A Strain of Belladonna Mottle Virus Isolated from Physalis heterophylla in Iowa

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ABSTRACT

In 1971, a mechanically transmissible agent was recovered in central Iowa from *Physalis heterophylla*, a common perennial weed. Studies indicated that the agent is a virus, limited in host range to members of the Solanaceae and *Chenopodium quinoa*. The virus is serologically related to European Belladonna mottle virus. The virion is ca. 29 nm in diam and contains approximately 37% RNA. Two particles are associated with infectivity following density-gradient centrifugation, a noninfective top component (54S) and an infective bottom component (114S). *Nicotiana glutinosa* is a good systemic host which yielded large amounts of purified virus.

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A perennial weed, *Physalis heterophylla* L., displaying virus like symptoms was found in a foundation seed corn field in central Iowa in 1971. Symptoms included a yellow mottle, dwarfing of developing leaves, and internode

shortening. The plant was collected and assayed onto a number of indicator hosts in the greenhouse. The symptoms produced were not typical of any other viruses we had worked with in *Physalis* spp.; therefore, this study was made to identify the causal agent. A preliminary report has been made of isolation of this virus (9).

MATERIALS AND METHODS.— Initial inoculations were made onto sweet corn, *Zea mays* L. 'Golden Bantam'; *Triticum aestivum* L. 'Lucas'; *Chenopodium quinoa* L.; *Nicotiana tabacum* L. 'Samsun NN' and 'Turkish'; *N. glutinosa* L.; and *Datura stramonium* L. The virus was maintained in *N. glutinosa* and *D. stramonium* in a greenhouse maintained at 25 C and 12-hr 15,064 lx (1400 ft-c) days; all other plants tested as possible hosts were assayed back to either of these to check for symptomless hosts. All mechanical transmissions were made by grinding infected plants in 0.01 M phosphate buffer (pH 7.0) or distilled water and inoculating assay hosts that had been dusted with 600-mesh silicon carbide.

Physical properties.— We determined the physical properties of the virus by grinding infected *N. glutinosa* in 0.01 M phosphate buffer, pH 7.0. Longevity in vitro (LIV) tests were made by maintaining expressed sap at 25 C and assaying at regular intervals for 21 days. The thermal inactivation point (TIP) was determined by heating 1.0-ml samples of clarified sap (diluted 1 vol in 9 vol 0.01 M phosphate buffer, pH 7.0) for 10 min, cooling, and assaying for virus onto *D. stramonium* and *N. glutinosa*. The dilution end point (DEP) was determined by making 10-fold serial dilutions of sap expressed from *N. glutinosa* and *D. stramonium* in buffer and inoculating to *N. glutinosa*.

Purification. — The virus was purified from *N. tabacum*, (Samsun NN and Turkish), *N. glutinosa*, and *D. stramonium* by modification of the method of Steere (10, 13). The procedure was amended by omitting butanol from the initial clarification step and emulsifying the homogenate with chloroform (25%, v/v) for 2 min.

Density-gradient centrifugation. — Particle density was estimated by equilibrium, cesium chloride (CsCl), density-gradient centrifugation. Cesium chloride gradient tubes were prepared from 3.5 M CsCl (optical grade) and centrifuged at 204,000 *g* for 18-24 hr. Gradient densities were standardized by using micule density markers (Microspheres, Inc., Palo Alto, Calif.). Fractions from each peak were assayed for infectivity after dialysis against phosphate buffer (0.01 M, pH 7.0) for 24 hr.

Sedimentation properties of viral components were measured by analytical ultracentrifugation of the purified virus in a Spinco Model E analytical ultracentrifuge using an An-D rotor with Schlieren optics at a 60° interference angle. Exposures were taken at 4-min intervals at a rotor speed of 31,410 rpm. Sedimentation rates were determined by the graphic method of Markham (8).

Serology.— Antisera to our virus isolate were produced by intravenous injections to rabbits with 1 mg of purified virus at four-day intervals for 12 days. Intramuscular boosters of 1 mg of purified virus in 0.5 ml of Freund's incomplete adjuvant were then given at two-week intervals, and blood was collected by cardiac puncture.

Microprecipitin tests (1) and tube precipitin tests were conducted at regular intervals to determine antisera titers.

Immunodiffusion tests were made in plates containing 0.5% Ionagar No. 2 and 0.02% NaN_3. The central well contained the purified unknown virus. The outer wells contained antisera specific for: European Belladonna mottle virus (BMV) (obtained from H. L. Paul, Braunschweig, Germany), tomato aspermy virus (two strains), turnip yellow mosaic virus (TYMV), bromegrass mosaic virus (BGMV), tobacco ringspot virus (TRSV), tomato ringspot virus (TomRSV), wild cucumber mosaic virus (WCMV), and cucumber mosaic virus (CMV).

Electron microscopy.— Purified preparations of the virus were negatively stained with phosphotungstate for observation with the electron microscope. Particle measurements were made on more than 400 particles from three different purifications. The instrument magnification was determined by using a carbon grating-replica grid of 54,800 lines/inch (obtained from E. F. Fullam Co.).

Chlorotic lesions from inoculated *D. stramonium* leaves were fixed with 3% glutaraldehyde in 0.05 M phosphate buffer (pH 7.2) for 4 hr, post-fixed with phosphate buffered 1% OsO_4 for 2 hr, dehydrated with ethanol-propylene oxide, and embedded in Araldite-Epon for sectioning. Thin-sections were cut with a diamond knife using an LKB-3 ultramicrotome, floated onto Formvar-coated grids, stained with Reynolds lead citrate, and observed with an RCA EMU-3G electron microscope.

RESULTS.— *Host Range.*— Our Physalis Mottle (PM) isolate produced numerous lesions on inoculated leaves of *D. stramonium*, *N. clevelandii* Gray, *N. rustica* L., *N. glutinosa*, *N. tabacum* (Samsun NN and Turkish), *Physalis angulata* L., *P. heterophylla* L., and *Solanum nigrum*.

The virus also moved systemic in all hosts previously mentioned, causing a severe yellow mottle and leaf distortion in *N. glutinosa* within 10 days of inoculation (Fig. 1). A yellow mottle was produced on systemically infected leaves of *P. angulata*, *P. heterophylla*, and *D. stramonium*. Systemically infected leaves of *N. clevelandii*, *N. rustica*, and *S. nigrum* were also mottled.

A chlorotic mottle also was produced on *N. tabacum* (Samsun NN and Turkish), but symptom expression was much slower than in other hosts, with a 3-week latent period before symptoms first appeared. Lesions were produced on inoculated leaves of *Lycopersicon esculentum* Mill. 'Bonny Best', and *C. quinoa*, but the virus did not move systemically. PM did not infect the following plants tested as possible hosts: *S. tuberosum*, *Z. mays*, *T. aestivum* Lucas, *C. amaranticolor* Coste and Reyn., *C. hybridum* L., *C. murale* L., *Gomphrena globosa* L., *Vinca rosea* L., *Dianthus caryophyllus* L., *Helianthus annuus* L., *Cucumis sativus* L. 'Natl. Pickling', *Glycine max* L. 'Amsoy', *Phaseolus vulgaris* L. 'Bountiful' and 'Scotia', *Pisum sativum* L. 'Wilt-resistant Perfection', *Vicia faba* L. 'Minor', *Vigna sinensis* Endl. 'Early Ramshorn', and *Daucus carota* L.

Physical properties.— Virus infectivity in vitro was maintained up to 21 days at 25 C. The virus was inactivated after heating for 10 min at 82 C, but not at 80 C. The DEP was between 10^{-6} and 10^{-7} from *N. glutinosa* and *D. stramonium* when assayed onto either *D. stramonium* or *N. glutinosa*.

Purification.— Initial attempts to purify the virus from *N. glutinosa* by the method of Steere (10, 13) resulted in large amounts of host contaminants sedimenting with the virus. This procedure also resulted in low virus yields. Omitting butanol from the clarification step and emulsifying

the homogenate with chloroform allowed for removal of host contaminants and resulted in a purified virus preparation of high yield after two cycles of differential centrifugation. Purified virus was stable in either 0.01 M

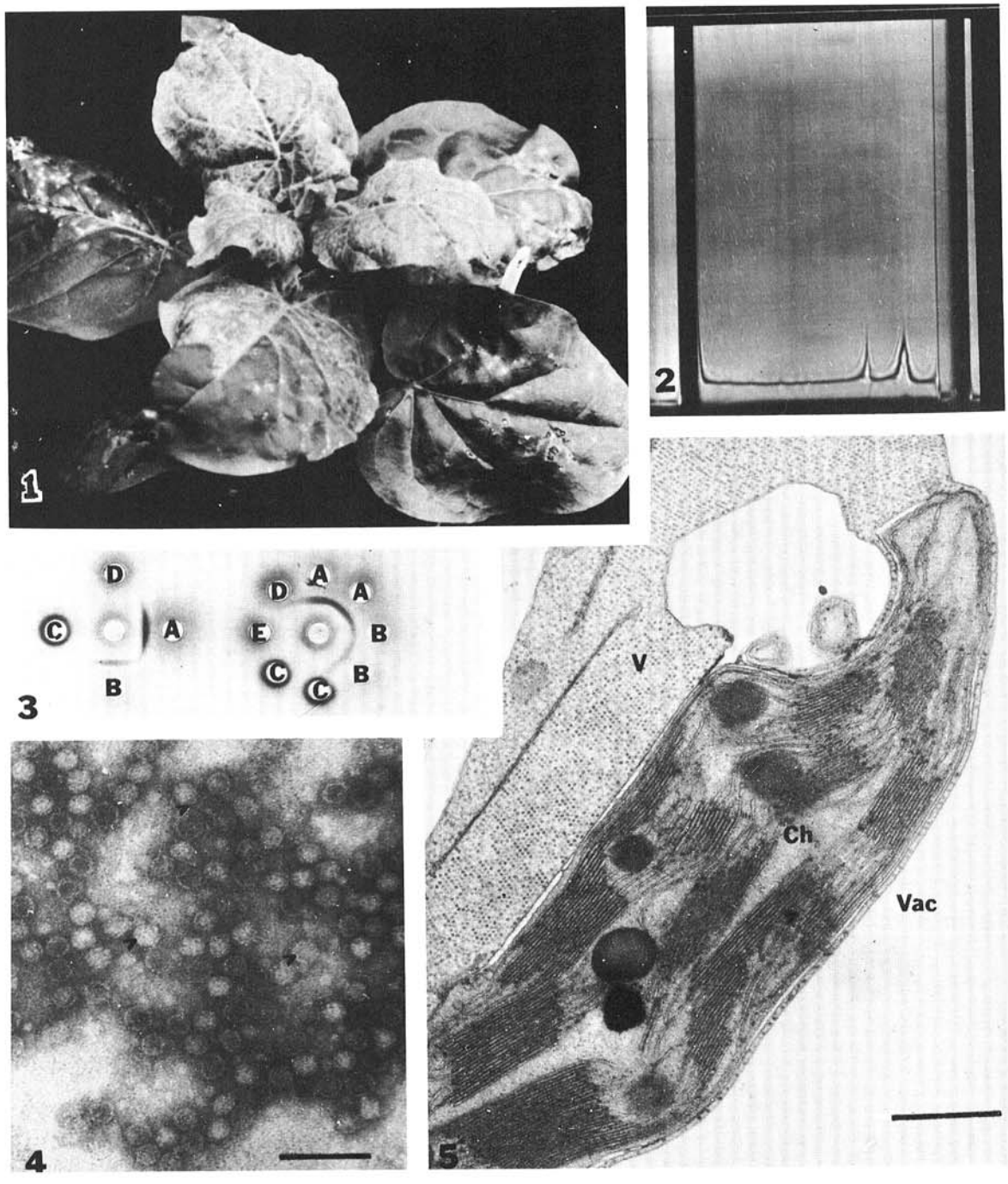

Fig. 1-5. 1) Necrotic local lesions and systemic yellow mottle symptoms induced by the Physalis mottle strain of Belladonna mottle virus (PM) on *Nicotiana glutinosa*. **2)** Schlieren pattern at 31,410 rpm of purified Physalis mottle strain at 20 C in distilled water. (Top of cell to right). **3)** Immunodiffusion test of purified Physalis mottle antigens (PM) in the center well reacted against antisera specific for (A) PM, (B) Belladonna mottle virus, (C) turnip yellow mosaic virus, (D) wild cucumber mosaic virus, (E) normal serum. **4)** Negatively stained preparation of Physalis mottle showing apparent hollow virus capsids (particles with dark center) and intact particles (arrows) (bar=100 nm). **5)** Physalis mottle inclusion in mesophyll cell of *Datura stramonium* leaf. Crystalline virus (V) inclusion found adjacent to chloroplasts (Ch) in vacuole (Vac) of a mesophyll cell in an inoculated leaf (bar=500 nm).

phosphate buffer or distilled H_2O. The best host for purifying virus from was *N. glutinosa*, with yields of 1250 mg/kg of tissue. *D. stramonium* yielded comparable amounts of virus, but was more difficult to propagate in the greenhouse. *N. tabacum* (Samsun NN and Turkish) also gave high yields, but required longer periods for virus increase and symptom expression than did either *N. glutinosa* or *D. stramonium*. Purified preparations remain infective at least 1 yr when stored at 4 C.

Density-gradient centrifugation.— Cesium chloride and sucrose gradients of virus purified from all hosts always yielded two light-scattering zones. The two zones always present comprised the top (T) and bottom (B) components of the polyhedral particle. The density of the top component was approximately 1.27, and that of the bottom component 1.42, as determined with the aid of density-gradient micules.

Virus infectivity always was associated with the bottom peak; if the top component was collected from cesium gradients and rerun by itself, no band could be detected in subsequent gradients, but rather, a layer of material could be found at the top of the centrifuge tube. If, on the other hand, the bottom component were run on a second gradient, two zones always appeared on the second gradient.

The ultraviolet-absorption spectrum of the mixed preparation was $A_{260/280} = 1.51$; the top component $A_{260/280} = 1.06$; and the bottom component $A_{260/280} = 1.63$. Purified virus yielded two peaks, 54S and 114S (Fig. 2), comparable to the top and bottom components observed in density-gradient analyses.

Virus nucleic acid present within the virions was determined to be RNA by its positive reaction with orcinol (12) and negative reaction with diphenylamine (4). Purified preparations composed of a mixture of top and bottom components, contained approximately 37% RNA as estimated from ultraviolet scans (14).

Serology. — The isolate from *Physalis* elicited a strong immune response in injected rabbits with a microprecipitin titer of 1/1024 after three intravenous injections followed by two intramuscular adjuvant boosters. Immunodiffusion tests showed homologous reactions between PM antigens and European BMV antiserum; no reaction occurred with other antisera tested (Fig. 3).

When BMV antiserum was placed in the center well and BMV and PM antigens placed in surrounding wells, spurs were formed, indicating a heterologous reaction between the two viruses. These data, accompanied by microprecipitin reactions of 1/16 between BMV AS of a titer of 1/512 and PM antigens, and 1/32 between PM AS of a titer of 1/512, and BMV antigens suggest a distant relationship between the two viruses.

Electron microscopy.— Measurements of negatively stained preparations showed that the normal diameter of the polyhedral viral particle is 29 nm (Fig. 4). Thin-sections of lesion areas revealed virus particles that formed distinct crystalline aggregates adjacent to mesophyll chloroplasts (Fig. 5). Particle-size correlates closely with that measured from purified preparations.

DISCUSSION.— Our host range studies have shown that PM has a limited host range nearly identical to that of BMV. Symptom expression on those hosts studied also correlates quite closely to that reported for BMV (10, 11).

Physical properties of PM are in close agreement with those of other members of the Andean potato latent virus group and BMV (2, 6, 7, 11). Many of these properties also are shared by TYMV; no serological relation, however, could be detected between PM and TYMV in our studies, although a relationship has been found between TYMV and other members of this group by others using a more sensitive test (3).

Spur formation was noted in Ouchterlony agar-diffusion tests between BMV and PM, and titers obtained with heterologous antisera in microprecipitin tests were low. This indicates a distant, although definite, serological relationship between the two viruses.

Estimates of the densities of the two components of the virus with the aid of microdensity beads in the preparative ultracentrifuge, placed them very close to turnip yellow mosaic (2).

Studies were not conducted to determine the distribution of PMV in native *P. heterophylla* in central Iowa, but the virus has since been isolated (1972) from this host by another investigator in our laboratory.

Since PM seems to have a restricted host range, its importance as a potential pathogen of agronomic crops may not be significant, even though the distribution *P. heterophylla* covers all the United States east of the Continental Divide (5).

Based on physico-chemical properties of PM, we have placed it in the Andean potato latent virus subgroup of the turnip yellow mosaic virus group, and it should be properly identified as a Physalis mottle strain of Belladonna mottle virus.

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