

## Two Types of Inclusions in Maize Infected with Maize Stripe Virus

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Research was conducted while the senior author was on leave as a Fulbright Scholar from the Department of Economic Entomology, Faculty of Agriculture, Cairo University, Giza, Egypt.

Cooperative investigation of USDA-ARS and OSU-OARDC. Submitted as Journal Article 155-83 by the OSU-OARDC.

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We gratefully acknowledge the technical assistance of Frances Butts, Elke Kretschmar, and Anna M. Gerig. We thank R. E. Whitmoyer for preparing the included micrographs, and W. G. Langenberg for generously supplying the gold-labeled goat antirabbit IgG complex.

Salaries and research support were provided by USDA-ARS and by State and Federal funds appropriated to the OSU-OARDC.

Accepted for publication 16 July 1984.

### ABSTRACT

Ammar, E. D., Gingery, R. E., and Nault, L. R. 1985. Two types of inclusions in maize infected with maize stripe virus. *Phytopathology* 75:84-89.

Two types of inclusions were consistently found in the epidermis, mesophyll, vascular parenchyma, and phloem elements of maize stripe virus (MStpV)-infected maize (*Zea mays*) leaves, but not in similar tissues from healthy or maize mosaic virus-infected plants. One type consisted of long, narrow bundles of filamentous, electron-opaque (FEO) material. The bundles were from 30 to 400 nm wide and up to 3.5  $\mu$ m long and were composed of fine filaments in a paracrystalline array with a periodicity of 3.5-3.7 nm. The FEO inclusions were usually surrounded by a membrane and were found in the cytoplasm and cell vacuole. The other type of

*Additional key words:* *Peregrinus maidis*, ultrastructure, rice stripe virus, European wheat striate mosaic virus, colloidal gold, immunocytological labeling.

inclusion consisted of irregularly shaped masses of amorphous, semi-electron-opaque (ASO) material that generally was not enclosed by a membrane. Cells were found that contained both inclusion types. The ASO inclusions were much more abundant in leaves 4-5 wk after inoculation than at 1-2 wk; the incidences of FEO inclusions were similar at both times. Antibodies to the MStpV noncapsid protein bound to the FEO inclusions, antibodies to the MStpV nucleoprotein bound to an unidentified cytoplasmic constituent, and neither antibody bound to ASO inclusions.

Maize stripe (MStpV) and rice stripe viruses are members of a new plant virus group (4,5). Plants infected with these viruses contain a fine-stranded nucleoprotein about 3 nm in diameter, and large amounts of a noncapsid protein (4,7,8). Despite occurring in high concentrations in infected tissue, neither the nucleoprotein nor the noncapsid protein have been located in MStpV-infected tissue. Previous studies of thin sections of MStpV-infected maize (*Zea mays* L.) have revealed no typical virus or mycoplasma-like structures (2,6,13) although cytoplasmic inclusions of low electron density have been reported for MStpV-infected tissue (2).

In this paper we describe two cellular inclusions associated with MStpV-infected but not maize mosaic virus-infected or healthy maize leaves. One of these is probably the low-density inclusion, but the other has not previously been reported. We also report attempts to characterize the inclusions by immunocytological labeling with colloidal gold-labeled antibodies.

### MATERIALS AND METHODS

Leaf pieces from corn plants (*Zea mays* 'Aristogold Bantam Evergreen' and 'Golden Cross Bantam') showing symptoms of MStpV either 1-2 or 4-5 wk after inoculation were prefixed overnight in cold 2.5% glutaraldehyde in 0.1 M potassium phosphate buffer (pH 7.4) and postfixed in 1% OsO<sub>4</sub> in the same buffer for 3 hr. After staining overnight in 1% uranyl acetate, the leaf pieces were dehydrated in an ethanol-acetone series, and imbedded in Spurr's medium. Ultrathin sections were cut with a

diamond knife, stained with 0.5% uranyl acetate and 0.1% lead citrate, and examined in a Philips 201 electron microscope.

The MStpV isolate and the *Peregrinus maidis* (Ashmead) vector used to inoculate maize plants were obtained from Florida (3).

Control leaves from healthy and maize mosaic virus (MMV)-infected plants were similarly processed and examined. MMV, also vectored by *P. maidis*, was obtained from Hawaii.

For immunocytological labeling, leaf pieces of MStpV-diseased or healthy control plants were processed for electron microscopy as described above except that postfixation in OsO<sub>4</sub> and the first uranyl acetate staining were omitted. Sections were etched and immunocytologically labeled with antisera to MStpV nucleoprotein or noncapsid protein followed by gold-labeled goat antirabbit IgG as described by Lin and Langenberg (9) except that: 1% ovalbumin was used to rinse the sections instead of 3.3% normal goat serum, the antisera were used at 1:10 dilutions instead of 1:100, and the sections were incubated for 1 hr with gold-labeled goat antirabbit IgG complex instead of 10 min. After labeling, sections were stained 10 min in 5% uranyl acetate, rinsed with water, stained 5 min in 0.1% lead citrate, rinsed with water, dried, and examined.

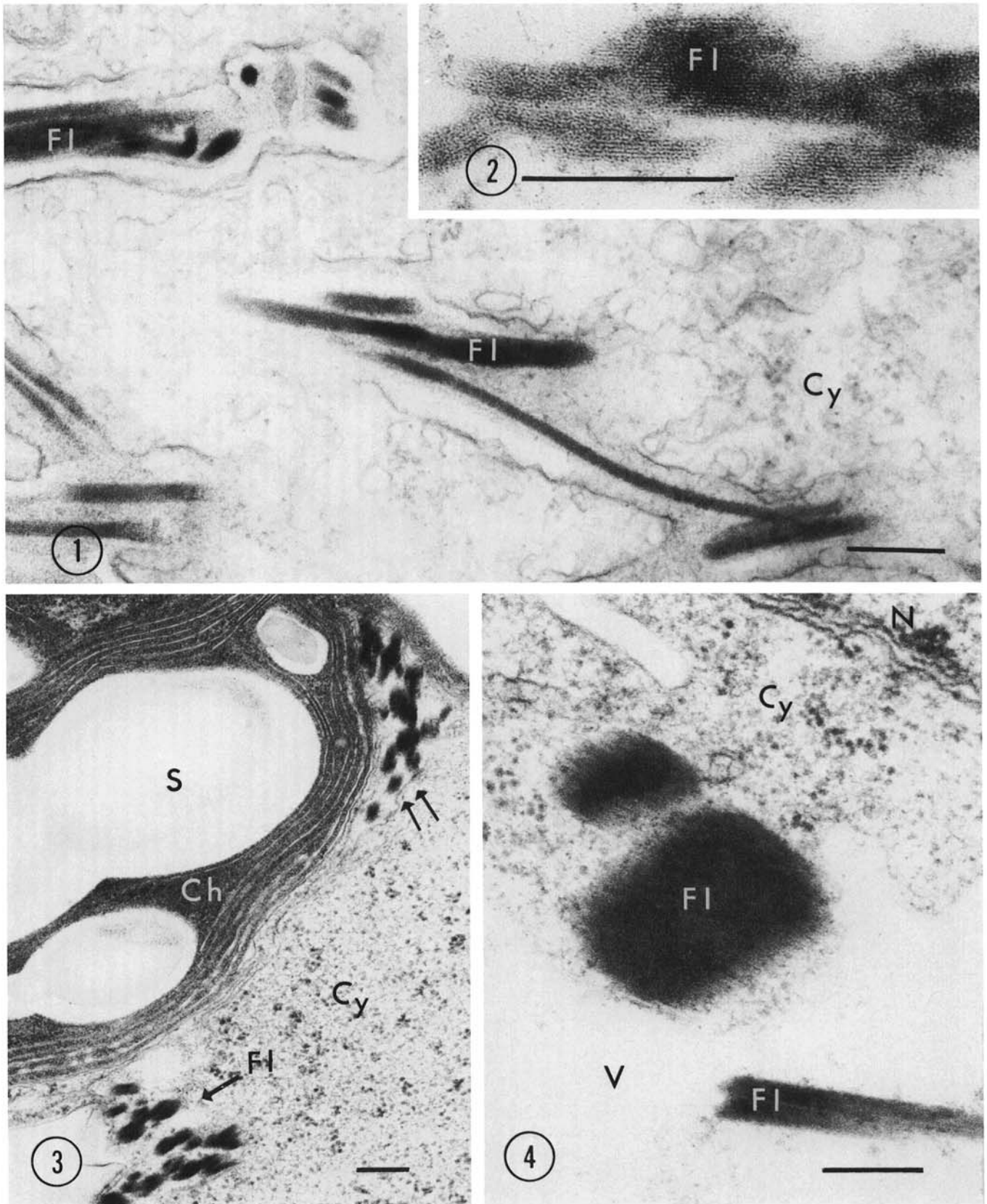
### RESULTS

**Intracellular inclusions.** Two types of inclusions were consistently found in leaves from MStpV-infected corn plants but not in leaves from healthy or MMV-infected plants. The inclusions were designated filamentous, electron-opaque (FEO) or amorphous, semi-electron-opaque (ASO).

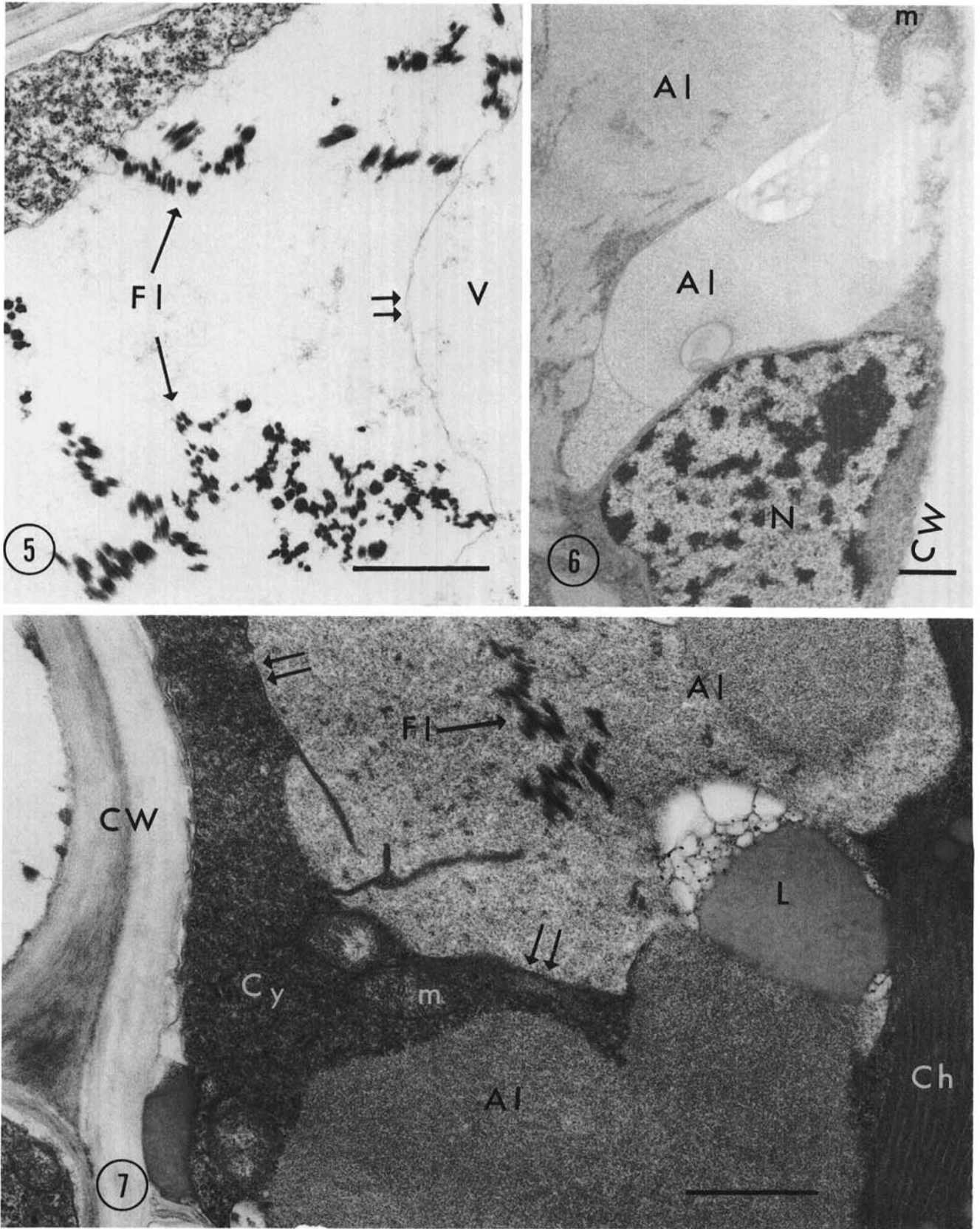
The FEO inclusions were long, narrow bundles that were either straight or curved (Figs. 1-5), from 30 to 400 nm wide, and up to 3.5  $\mu$ m long. Filamentous units, closely packed in paracrystalline arrays with a periodicity of 3.5-3.7 nm, were clearly discernible in some micrographs of these bundles (Figs. 1 and 2) where the plane of sectioning was parallel to the long axis of the filaments. FEO inclusions were found in phloem elements (Fig. 1), the epidermis,

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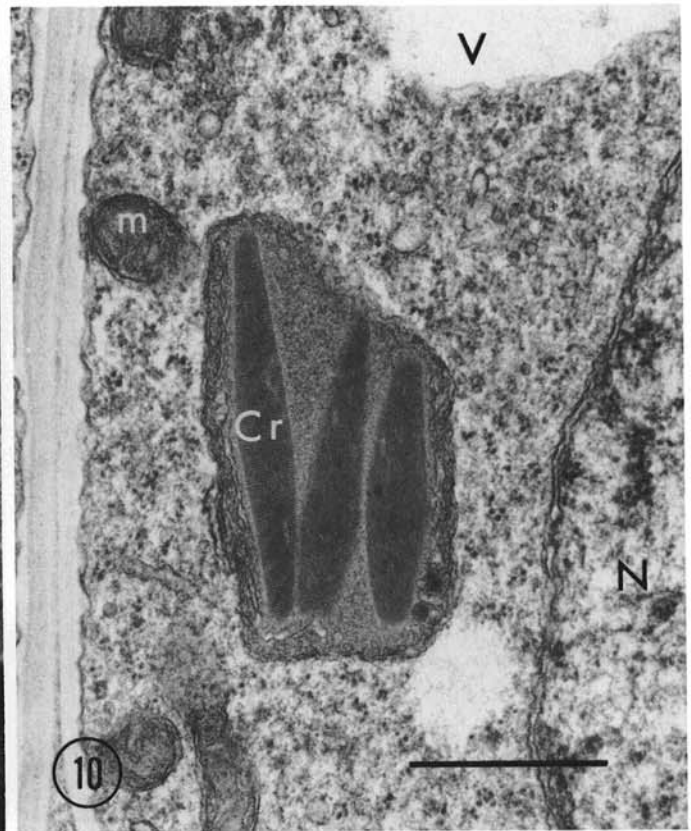
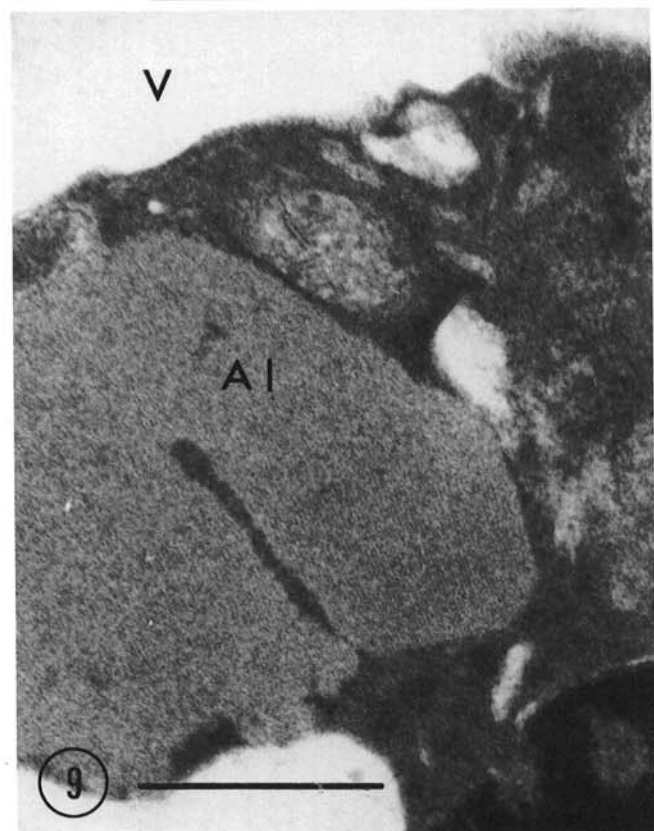
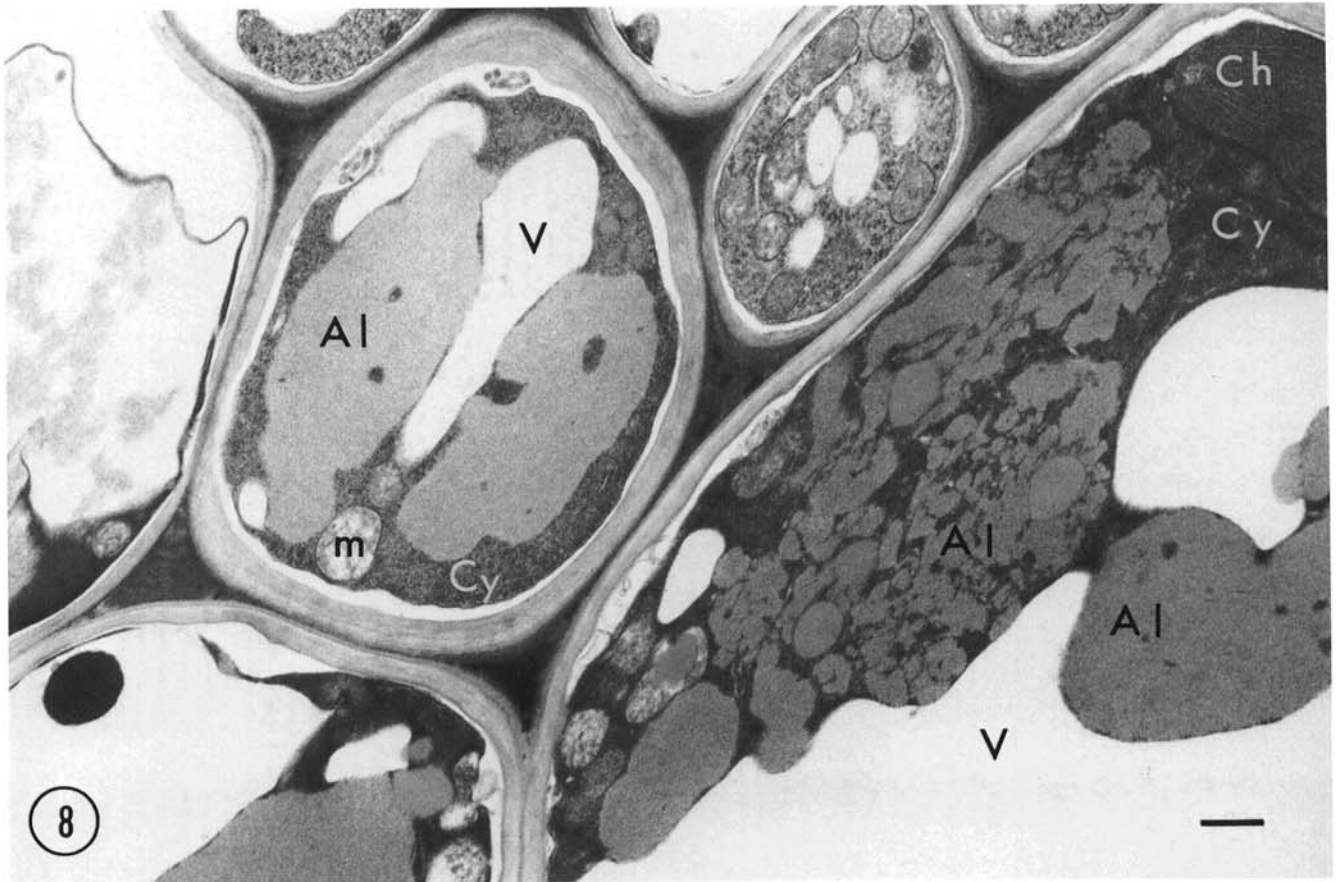


**Figs. 1-4.** Filamentous electron-opaque (FEO) inclusions in MStpV-infected maize leaves: **1**, Bundles of FEO inclusions (FI) membrane-bounded in the cytoplasm of a phloem cell. **2**, Higher magnification of FEO bundles (FI) showing a paracrystalline filamentous structure in an epidermal cell. **3**, FEO inclusions (FI) bounded by membranes (double arrows) in the cytoplasm of a parenchyma cell. **4**, FEO inclusions (FI) apparently not bounded by membranes in the cytoplasm and vacuole of a parenchyma cell. Ch = chloroplast, Cy = cytoplasm, FI = FEO inclusion, N = nucleus, S = starch grain, and V = vacuole. Bars = 200 nm.



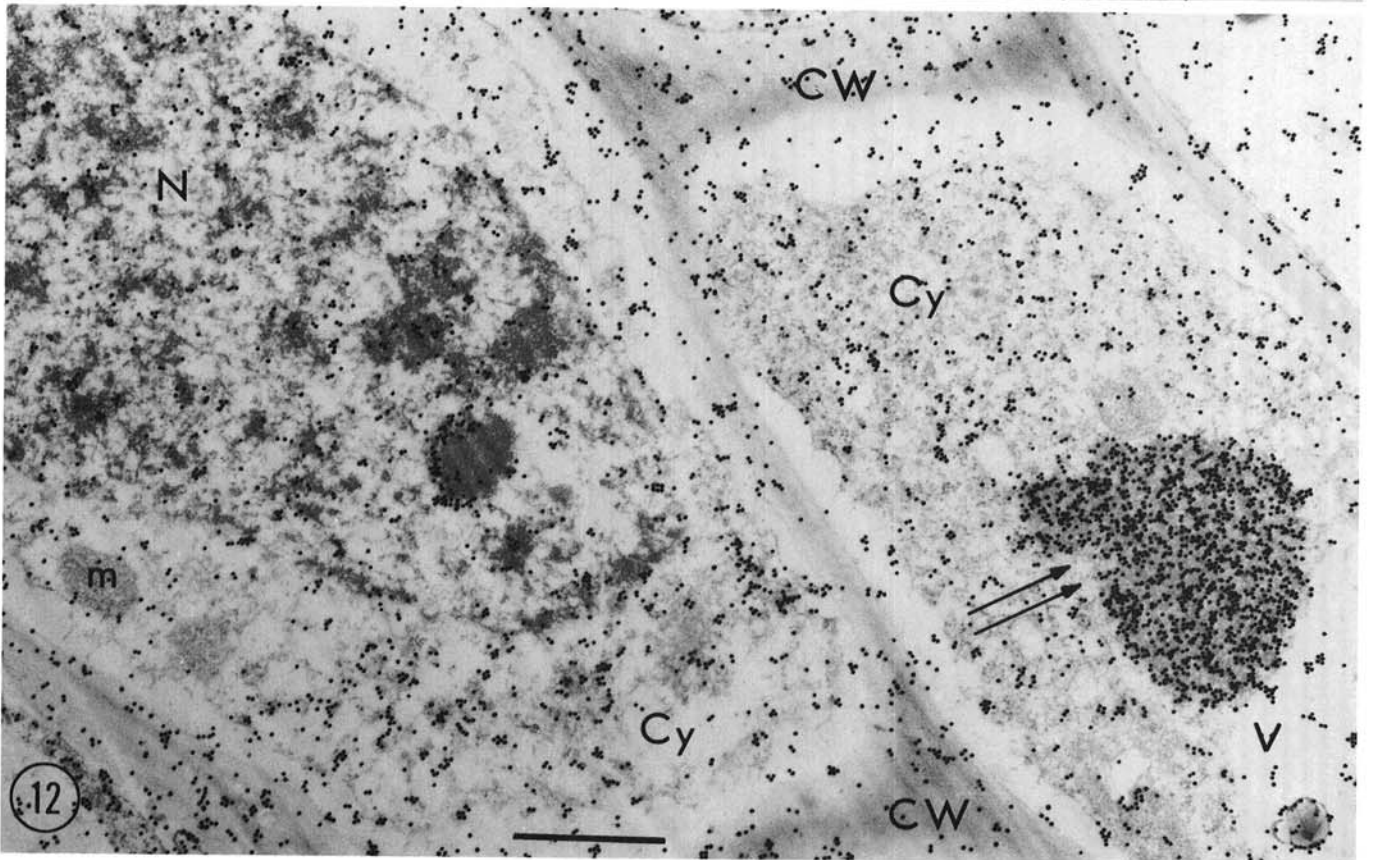
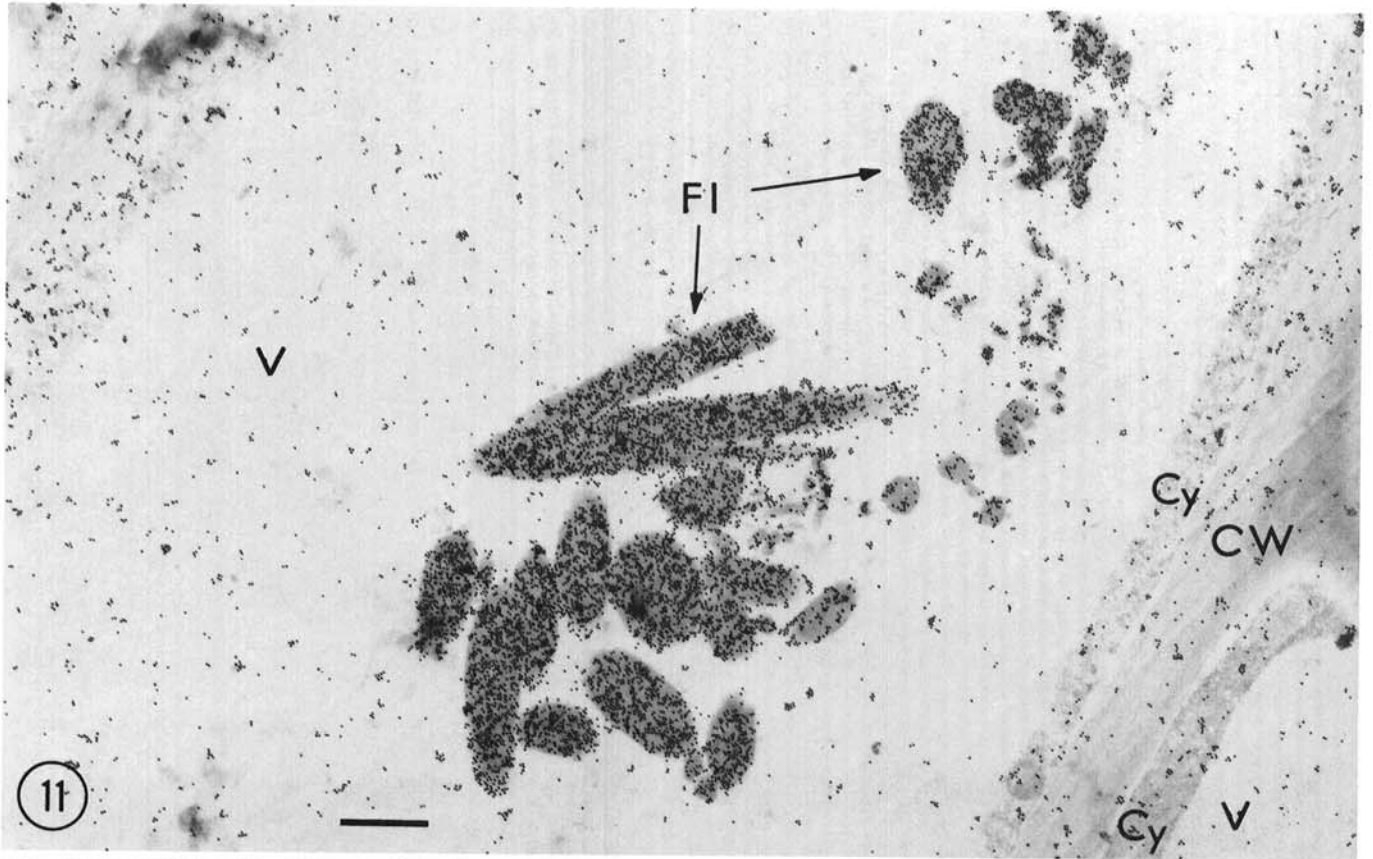
**Figs. 5-7.** Filamentous electron-opaque (FEO) and amorphous semi-electron-opaque (ASO) inclusions in MSTpV-infected maize leaves: **5,** Bundles of the FEO inclusions (FI) in a large vacuolelike structure surrounded by a membrane (double arrows) in a parenchyma cell. **6,** Large masses of the ASO inclusions (AI) occupying most of the cell space of an epidermal cell. **7,** FEO inclusions (FI) surrounded by large masses of the ASO inclusions (AI) in a parenchyma cell. The ASO inclusions are not bounded by membranes except in a few areas (double arrows). AI = ASO inclusions, Cy = cytoplasm, CW = cell wall, FI = FEO inclusions, L = lipid, m = mitochondrion, N = nucleus, and V = vacuole. Bars = 800 nm.





**Figs. 8-9.** Amorphous semi-electron-opaque (ASO) inclusions in MStpV-infected maize leaves: **8**, Large masses of the ASO inclusions (AI) in phloem (left) and vascular parenchyma (right) cells. **9**, Part of an ASO inclusion (AI) showing crystalline or paracrystalline structure.

**Fig. 10.** Polygonal crystals (Cr) embedded in a granular matrix surrounded by membranes. This is from a healthy plant although similar structures were also seen in diseased plants. AI = ASO inclusion, Ch = chloroplast, Cr = crystal, Cy = cytoplasm, m = mitochondrion, N = nucleus, and V = vacuole. Bars = 600 nm.



**Figs. 11-12.** Immunocytological labeling of sections from MStpV-infected maize leaves. **11.** Treated with MStpV noncapsid protein antiserum. **12.** Treated with MStpV nucleoprotein antiserum (double arrows indicate labeled cytoplasmic mass). Cy = cytoplasm, CW = cell wall, FI = FEO inclusion, m = mitochondrion, N = nucleus, and V = vacuole. Bars = 800 nm.

and vascular and mesophyll parenchyma (Figs. 2-5). FEO bundles, either individually or in groups, were usually surrounded by a membrane (Figs. 1, 3, and 5), but occasionally were seen free in the cytoplasm (Fig. 4) or cell vacuole (Figs. 2 and 4). The FEO inclusions were not found within or in close association with the nucleus, chloroplasts, or mitochondria. Their abundance in leaves inoculated 1-2 or 4-5 wk before fixation was similar.

The ASO inclusions were large, irregularly shaped masses of granular or fine, fibrous material of low electron density (Figs. 6-8) that in rare instances appeared crystalline with a periodicity of about 11 nm (Fig. 9). They were found in the epidermis (Fig. 6), mesophyll, and vascular parenchyma (Figs. 7 and 8) and in phloem elements (Fig. 8). ASO inclusions were generally not separated from the adjacent cytoplasm by membranes, but masses of these inclusions were occasionally separated from each other or from the cell vacuole by a limiting membrane (Figs. 6-8). Material appearing similar to the lipid usually found in chloroplasts was frequently found either in or around the ASO inclusions (Fig. 7). ASO and FEO inclusions were occasionally found together in the same cell (Fig. 7). In leaves inoculated 1-2 wk before fixation, ASO inclusions occurred in fewer cells and occupied less area per cell than in leaves inoculated 4-5 wk before fixation.

A third type of intracellular cytoplasmic inclusion was found in leaves of healthy and MStpV- or MMV-diseased plants, although more commonly in diseased than healthy leaves, and more commonly in phloem than parenchyma cells. These inclusions were polygonal crystals with an internal periodicity of about 10 nm. They were usually embedded in a granular, semi-electron-opaque matrix surrounded by membranes (Fig. 10).

No virus or mycoplasma-like structures were seen in MStpV-diseased leaves. As expected, numerous rhabdovirus-like particles were seen in MMV-diseased leaves.

**Changes in cell ultrastructure after MStpV infection.** In leaves inoculated with MStpV 1-2 wk before fixation, no ultrastructural changes in cells were noticed except for the presence of FEO and, less frequently, ASO inclusions. However, in leaves of plants inoculated 4-5 wk before fixation, several ultrastructural changes were seen, apparently a result of the large volume occupied by ASO inclusions. Cell vacuoles were often smaller in large cells (Fig. 8) and nearly absent in small cells (Figs. 6 and 7). Compared to healthy controls, the cytoplasm was more electron-dense (Figs. 6-8), and the chloroplasts usually contained fewer well-organized stacks of grana lamellae and starch grains (Figs. 7 and 8).

**Immunocytological labeling.** Noncapsid protein antibodies specifically attached to FEO inclusions (Fig. 11); nucleoprotein antibodies attached to some unidentified, electron-opaque cytoplasmic masses (Fig. 12). Unexpectedly, neither type of antibody attached to the ASO inclusions. The non-osmic acid treated tissue processed for immunocytological labeling (Figs. 11 and 12) showed less structural detail than osmic acid treated tissue processed for ultrastructural examination (Figs. 1-10).

## DISCUSSION

The ASO inclusions were probably the same as the low-density inclusions reported by Bradfute and Robertson (2), but the FEO inclusions have not been reported before from MStpV-diseased plants. The absence of FEO and ASO inclusions in healthy or MMV-infected plants suggests that both types of inclusion are specific to MStpV infections and do not represent a general response to disease in maize plants.

Immunocytological labeling indicated that the FEO inclusions contained noncapsid protein, but the role of the ASO inclusions in

the disease remains unclear since they bound neither noncapsid protein nor nucleoprotein antibodies. The identity of the electron-opaque, cytoplasmic masses that bound nucleoprotein antibodies was not determined, because of the poor preservation and contrast in sections used for immunocytological labeling.

Inclusions similar to FEO, although larger in diameter, were found in wheat (*Triticum aestivum* L.) plants infected with European wheat striate mosaic virus (EWSMV) (1). This virus shares the following characteristics with MStpV and rice stripe virus: transmission by delphacid planthoppers; transovarial transmission; slow, heterodisperse sedimentation in sucrose density gradients (11); and large quantities of a noncapsid protein in infected tissue (R. E. Gingery, unpublished). Also, no typical virus or mycoplasma-like structures have been observed in EWSMV-infected plants (1,11). Thus, EWSMV may be another member of the MStpV-RSV group of plant viruses. Rice hoja blanca virus (RHBV), which also is transovarially transmitted by a delphacid, probably belongs to this group (10), although in earlier reports a filament of larger diameter (8-10 nm) was associated with the disease (12). Bundles of threadlike particles that frequently occupied much of the cell volume were observed in RHBV-infected plant and vector cells (12). The relationship, if any, between these bundles and FEO or ASO inclusions is unknown. The volume and location of these bundles and ASO inclusions were similar, but we have never observed threadlike particles in ASO inclusions. On the other hand, the electron-opacity and ultrastructure of these bundles are more comparable to FEO- and EWSMV-associated inclusions than to ASO inclusions.

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