Maclura Mosaic Virus—An Elongated Plant Virus of Uncertain Classification

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The financial support of the Deutscher Akademischer Austauschdienst and the Deutsche Forschungsgemeinschaft and the reliable technical assistance of Mrs. Beate Hane, Miss Petra Rähse, and Mrs. U. Herzberg are gratefully acknowledged.

Accepted for publication 25 November 1978.

ABSTRACT

PLEŠE, N., R. KOENIG, D. E. LESEMANN, and R. F. BOZARTH. 1979. Maclura mosaic virus—An elongated plant virus of uncertain classification. Phytopathology 69:471-475.

An isolate of Maclura mosaic virus (MacMV) obtained after four successive passages through single lesions on *Chenopodium amaranticolor* had flexuous elongated particles with a normal length of 672 nm and a sedimentation velocity relative to marker viruses of 155S. Infected cells always contained cylindrical (pinwheel-type) inclusions, which suggests that MacMV may be a potyvirus. Other properties of MacMV, however, were not typical of potyviruses: its coat protein had a molecular mass of 45,000–48,000 daltons; the uncorrected and corrected values of $A_{280/260}$ were 0.94 and 1.04, respectively; the buoyant density in cesium chloride was 1.307 g/cm^3; and peculiar granular structures aligning the surface of the particles were sometimes seen in crude sap preparations but not in purified preparations. The virus showed no pronounced reactions with antisera to one tobamo virus, 10 potex viruses, 10 carla viruses, two clostero viruses, or 15 potyviruses.

Additional key words: Buoyant density, plant virus classification, surface structures of plant viruses, UV-absorption spectrum.

Maclura mosaic virus (MacMV) was described first by Plešе and Miličić (25). In further studies Plešе and Štefanac (26) reported the host range, properties in crude sap, aphid transmissibility, and a normal length (600–650 nm) of the rod-shaped particles. They observed cylindrical (pinwheel-type) inclusions and suggested that MacMV might be a potyvirus. This article describes further properties of the virus.

MATERIALS AND METHODS

Purification of MacMV was achieved by homogenizing 100 g of leaves from systemically infected *Nicotiana clevelandii* Gray in 100 ml of 0.5 M borate buffer, pH 7.8, containing 0.2% ascorbic acid and 0.2% sodium sulfite. The supernatant solution obtained after low speed centrifugation was stirred with 0.4 volume of chloroform. The mixture was centrifuged 30 min at 6,000 *g* and the virus in the aqueous phase was subjected to two high and low speed cycles of centrifugation.

Sucrose density gradient (SDG) centrifugation was at 35,000 rpm on 5-ml 10–40% linear SDG in a Beckman SW-39 rotor for 2 hr at 8 C. Gradients were analyzed on an ISCO density gradient fractionator and UA-5 ultraviolet absorption monitor. Cesium chloride density gradient analyses were made in the same equipment. Centrifugation was for 16 hr at 35,000 rpm and 25 C. Gradients were fractionated into 0.3-ml fractions and the density of each fraction was determined from 10-μl aliquots by refractometry using the formula ρ CsCl = n × 10.4091 − 12.8812 (4).

Ultraviolet absorption spectra were measured in an Unicam SP 8-100 spectrophotometer. Scans from 220 to 400 nm were corrected for light scattering by the method of Englander and Epstein (9).

Sedimentation velocity (S_{rel}) relative to that of top component (53S) and bottom component (113S) of belladonna mottle virus (24) and tobacco mosaic virus (194S) (29) was estimated by SDG centrifugation of purified virus.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed in different gel concentrations as described previously (16,17). Serologic tests were made with the slide precipitin test (2).

Virus particles were studied in crude sap or purified preparations negatively stained with 1% neutral sodium phosphotungstate or 1% uranyl acetate. For particle length measurements virus suspensions were mixed with crude sap from tobacco mosaic virus-infected *Nicotiana tabacum* L. 'Samsun.' The TMV particles served as length standards and were assumed to be 300 nm long.

Ultrathin sections were cut from infected and healthy leaves of *N. clevelandii* embedded in Epon as previously described (19).

RESULTS

Host range. Most of the host plants reported by Plešе and Štefanac (26) were only locally infected by MacMV. We found that the virus systemically infects *N. clevelandii* and induces mild mosaic symptoms. In this host the virus reaches fairly high concentrations. It was therefore well suited as a source for virus purification and for studies on particle morphology and cytopathological effects. *Nicotiana acuminata* Grah. and *Nicotiana bigelovii* S. Wats also were infected systemically and developed mild mosaic symptoms.

Particle morphology. Flexuous elongated particles (650–710 nm) were present in crude sap from MacMV-infected *N. clevelandii*, *Tetragonia expansa* Murr., *Chenopodium quinoa* Willd., *C. amaranticolor* Coste & Reyn., and in purified virus preparations (Fig. 1). The mean of the normal lengths obtained from a population of 618 particles from *N. clevelandii* was 672 ± 23 nm. This value is in reasonable agreement with the values of 600 nm and 650 nm reported for MacMV from leaf dip preparations of *C. amaranticolor* and partially purified preparations from *T. expansa*, respectively (26). Fixation of purified preparations and crude sap with 2% glutaraldehyde did not significantly alter the particle dimensions. The particle length of purified MacMV was not affected by EDTA or by Mg^{++} ions (11). Fine structures were not observed with NaPT-stained particles (Fig. 1). In uranyl acetate (Fig. 2) and uranyl formate a cross-banding was sometimes faintly visible.

Images of virus particles with associated granular structures (Figs. 3 and 4) occasionally were obtained with the drop dispersion method of I. Roberts, Scottish Horticultural Research Institute, Dundee (*personal communication*). A small amount of

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crude sap from MacMV-infected *T. expansa* or *N. clevelandii* was dropped onto a water surface covered by a fine layer of talcum powder. At the edge of the clear area that formed where the drop spread out, a carbon-formvar-coated grid was touched to the water surface, withdrawn, drained, and negatively stained. In some of these preparations the virions occurred in small bundles and distinct small granular structures aligned the surface of the particles at a distance of 5–6 nm (Fig. 3). The center-to-center distance between the granules was about 8 nm. In the space between virions within a bundle the granules apparently formed aggregates with a regular line pattern in which the periodicity was about 8 nm (Figs. 3 and 4). This periodicity differed from that of the rectangular plates of the cylindrical inclusions that also were found in these preparations (Fig. 5) and showed a line pattern with a 6 nm periodicity. The dimensions of fraction I protein particles were in the range of 10 nm (Fig. 5). Thus, the size of the small granular structures associated with MacMV particles differs from that of the cylindrical inclusion protein and fraction I protein.

The granules aligning the virions were observed only rarely when grids were sprayed either with a mixture of crude sap and sodium

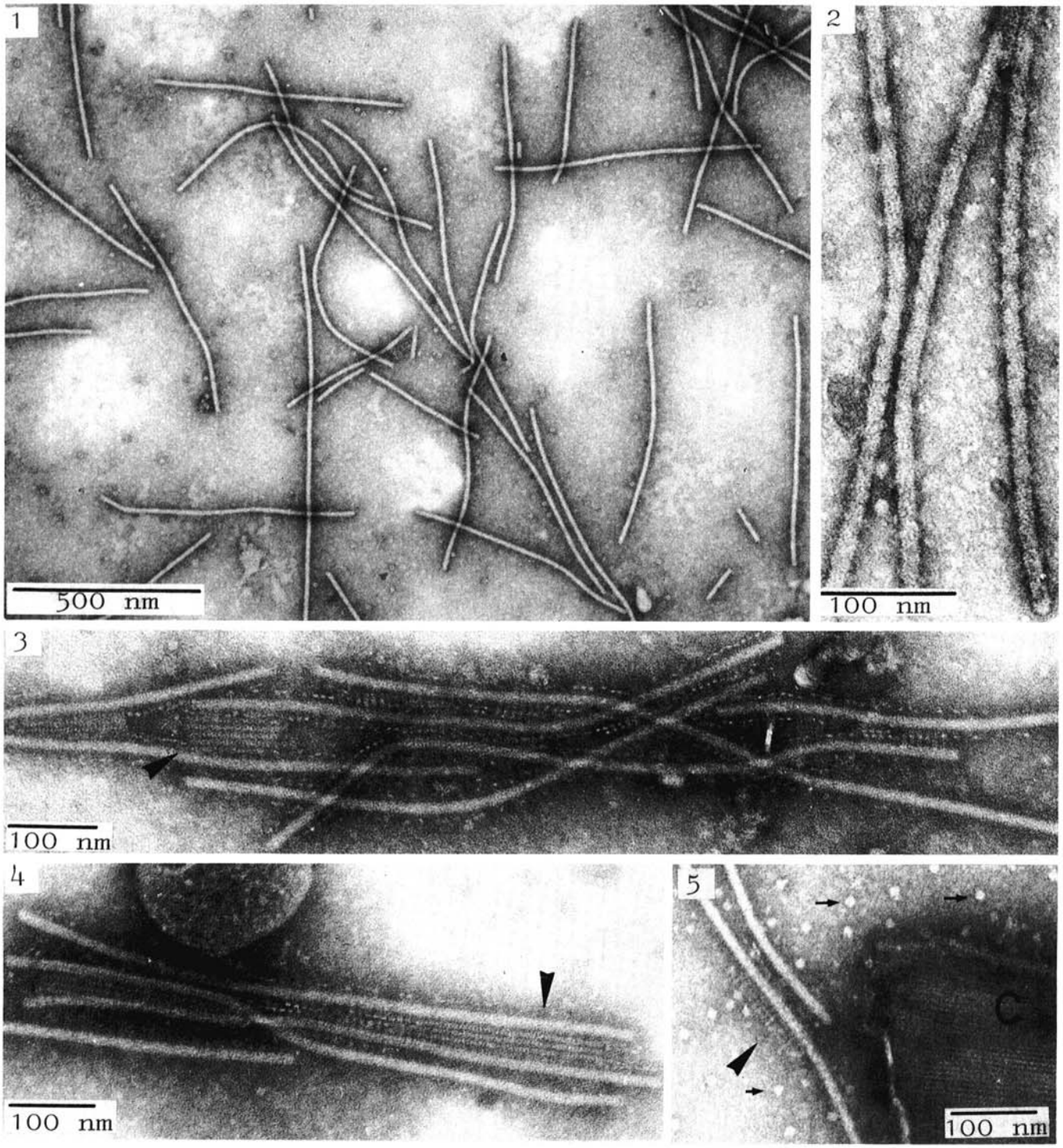

Fig. 1–5. Negatively stained particles of Maclura mosaic virus. **1)** Purified preparation from *Tetragonia expansa* stained in sodium phosphotungstate. **2)** Purified preparation from *Nicotiana clevelandii* stained in uranyl acetate. **3-4)** Virus particles from crude sap of *T. expansa* prepared by the drop dispersion method and stained in sodium phosphotungstate show virus-associated granules (arrows indicate aggregates of granules). **5)** Prepared as for Fig. 3, 4 but from *N. clevelandii*. Note an aggregate of virus-associated granules (large arrow) overlapping a virus particle, part of cylindrical inclusion (C) for comparison, and fraction I proteins (small arrows).

phosphotungstate or touched with crude sap and then washed and stained with sodium phosphotungstate. They never were observed on virions from purified preparations.

Properties of MacMV particles. Virus preparations obtained from clarified plant sap after two cycles of high and low speed centrifugation yielded three bands when centrifuged on SDG. A band that sedimented at about 70–80S had the properties of phytoferritin (13). The second band was sharp; nonaggregated virus particles were observed by electron microscopy. A third broad band contained aggregated virions. Further analysis of the nonaggregated virus by cocentrifugation with marker viruses yielded $S_{rel} = 155$. Cesium chloride density gradient centrifugation of the nonaggregated and the aggregated virus particles yielded sharp bands at ρ CsCl = 1.307 ± 0.002 g/cm^3.

Ultraviolet absorption analysis of the nonaggregated virions after dialysis against 0.05 M sodium phosphate buffer, pH 7.0, yielded a typical virus spectrum with a maximum absorbancy at 269 nm and a minimum absorbancy at 247 nm. The values of A_{280}/A_{260} ratios before and after correction for light scattering were 0.94 and 1.04, respectively.

In SDS-PAGE the virus yielded a single protein band and a molecular mass of 45,000 daltons was calculated by the method of Shapiro et al (27). This value was independent of the polyacrylamide concentration in the range of 1–10%. Therefore, the protein behaves normally in SDS-polyacrylamide electrophoresis. A molecular mass of 48,000 daltons was calculated by the method of Hedrick and Smith (12).

Serologic properties. An antiserum to MacMV with a homologous titer of 1:512 was obtained. Since neither particle morphology nor protein molecular mass determinations clearly indicated a specific virus group, the virus was tested with antisera to elongated viruses of several groups. The following results were obtained:

Tobamovirus group. No reaction with antiserum to tobacco mosaic virus was observed

Potexvirus group. No reactions were observed with antisera to Dioscorea latent, cactus X, clover yellow mosaic, cymbidium mosaic, potato X, potato aucuba mosaic, narcissus mosaic, Nerine X, white clover mosaic, or zygocactus viruses.

Carlavirus group. No reactions were observed with antisera to cactus 2, carnation latent, cowpea mild mottle, chrysanthemum B, mulberry latent, Nerine latent, Passiflora, potato M, or potato S viruses. A weak reaction to a dilution of 1:4 to 1:8 was observed with an antiserum to poplar mosaic virus.

Potyvirus group. No reactions were observed with antisera to bean common mosaic, bidens mottle, clover yellow vein, gloriosa stripe mosaic, lettuce mosaic, narcissus yellow stripe, pea necrosis, pea seedborne mosaic, plum pox, potato Y, tobacco etch, or turnip mosaic viruses.

Two antisera to a bean and a lupinus isolate of bean yellow mosaic virus each gave weak reactions up to a dilution of 1:4. The homologous titers of these sera were 1:2,000. The antiserum to MacMV did not react with a bean yellow mosaic virus isolate from lupinus.

Closterovirus group. No reactions were observed with antisera to apple stem grooving or carnation yellow fleck viruses.

Viruses with uncertain group membership. No reactions were observed with antisera to agropyron mosaic, wheat streak mosaic, or rice necrosis mosaic viruses.

Cytopathology. Infected tissues of *N. clevelandii* contained cells with large masses of cytoplasm (Figs. 6 and 7), which showed conspicuous elements of cylindrical inclusions (8). In addition, filamentous structures, vacuoles of variable size, lipid droplets, small osmiophilic globules, microbodies, mitochondria, and ribosomes were seen in these areas. Cylindrical inclusions showed "pinwheel" structures with radiating plates and laminated aggregates (Fig. 6), according to the subdivision II as defined by Edwardson (8). They were similar or identical to those described by Plese and Stefanac (26) from MacMV-infected *Maclura pomifera* and *Tetragonia expansa.* No tubes or scrolls were seen. Filamentous structures about 20 nm thick were found to be closely associated with "pinwheel" plates and laminated aggregates (Fig. 7) and also were seen in larger three dimensional aggregates (Fig. 7). Consideration of their dimensions indicates these filaments probably are not virus particles.

DISCUSSION

Based on its length of 672 nm, MacMV could be a carlavirus, a closterovirus, or perhaps a potyvirus. The apparent flexibility of the particles suggests that it is not a carlavirus. Also, aggregates of virus particles occurring in cells infected by carlaviruses (7) were never seen with MacMV. Cytopathological effects typical for closteroviruses—such as specific alteration of the phloem with accumulations of virus masses and appearance of small vesicles (10,21)—were not observed with MacMV. The occurrence of large numbers of cylindrical ("pinwheel"-type) inclusions in infected cells indicates that MacMV may be a potyvirus (8).

However, in addition to its rather short particle length, MacMV has other properties that are not typical of potyviruses. Its protein molecular mass of 45,000–48,000 is unusually high for any elongated plant virus. The coat protein molecular masses of tobamo-, potex-, carla-, clostero-, and potyviruses range from 17,000 to 36,000 daltons. Proteins with higher molecular masses have been reported only for dasheen (1) and poplar mosaic viruses (3). However, unlike MacMV protein, these proteins behave anomalously in SDS-PAGE. Their migration rates relative to those of marker proteins vary with gel concentration and therefore the calculated molecular masses are probably too high. These two viruses also have normal-behaving proteins with molecular masses ~30,000. The unusually high molecular mass of the MacMV coat protein could be due to dimerization. However, coat protein molecular masses of potyviruses range from 28,000 to 36,000 daltons (14,15,20).

The uncorrected and corrected values of A_{280nm}/A_{260nm} for MacMV (0.94 and 1.04, respectively) are unusually high for potyviruses (1,20) and elongated viruses in general. This could mean that MacMV has either an unusually low RNA content of 3–4% (18,22) or a high content of aromatic amino acids (23) or both. The observation that MacMV has a buoyant density lower than that of potyviruses (15) also suggests that MacMV has a low RNA content. This may be a result of the large size of the protein subunit. However, the sedimentation velocity of MacMV, 155S, is well within the range of values reported for typical potyviruses (15,20).

In crude plant sap preparations peculiar small structures occasionally were seen in a regular alignment along the surface of the virus particles (Figs. 3 and 4). Because these structures were never found in purified preparations, they are probably not responsible for the unusually high protein molecular mass. The granular structures resemble those described for two carlaviruses; ie, cowpea mild mosaic (5) and narcissus latent viruses (6). However, differences also are apparent in the published photo micrographs; therefore it seems uncertain whether these structures indicate a relationship of MacMV with the carlavirus group. Such structures have not been described for potyviruses to our knowledge.

Particles of MacMV stained with uranyl salts sometimes show a faint cross-banding that is not typical of potyviruses (28).

Serology has been of little value for classifying MacMV among plant viruses. The only reactivities found with this virus were with one antiserum to poplar mosaic, probably a carlavirus, and with two antisera to different isolates of bean yellow mosaic, a potyvirus. These reactivities were too weak, however, to indicate definite relationships of MacMV.

The properties of MacMV that were not typical of potyviruses lead us to question whether the cylindrical inclusions in infected cells were indeed induced by this virus or by a contaminant. There were no indications of a mixed infection. Our isolate was obtained after four successive passages through single lesions on *C. amaranticolor.* The cylindrical inclusions were observed in four plant species that were infected by MacMV; ie, *M. pomifera, N. clevelandii, T. expansa,* and *C. quinoa* and seemingly were indeed induced by MacMV. Possibly, the induction of such inclusions is

not a unique property of potyviruses. To date it is uncertain whether mite-transmitted viruses such as agropyron mosaic and ryegrass mosaic and soilborne viruses such as oat mosaic and rice necrosis mosaic should be classified as members of deviating subgroups of the potyviruses or as new independent groups of plant viruses that characteristically induce "pinwheel"-type inclusions.

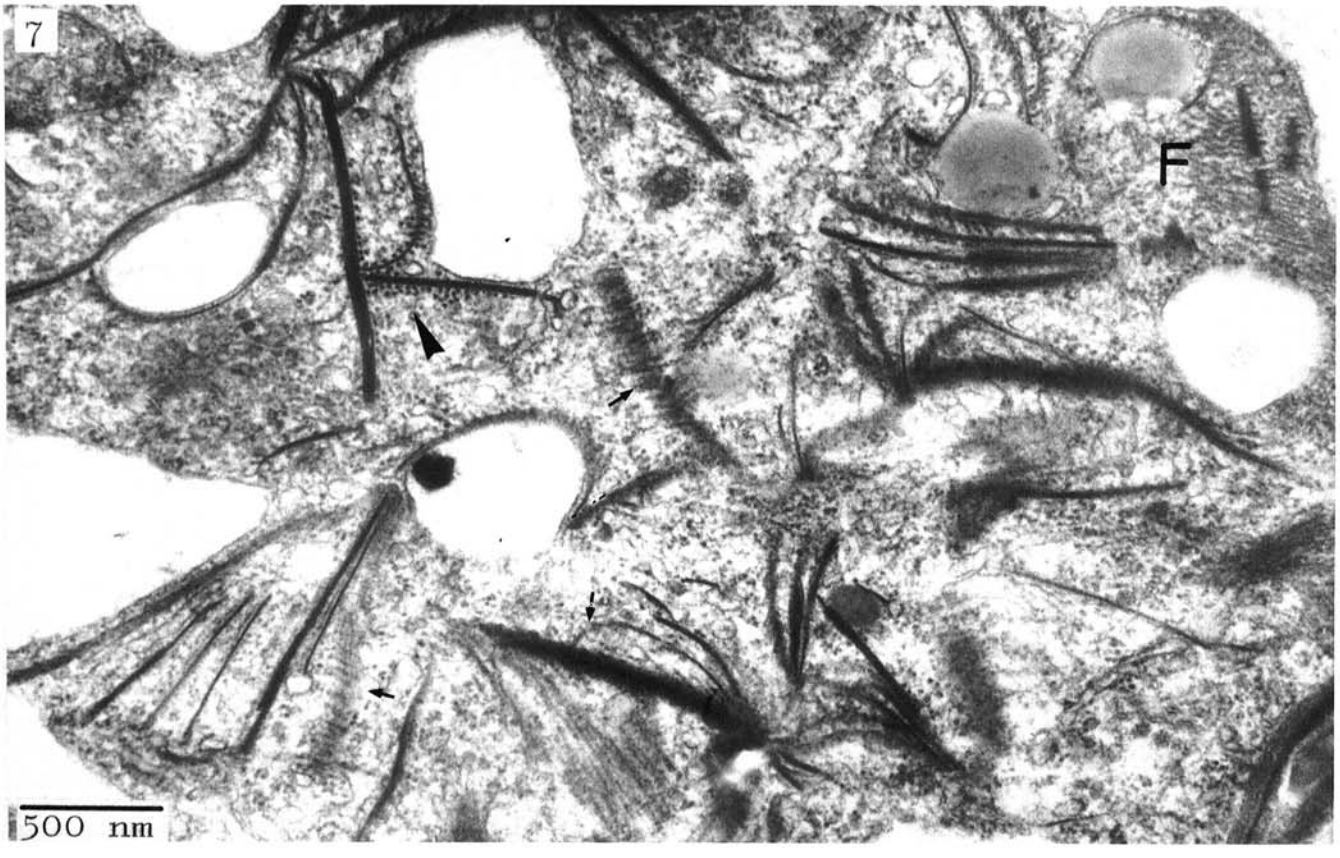

Fig. 6 and 7. Ultrathin sections from Maclura mosaic virus infected *Nicotiana clevelandii*. **6)** Portion of cellular inclusion with cylindrical inclusion elements (pinwheels, laminated aggregates). **7)** Part of MacMV-induced inclusion showing filaments associated with cylindrical inclusion elements which are seen in cross section (large arrow) and oblique section (small arrows). Note also aggregate of filaments (F).

Maclura mosaic virus may be a member of another group of "pinwheel"-inducing plant viruses with an uncertain relationship to the potyvirus group.

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