, possibly due to low titer sera. Strong reactions consisting of heavy precipitant bands occurred between PlMV and antisera of the andean potato latent viruses (APLV, DMV, and OYMV).

DISCUSSION.—Physical, chemical, and biological properties of PlMV indicate that it is a member of the tymovirus group. Furthermore, PlMV belongs to the b-subgroup which includes BMV and the andean potato latent viruses: APLV, DMV, and OYMV (5). This conclusion is based on the similarity of base ratios and

serological reactions. There are also several common host plants shared by one or more of the b-subgroup and PlMV including *Glycine max* (OYMV), *Nicotiana clevelandi* (all), *Petunia hybrida* (APLV, BMV) and *Pisum sativum* (OYMV). Other plants, however, are infected by the b-subgroup viruses but not by PlMV: *Chenopodium amaranticolor* (APLV), *C. quinoa* (APLV), *Datura stramonium* (APLV, BMV, DMV, OYMV), *Gomphrena globosa* (APLV), *Lycopersicon esculentum* (APLV, BMV, DMV, OYMV), *Trifolium pratense* (OYMV) and *T. repens* (OYMV). A dissimilar host range and uncommon antigens, indicated by serology, differentiate PlMV from the viruses described by Gibbs et al. (3).

Berck & Querfurth (2) found a serological relationship between TYMV and the andean potato latent viruses which is confirmed here. A virus isolated in Iowa was reported by Moline & Fries (8) to be related to BMV. PlMV reacted with BMV antiserum, but not to the Iowa antiserum which was relatively weak.

PlMV was not serologically related to either WCMV or DYMV, both of which seem to be in subgroup a. In addition, DYMV induced systemic chlorosis in pea varieties resistant to PlMV.

From available data (1), scrophularia mottle virus also appears to be related to PlMV.

It seems significant that a pea plant was found naturally infected with PlMV. As the major virus diseases of pea become less important due to resistant varieties, some of the diseases heretofore considered minor will be detected more frequently.

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