Ultrastructural Host Cell Changes Associated With Tomato Yellow Mosaic

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ABSTRACT

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Leaf phloem cells of tomato and *Nicotiana glutinosa* plants affected by tomato yellow mosaic (mosaico amarillo del tomate [MAT]) showed striking changes in nuclear morphology 6 days after inoculation either mechanically or by infectious whiteflies. The main changes were hypertrophy of the nucleus, which contained large masses of viruslike particles (18–20 nm in diameter), and often fibrillar rings also were observed. The viruslike particles were observed as large masses in the nuclei of infected phloem cells of both hosts. In *N. glutinosa*, however, they also occurred in the nuclei of mesophyll cells. Occasionally fibrillar rings were found in the nuclei of epidermal cells of *N. glutinosa*, but not in tomato. Comparable tissues from healthy plants were free of nucleopathy. The symptoms and signs of MAT resemble those reported for other whitefly-transmitted viruses such as bean golden mosaic virus and euphorbia mosaic virus.

Additional key words: geminivirus, *Bemisia tabaci.*

In cultivated areas of Venezuela, tomatoes (*Lycopersicon esculentum* L.) are affected by several diseases caused by viruses or viruslike agents. Of these, tomato yellow mosaic (mosaico amarillo del tomate [MAT]) is the most prevalent (5). The causal agent of MAT is whitefly (*Bemisia tabaci*)-transmitted and also infects tobacco (*Nicotiana tabacum* L.) under field conditions. Mechanical transmission of the MAT agent to tomato and tobacco is possible, but difficult (7). Similarities in physical properties, mode of transmission, and dependence on the same vector indicate that the MAT agent and other whitefly-transmitted viruses should be classified in the geminivirus group. Recently, viruslike particles were found: in the nuclei of young vascular cells in *Phaseolus vulgaris* L. infected with bean golden mosaic virus (BGMV) (3), in *Euphorbia heterophylla* L. infected with euphorbia mosaic virus (EMV) (4), in *L. esculentum* affected by yellow dwarf disease, and in *N. tabacum* affected by leaf curl (6). These viruses are economically important in tropical countries (1) and very interesting from the biological point of view, since one of the viruses (BGMV) contains a single-stranded DNA genome (2). The research reported here was done to determine whether tomato and *N. glutinosa* plants infected by the MAT agent exhibit the ultrastructural changes characteristic of those infected by the gemini viruses.

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MATERIALS AND METHODS

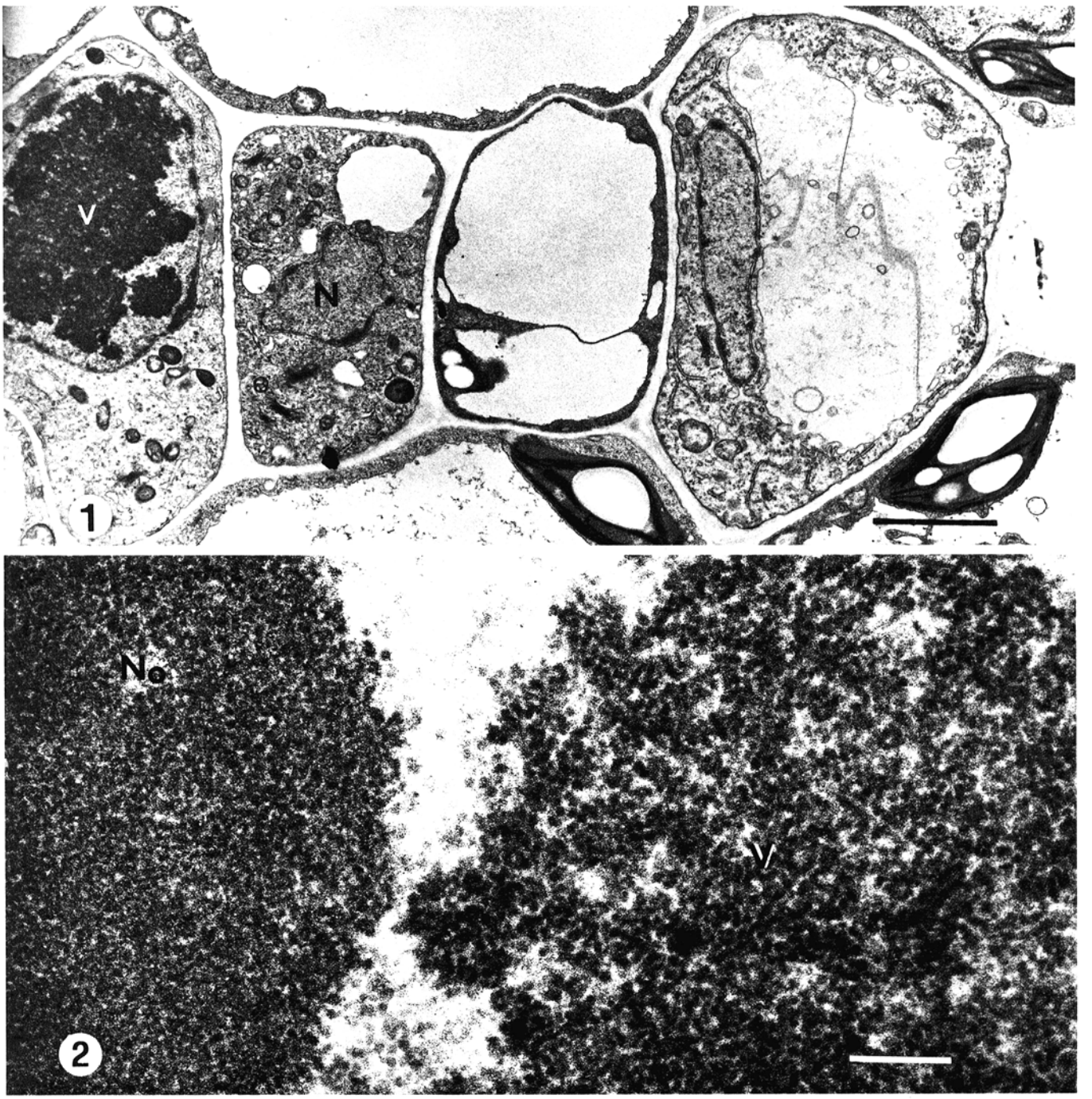
Young tomato and *N. glutinosa* plants grown in a growth chamber (32 C, 5,000 lux, 14-hr day length) were mechanically inoculated as described elsewhere (7). Plants showed symptoms of the disease 7 days after inoculation. Several samples were taken from the same systemically infected young leaf of three different tomato and *N. glutinosa* plants 5, 6, 8, 9, and 13 days after inoculation. Uninoculated healthy plants growing under the same conditions were used as controls. Tissue was fixed with 2% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.0, and postfixed in 2% osmium tetroxide. After dehydration in an ethanol series, the tissue was embedded in Epon 812 resin, sectioned 1.0-μm thick with a diamond knife, and stained with 1% toluidine blue for light microscopy. Thin sections for electron microscopy were stained with uranyl acetate and lead citrate and examined in a JEOL JEM 100 B electron microscope.

RESULTS

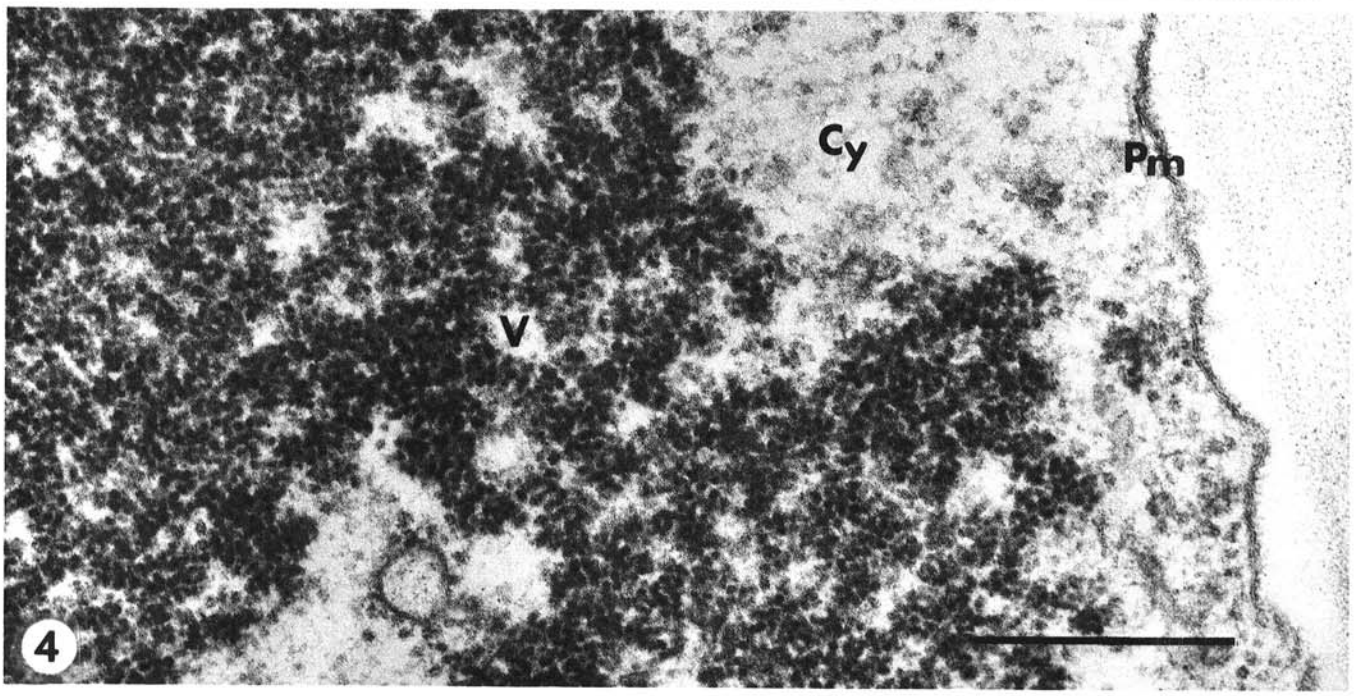
Six days after inoculation of the bottom leaves of tomato plants, major changes associated with MAT infection were found in the nuclei of the vascular bundle cells of systemically infected young leaves. The most striking feature was the appearance of electron-dense masses of viruslike particles (18–20 μm in diameter) in the nuclei of young phloem cells (Figs. 1 and 2) and immature sieve-tube elements (Figs. 3 and 4). The viruslike particles differed

somewhat from the particles in the granular area of the nucleolus (Fig. 2), being slightly smaller and appearing somewhat paracrystalline (Figs. 2 and 4). In immature sieve elements, masses of viruslike particles could be found either within or outside the nucleus (Figs. 3 and 4). Collapsed, necrotic, and apparently dead cells that appeared as electron-dense strands in the intercellular spaces occurred in some vascular bundles (Fig. 3). At high magnification such electron-dense strands often revealed the presence of viruslike particles.

The changes in *N. glutinosa* infected by the MAT agent were similar to those observed in tomato. However, a greater number of phloem cell nuclei with masses of viruslike particles were encountered in *N. glutinosa*. These were, therefore, easily localized with light microscopy in the nuclei of phloem cells of *N. glutinosa* (Fig. 5). These masses were confirmed to be viruslike particles when serial sections of the same tissue were examined by electron microscopy. In addition, nuclei containing viruslike particles also were found in a few mesophyll cells of *N. glutinosa* (Fig. 6) but not in those of tomato. Fibrillar rings, similar in structure to those described in BGMV- and EMV-infected cells (3,4), were numerous in *N. glutinosa* (Fig. 6) and to a lesser extent in tomato tissue. The rings, which were composed of extremely compact fibrils, occurred in the nuclei of infected cells. Epidermal cells appeared to be free of the masses of viruslike particles, but the fibrillar rings were present in some of the epidermal cells of *N. glutinosa* (Fig. 7). Occasionally, some of the fibrillar rings showed dense granules, 20–40 nm in diameter, in association with the inner and outer boundaries of fibrillar rings (Fig. 8). Serial sectioning of tissue with fibrillar rings



Figs. 1 and 2. Ultrastructural effects of infection of tomato leaf tissue 6 days after inoculation with the causal agent of mosaico amarillo del tomate (MAT). Section through very young phloem tissue. **1,** Phloem parenchyma cell with hypertrophied nucleus containing a large mass of viruslike particles (V). Adjacent cell with normal nucleus (N). Scale bar represents 3 μm. **2,** Magnified view of a portion of a similar mass of viruslike particles (V) that are discrete (average diameter of 18–20 nm) compared with the nondiscrete and more electron-dense nucleolus (No). Scale bar represents 200 nm.



Figs. 3 and 4. Section through a young sieve element in a vascular bundle of a tomato leaf infected by the MAT agent 8 days after inoculation. **3,** A mass of viruslike particles (V) is located in the cytoplasm outside the nucleus (N). The dark mass in the area adjacent to the infected cell also is composed of viruslike particles (arrow). Scale bar represents 3 μm. **4,** Enlargement of the mass of viruslike particles (V) in the cytoplasm (Cy) of a maturing cell. Pm = plasma membrane. Scale bar represents 500 nm.

indicated that these rings were spherical structures with an inner part less electron-dense than the surrounding nucleoplasm. Although in most cases the fibrillar rings were found as single structures in the nucleus, association of several rings forming complex structures also was observed (Fig. 9). Masses of viruslike particles were found in a few nuclei starting 6 days after inoculation and their number seemed to increase with time. However, we only checked leaf tissue 5–8 days after inoculation because it was more difficult to find young phloem cells in older tissue. Furthermore, masses of viruslike particles were found outside the nucleus only in leaf tissue 8 days after inoculaion. None of the changes and structures described above were seen in comparable tissue from healthy plants.

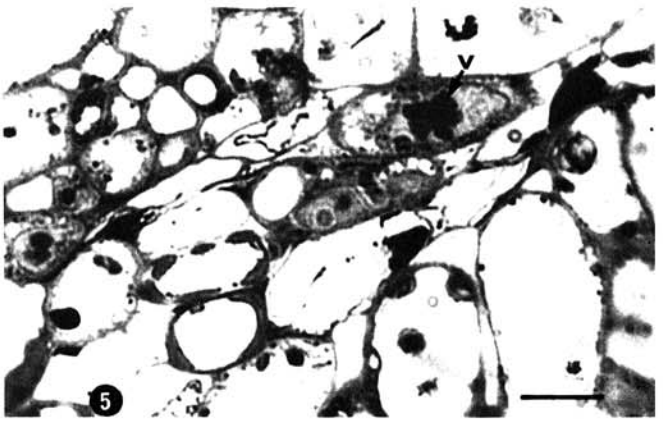
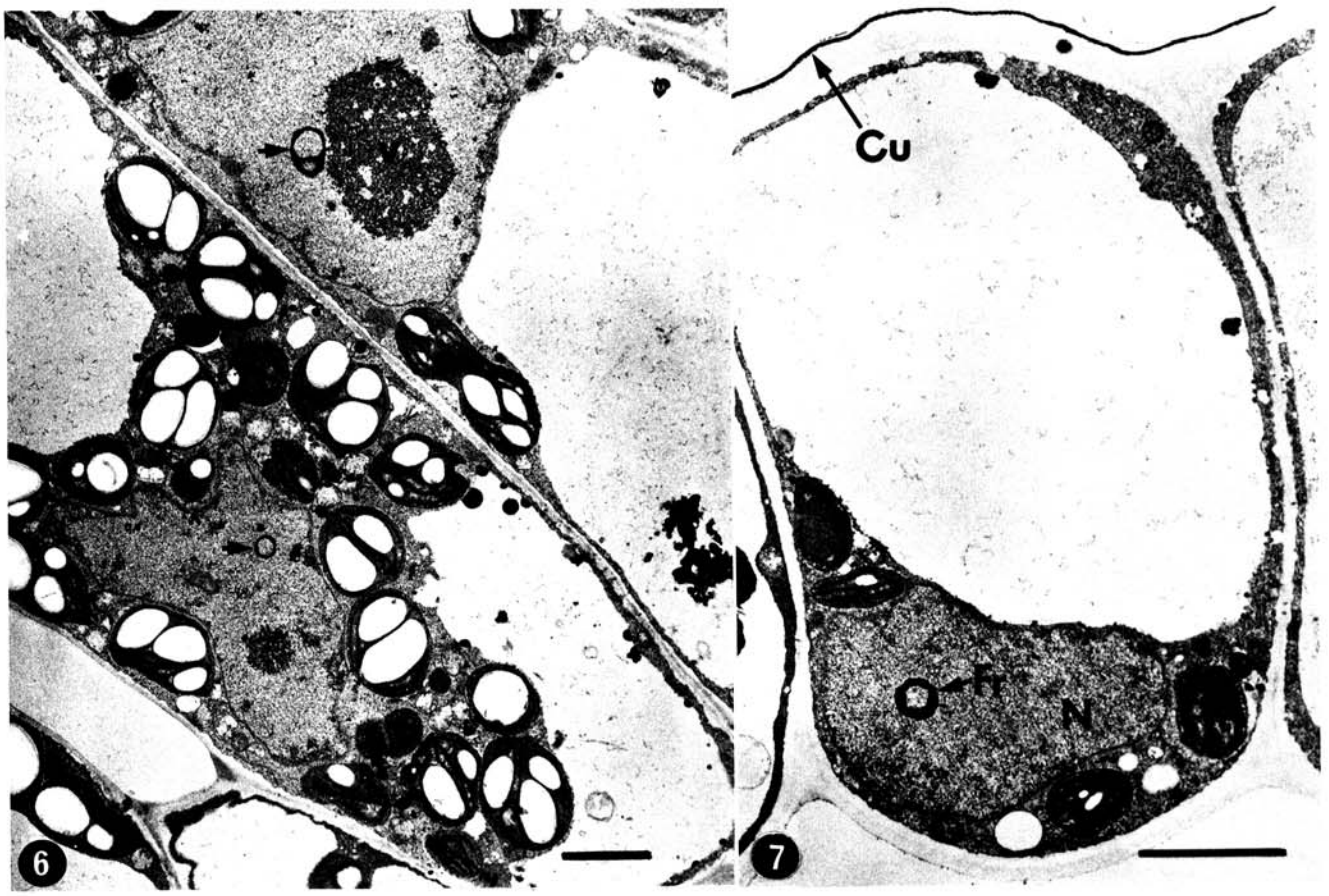


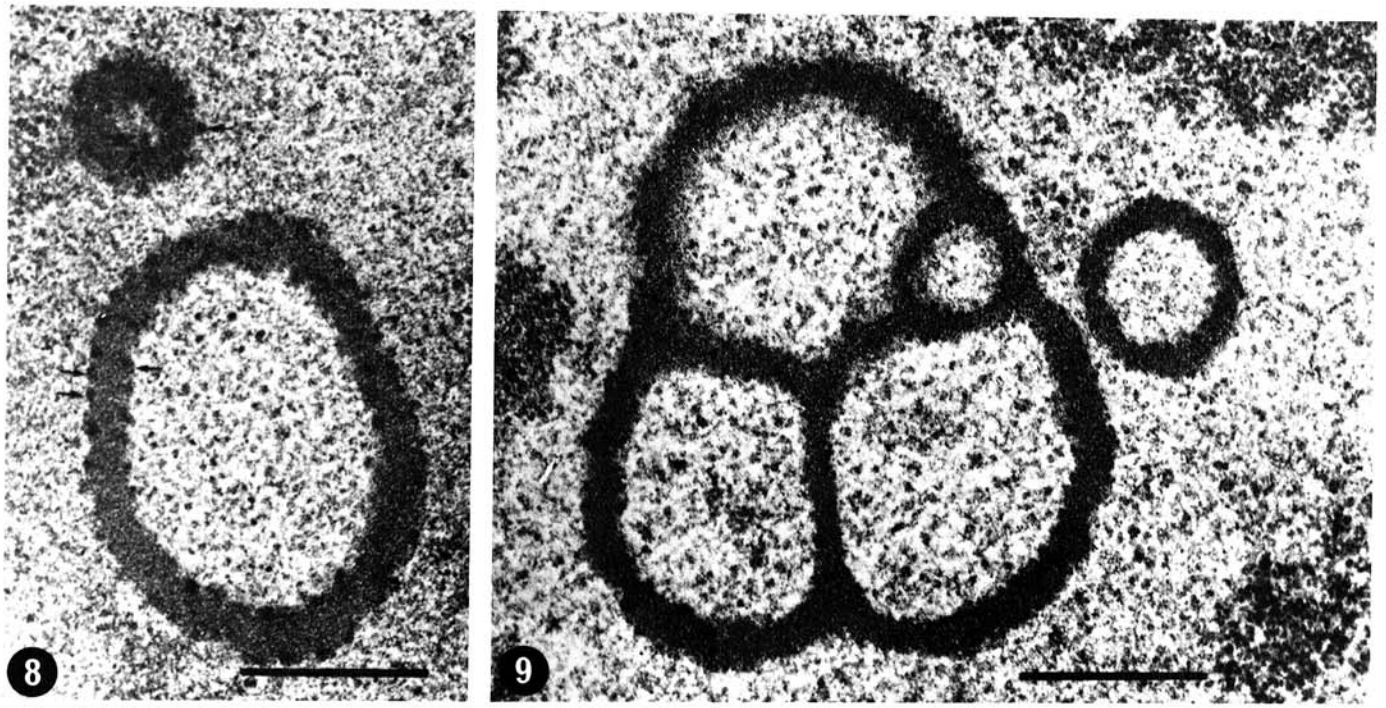
Fig. 5. Light micrograph of a thick section through a vascular bundle in a leaf of *Nicotiana glutinosa* 7 days after inoculation with the MAT agent. A mass of viruslike particles (V) is located in the nucleus of a phloem parenchyma cell. Scale bar represents 10 μm.

DISCUSSION

The morphological changes and sizes of the MAT agent particles observed in the immature phloem cells of vascular bundles of infected tomato and *N. glutinosa* plants were similar to those reported for other whitefly-transmitted viruses; eg, BGMV (3), EMV (4), and tobacco leaf curl virus (TLCV) (6). The presence of viruslike particles in the cytoplasm could be associated with the formation of functional sieve elements. During this process the nuclear membrane is broken and therefore the masses of viruslike particles pass into the cell cytoplasm. A major difference between the MAT agent that we studied and the whitefly-transmitted viruses (3,4,6) is the presence of masses of viruslike particles of the former in the nuclei of mesophyll cells in infected *N. glutinosa* and their restriction to the phloem cells in BGMV- and EMV-infected plants (3,4). Although the fibrillar rings were mostly associated with the masses of viruslike particles, they occasionally also were found in cell nuclei with no detectable viruslike particles. Since the rings were never found in tissue from healthy plants they probably are associated with the earlier stages of viral multiplication. Recently, Kim et al (3) showed that fibrillar rings in *P. vuglaris* infected with BGMV contain DNA. This fact, plus the presence of particles associated with the inner and outer part of the rings and having diameters similar to those of the virus particles and of variable length, seems to substantiate suggestions made by other authors that the fibrillar rings may be the sites of virus assembly (3). From the histological point of view it seems that whitefly-transmitted viruses multiply mainly in young phloem cells. The difficulty in extracting virus from this tissue could be the reason why these viruses are difficult to transmit mechanically. Thus, mechanical transmission of these viruses may depend upon the capacity of the epidermal and mesophyll cells to sustain virus multiplication. The MAT agent was transmitted more easily to *N. glutinosa* than to tomato and this could be explained by the fact that some mesophyll cells (Fig. 6) of *N. glutinosa*, but not of tomato, were infected.



Figs. 6 and 7. Section through a *Nicotiana glutinosa* leaf 8 days after inoculation with the MAT agent. **6,** Mesophyll cells containing masses of viruslike particles (V) in the nucleus together with fibrillar rings (arrows). **7,** Epidermal cell showing cuticle (Cu) and thickening of outer wall. A fibrillar ring (Fr), usually associated with cell infection, is located in the nucleus (N). Scale bars represent 3 μm.



Figs. 8 and 9. Sections through a mesophyll cell of *Nicotiana glutinosa* 8 days after inoculation with the MAT agent. **8,** Fibrillar rings in the nucleus of an infected cell. Note the particles in the outer and inner part of the ring (arrows). Particle sizes are within the range of virus particles. **9,** A complex structure formed by association of several fibrillar rings in the nucleus. Scale bars represent 500 nm.

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