Tubular Helical Structures and Fine Filaments Associated with the Leafhopper-borne Maize Yellow Stripe Virus

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ABSTRACT

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A new disease agent, designated maize yellow stripe virus (MYSV) and transmitted in a persistent manner by the leafhopper *Cicadulina chinai*, is associated with three types of symptoms on infected plants: fine stripe, coarse stripe, and chlorotic stunt. Light and electron microscopy of naturally or experimentally infected maize or sorghum leaves showing any of these three symptoms revealed the presence of large, amorphous, intracytoplasmic inclusions in phloem elements, vascular parenchyma, bundle sheath, and mesophyll cells. These inclusions contained masses of long, flexuous, tubular structures, approximately 34 nm in diameter, apparently composed of helically wound filaments 5–7 nm thick. These structures commonly were associated with or sandwiched between aggregated mitochondria, some of which were degenerated. Some of the cells containing tubular structures also contained masses of loosely or densely packed fine fibrils. Purified preparations obtained from naturally infected leaves had typical nucleoprotein ultraviolet absorbance spectra and contained fine filaments 4–8 nm in diameter. Crystallized, apparently nonvirion protein also was purified from these leaves and was serologically unrelated to the noncapsid protein of maize stripe virus (MStV). Crude extracts from infected leaves did not react with antisera to the capsid protein of MStV or to several other maize viruses and spiroplasma in enzyme-linked immunosorbent assay. Similarities and differences between MYSV and tenuiviruses (rice stripe virus group) are discussed.

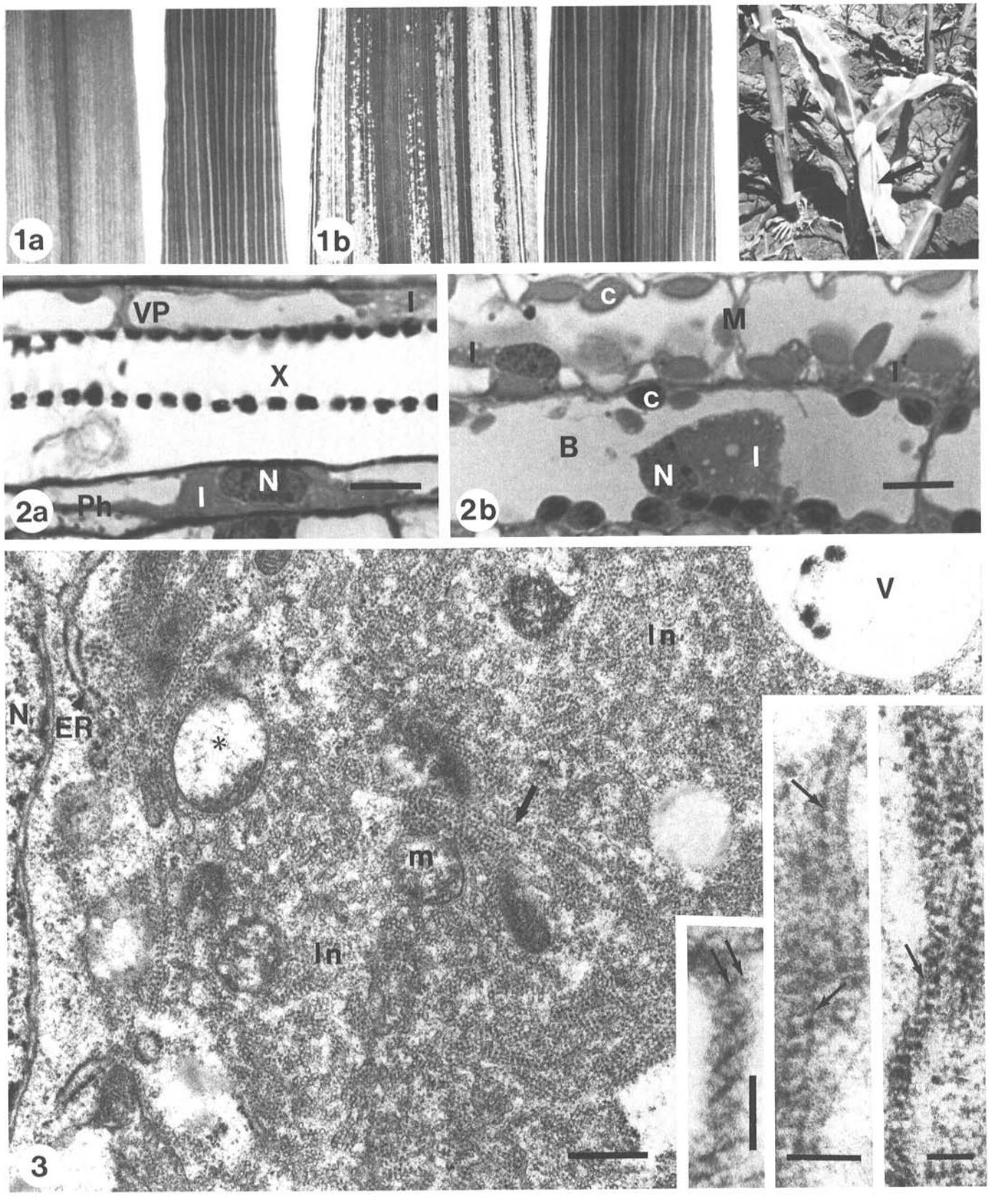
In a survey of maize virus and viruslike diseases in Egypt during 1981 and 1982, a new disease, designated maize yellow stripe, was discovered (6). The agent causing this disease is transmitted in a persistent manner by the leafhopper *Cicadulina chinai* Ghauri, but not by *C. bipunctella zeae* China, four other leafhopper and planthopper species, or mechanical means (5,6). In addition to maize (*Zea mays* L.), this agent infects sorghum (*Sorghum bicolor* (L.) Moench), wheat (*Triticum aestivum* L.), and barley (*Hordeum vulgare* L.). During 1984 and 1985, incidence of maize yellow stripe was very high, particularly in late-sown maize plants in Giza and some other areas of Egypt (1,4). Three types of symptoms are associated with this disease: fine stripe, coarse stripe, and chlorotic stunt (Fig. 1). When *C. chinai* were fed on maize leaves showing either fine or coarse stripe and then allowed to inoculate test plants, individual test plants developed both symptoms, usually with fine stripe on the lower leaves and coarse stripe on the upper leaves (5). A preliminary ultrastructural study indicated that maize yellow stripe is associated with previously undescribed fine, helical, filamentous structures (3). In the present work, we further describe these structures and other cytopathological features of maize yellow stripe-diseased maize and sorghum leaves, using light and electron microscopy. Serological and purification methods were used to identify and characterize the disease agent, for which the name maize yellow stripe virus (MYSV) is proposed.

MATERIALS AND METHODS

Light and electron microscopy. Samples of maize leaves showing each of the three types of symptoms associated with maize yellow stripe were collected from naturally or experimentally infected plants growing under field or greenhouse conditions, respectively, in Giza, Egypt, in 1986 and 1987. Additionally, sorghum leaves showing coarse stripe symptoms were collected from nearby fields in 1987. For light and electron microscopy, leaf pieces were prefixed in cold 3% glutaraldehyde in 0.1 M potassium phosphate buffer, pH 7.4, for 1 to several days, postfixed in 1% OsO_4 for 3 hr, stained overnight in 1% uranyl acetate, dehydrated in an ethanol-acetone series, and embedded in Spurr's medium. Prefixation was done in Egypt or Ohio, but samples were further processed and examined in Ohio. Semithin sections (1–2 μm thick) for light microscopy (Figs. 2A and B) were cut with glass knives and stained with toluidine blue. Ultrathin sections for electron microscopy (Figs. 3–9) were cut with a diamond knife and stained with uranyl acetate and lead citrate. Pieces from healthy leaves were similarly processed and examined. Designation and identification of various cell types in maize leaf vascular bundles follow Evert et al (11).

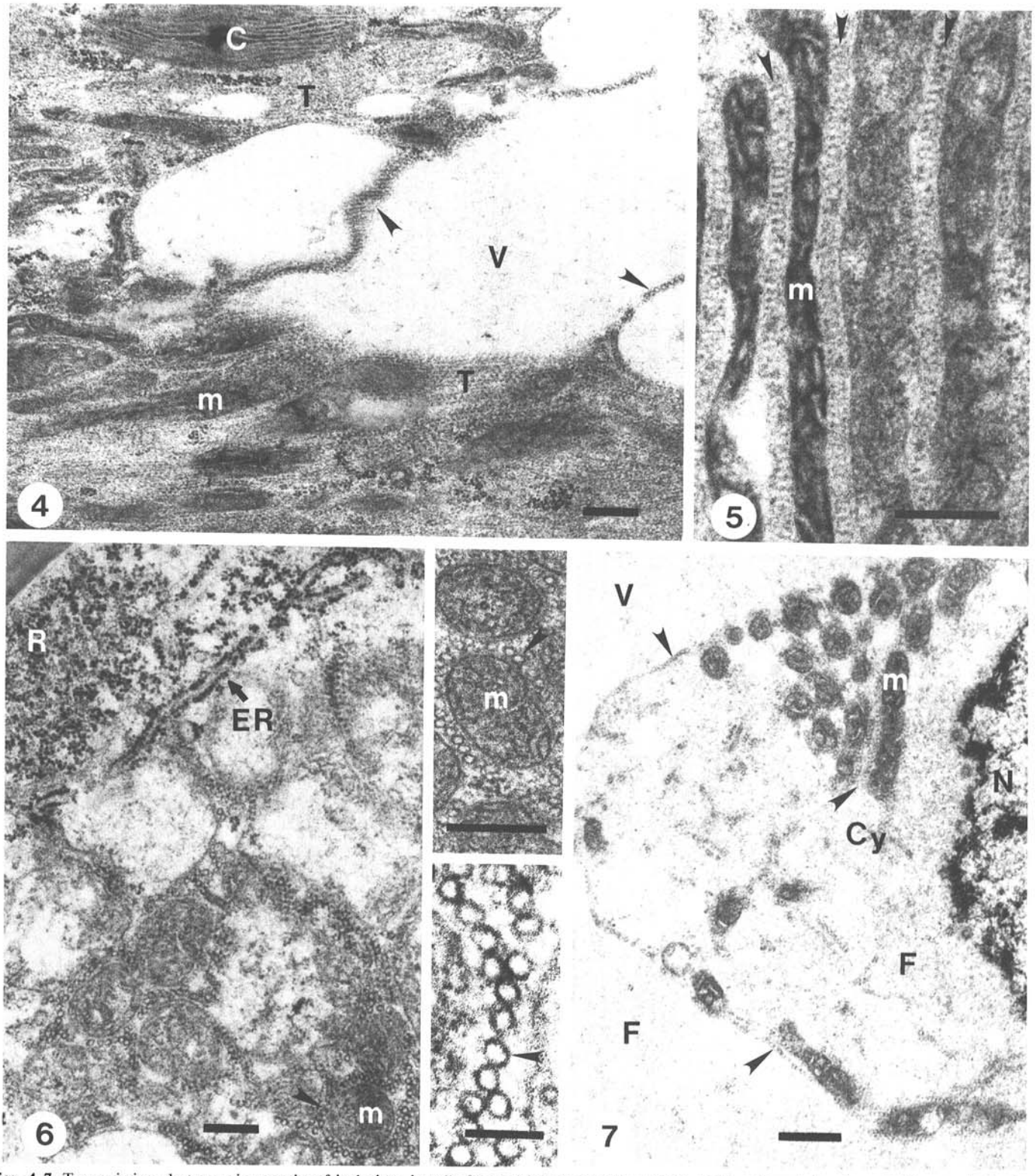
Purified or clarified extracts from naturally infected maize or sorghum leaves, prepared as described below, were negatively stained with 2% potassium phosphotungstate, pH 7.2, and examined by electron microscopy (Figs. 10 and 11) at magnifications up to ×100,000. A Phillips 201 electron microscope (Phillips Electronic Instruments, Inc., Mahwah, NJ) was used and was regularly calibrated with Fullam diffraction grating (Ernest F. Fullam, Inc., Latham, NY).

Purification. Diseased maize or sorghum leaves, naturally infected in the field in Giza in 1987 and showing any of the



Figs. 1–3. 1, Symptoms of maize yellow stripe virus (MYSV) on maize. **A,** Experimental infections: fine-stripe and coarse-stripe symptoms, respectively. **B,** Natural infections: fine-stripe, coarse-stripe, and chlorotic-stunt symptoms, respectively. Arrow indicates chlorotic, bent, apex. **2A and B,** Light micrographs of semithin sections from MYSV-infected maize leaves: large, amorphous inclusions (I) in the phloem elements (Ph), vascular parenchma (VP), mesophyll (M), and bundle sheath (B) cells. N = nucleus, X = xylem, c = chloroplast. Bars = 10 μm. **3,** Transmission electron micrograph of a large inclusion (In) in a parenchyma cell from MYSV-infected maize leaf, packed with masses of tubular helical structures (arrow and insets), in addition to mitochondria (m) and small vacuoles (V). * = degenerated mitochondria, ER = endoplasmic reticulum, N = nucleus. Double arrows in the inset indicate spirally wound tubular structures. Bar in main figure = 500 nm; bars in insets = 100 nm.

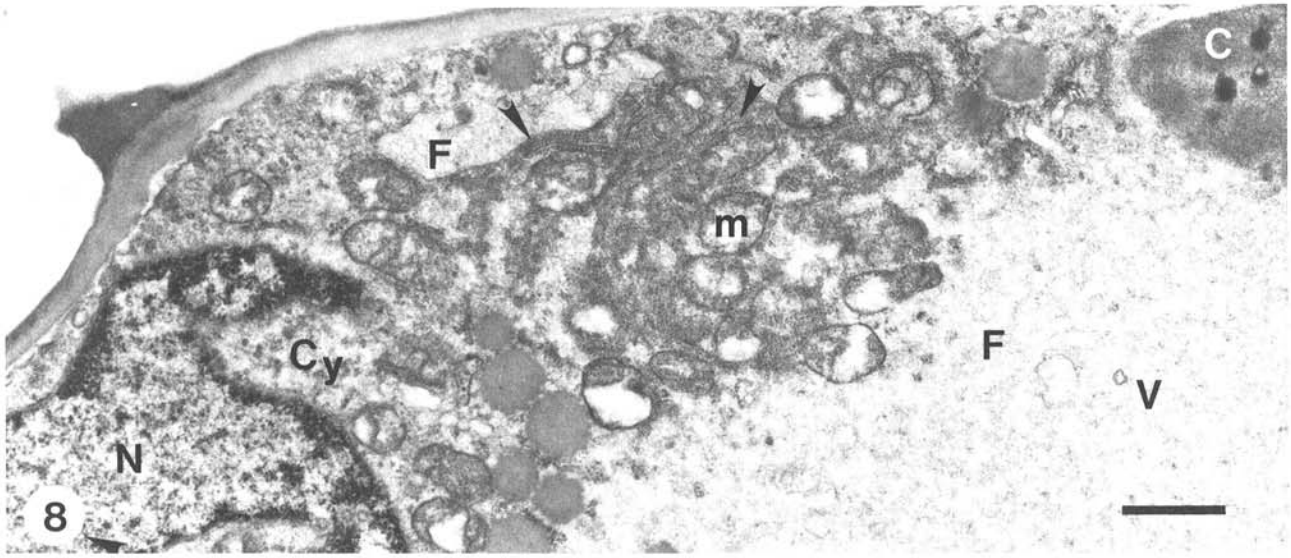
three symptoms associated with maize yellow stripe, were blended in 0.01 M potassium phosphate, 0.01 M ethylenediaminetetraacetic acid (EDTA), pH 7.0 (extraction buffer), plus 0.5% 2-mercaptoethanol (5 ml of buffer/g of tissue). The extract was pressed through two layers of cheesecloth and clarified by emulsification with 1/4 v chloroform. After low-speed centrifugation (6,000 *g* for 15 min), the aqueous layer was centrifuged at 100,000 *g* for 3 hr. The pellet was resuspended in extraction buffer, clarified by low-speed centrifugation, and centrifuged for 2 hr at 40,000 rpm through a 10–40% (w/w) linear sucrose gradient in extraction buffer in an SW41Ti rotor tube (Beckman Instruments, Inc., Palo Alto, CA). Centrifuged gradients were

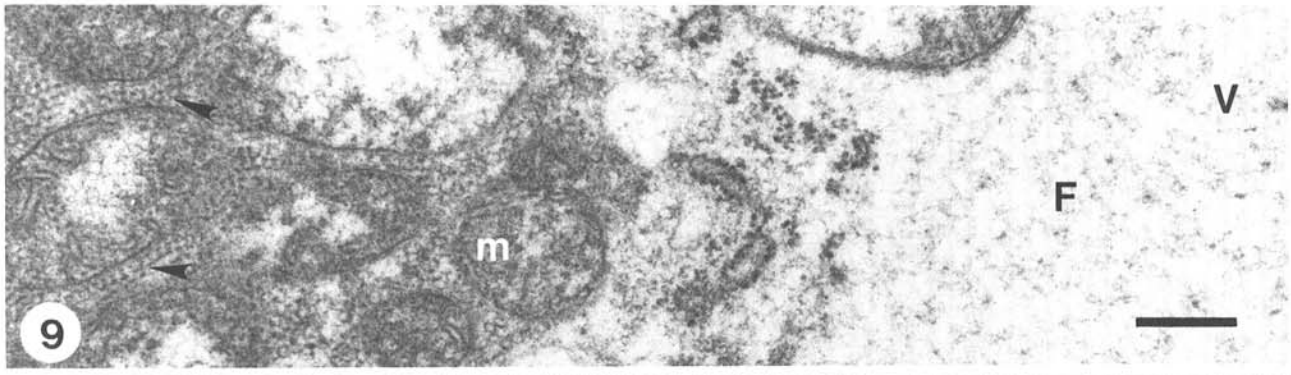


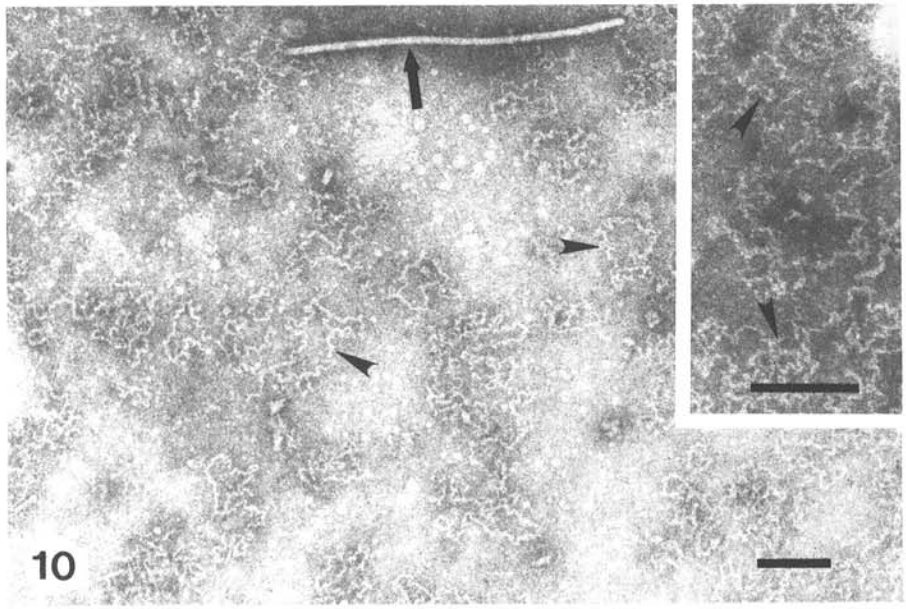
Figs. 4–7. Transmission electron micrographs of inclusions in cells from maize leaves infected with maize yellow stripe virus. **4,** Masses of tubular helical structures (T and arrowheads) in a bundle sheath cell, with mitochondria (m), chloroplast (C), and vacuoles (V). **5,** Longitudinal view of the tubular helical structures (arrowheads), sandwiched between elongated mitochondria (m). **6,** Cross sections of the tubular helical structures (arrowheads) surrounding mitochondria (m). ER = endoplasmic reticulum, R = ribosomes and polyribosomes. **7,** Aggregated mitochondria (m) in a mesophyll cell, with tubular structures (arrowheads) and fine fibrils (F) in the cytoplasm (Cy) and vacuole (V). N = nucleus. Bars = 300 nm (Figs. 4–6), 300 and 100 nm (Fig. 6 upper and lower insets, respectively), and 500 nm (Fig. 7).

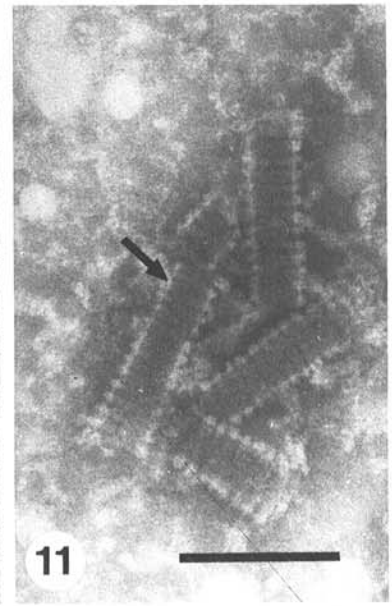
scanned at 254 nm, and an ultraviolet-absorbing zone about one-third of the distance from the top of the gradient was collected. Zones were diluted three- to fivefold with extraction buffer and centrifuged 2 hr at 45,000 rpm in the Beckman Type 50 rotor. Pellets were suspended in extraction buffer. Leaves from healthy maize plants were processed similarly and examined.

Nonvirion protein isolation. Field-collected diseased leaves also were subjected to a modified purification procedure for the non-capsid protein associated with maize stripe virus (MStV) (15). The phosphate-citrate buffers used were mixtures of 0.2 M K_2HPO_4 and 0.1 M citric acid. One gram of MYSV-infected tissue was ground in 4 ml of phosphate-citrate, pH 5.5. The extract was pressed through cheesecloth and centrifuged at 10,000 *g* for 30 min. The pellet was resuspended in 3 ml of phosphate-citrate,









Figs. 8–11. 8 and 9, Electron micrographs of inclusions in mesophyll cells of sorghum (Fig. 8) and maize (Fig. 9) infected with maize yellow strip virus. These cells contain aggregated mitochondria (m), tubular helical structures (arrowheads), and masses of fine fibrils (F) in the cytoplasm (Cy) and in non-membrane-bound vacuoles (V). C = chloroplast, N = nucleus. Bars = 1 μm (Fig. 8) and 300 nm (Fig. 9). **10,** Fine, filamentous particles (arrowheads) found in negatively stained, partially purified preparations of maize yellow stripe-diseased leaves. Arrow indicates a maize dwarf mosaic virus particle (found in a doubly infected sample), included here as a scale of internal size. Bars = 100 nm. **11,** Tubular structures (arrow), apparently composed of helical filaments, found in clarified extracts of maize yellow stripe-diseased leaves. Bar = 100 nm.

pH 7.5, and centrifuged at 12,000 *g* for 1 hr. The supernatant was recovered and adjusted to pH 5.5 with 0.1 M citric acid, and the protein was allowed to crystallize overnight at 4 C. The crystals were pelleted by centrifugation at 12,000 *g* for 30 min and redissolved in 3 ml of phosphate-citrate, pH 7.5.

The nonvirion protein was tested serologically in an agar-gel double-diffusion assay (16) using antiserum to the MStV noncapsid protein.

Protein polyacrylamide gel electrophoresis was done by the procedure of Laemmli (18) in 10% gels.

Serology. Field-collected maize leaves showing each of the three symptom types associated with MYSV infection were assayed by an indirect protein A sandwich enzyme-linked immunosorbent assay (PAS-ELISA) (10) or $F(ab')_2$-ELISA (9). To prepare extracts for assays, diseased or healthy leaf samples were ground in 0.25 M Tris, pH 8.0, 0.15 M NaCl, 0.05% Tween 20 (TBS-T) and then filtered through a double layer of fine-mesh cheesecloth. In PAS-ELISA, the pathogen antisera and trapping and detecting antiserum dilutions were, respectively: corn stunt spiroplasma (CSS) (1:6,000 and 1:100); maize chlorotic dwarf virus (MCDV) (1:6,000 and 1:400); maize chlorotic mottle virus (MCMV) (1:1,000 and 1:3,000); maize dwarf mosaic virus (MDMV) strains A, B, or O (1:8,000 and 1:256); maize streak virus (MSV) (1:800 and 1:400); and MStV (1:1,000 and 1:400). In $F(ab')_2$-ELISA, the pathogen antisera and detecting antiserum dilutions were, respectively: MCDV (1:1,600); MDMV strains A, B, or O (1:400); maize mosaic virus (MMV) (1:200); and MStV (1:400). For all antisera, an $F(ab')_2$ concentration of 1 μg/ml was used for coating well surfaces. The remainder of both assays involved coupling detecting antibodies bound to the trapped pathogens with biotinylated protein A and detecting biotinylated protein A with streptavidin-conjugated horseradish peroxidase. All pathogen antisera were produced in our laboratories (The Ohio State University, Ohio Agricultural Research and Development Center, Wooster).

Extracts were tested in duplicate and judged positive when the difference between means of the A_{405nm} for the test sample and controls was greater than three standard deviations of the control mean. Controls consisted of the extracts from healthy and heterologous pathogen-infected leaves and TBS-T.

RESULTS

Cytopathology and ultrastructure in situ. In semithin and thin sections of leaves showing maize yellow stripe symptoms from natural or experimental infections (Fig. 1), large, amorphous inclusions were observed consistently by light and electron microscopy in the phloem elements, vascular parenchyma, bundle sheath, and mesophyll cells (Figs. 2–4). These inclusions were intracytoplasmic and sometimes larger than the nucleus, and, together with the nucleus, they frequently bulged into the central vacuole (Fig. 2B). In thin sections examined by electron microscopy, these inclusions usually contained masses of long, flexuous, tubular structures approximately 34 nm in outer diameter, apparently composed of helically wound filaments 5–7 nm thick (Figs. 3–5). It was difficult to determine the length of these tubuler helical structures, but where they stretched parallel to the sectioning plane, some appeared to be longer than 1.6 μm. In addition to these structures, the inclusions often contained aggregated mitochondria, some of which appeared to be in various stages of degeneration (Figs. 3–9). These mitochondria were intimately associated with the tubular helical structures. When the helical structures were sectioned longitudinally, they appeared as tubules made of spiral strands sandwiched between elongated mitochondria (Fig. 5); when they were sectioned transversely, they appeared as rings aligned in a row often encircling an entire circumference of a mitochondrion (Fig. 6). The inclusions also contained vacuoles of various sizes (Figs. 2B, 3, and 4), sometimes lined with the tubular helical structures (Figs. 4 and 7). In the vicinity of these inclusions, the cytoplasm was usually rich in ribosomes, polyribosomes, and rough endoplasmic reticulum (Fig. 6). In some of the cells containing tubular helical structures, masses of loosely or densely packed fine fibrils were found in the cytoplasm or in non-membrane-bound vacuoles (Figs. 7–9). Both the tubular structures and the fine fibrils were found in the samples examined from experimentally or naturally infected maize and sorghum leaves showing any of the three symptoms of MYSV and collected in 1986 and 1987. However, the frequency of the fine fibrils was much lower (22%) than that of the tubular helical structures (92%) on a total of 72 grids examined. None of the structures described above, inclusions, or cytopathological effects were found in cells of healthy maize or sorghum leaves, and no other viruslike or microorganismlike structures were observed in leaves showing any of the three symptoms associated with maize yellow stripe.

Purification. Scans of centrifuged sucrose density gradients containing purified preparations from maize or sorghum leaves with any of the three symptoms associated with maize yellow stripe showed an ultraviolet-absorbing zone about one-third of the distance from the top of the gradient (Fig. 12). No such peak was seen with similarly prepared centrifuged samples from healthy tissues. Spectral analyses of this sedimenting component revealed an absorbance peak at 260–265 nm, typical of a nucleoprotein. This component also was detected when purification was performed with 0.5 M potassium phosphate, pH 7.0, or extraction buffer plus 0.14 M NaCl. This material sedimented to about the same depth as brome mosaic virus (87 S) under similar conditions. Electron microscopy of negatively stained partially purified preparations from diseased, but not from healthy, leaves revealed masses of fine, flexuous filaments 4–8 nm in diameter (Fig. 10). In clarified extracts from diseased leaves, tubular structures, approximately 36 nm in diameter, were found (Fig. 11). These exhibited cross striations, indicating that they probably were composed of helically wound filaments, but they appeared shorter than the tubular helical structures found in infected cells (Figs. 3–5).

Nonvirion protein. Needle-shaped crystals identical in appearance to those of the noncapsid protein of MStV (15) were isolated from infected leaves. However, there was no reaction between antiserum to MStV noncapsid protein and concentrated sap from MYSV-infected tissue or protein crystals purified from such tissue and adjusted to a concentration of 0.34 mg/ml. Control reactions between this antiserum and sap from MStV-infected tissue or purified MStV noncapsid protein occurred at a dilution of 1:128 and a concentration of 0.04 mg/ml, respectively. In 10% polyacrylamide gels, the apparent molecular weight of the MYSV

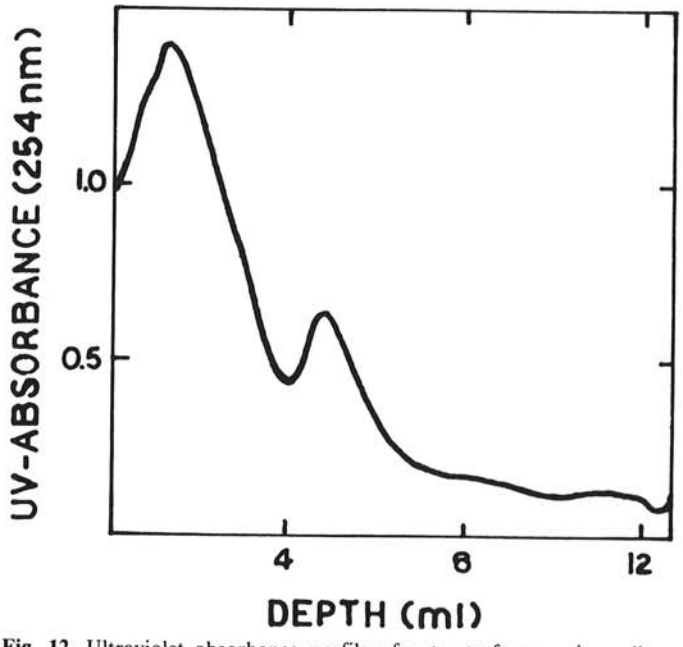


Fig. 12. Ultraviolet absorbance profile of extracts from maize yellow stripe-diseased leaves centrifuged through a 10–40% sucrose density gradient for 2 hr at 40,000 rpm in an SW41Ti rotor. The absorbance peak between about 4 and 6 ml was collected.

nonvirion protein was 13,800, compared with a molecular weight of 17,800 for the MStV noncapsid protein electrophoresed on the same gel.

Serology. Field-collected maize leaves showing each of the three symptom types associated with MYSV infection did not react with antisera to CSS, MCMV, MDMV-O, MSV, or MStV in PAS-ELISA or with antisera to MDMV-O, MMV, or MStV in $F(ab')_2$-ELISA. Antiserum to MCDV reacted slightly with extracts from leaves showing the fine-stripe and chlorotic-stunt symptoms in PAS- but not $F(ab')_2$-ELISA, whereas antisera to MDMV-A and -B reacted slightly with extracts of leaves showing these symptoms in $F(ab')_2$- but not PAS-ELISA. No serological reactions were obtained with leaf samples showing the coarse-stripe symptoms. Each of the two ELISAs for the three pathogens was conducted using extracts from different sets of diseased leaves collected on different dates. Electron microscopy of purified extracts and thin sections from the MDMV ELISA-positive samples revealed potyviruslike particles (Fig. 10) and cylindrical inclusions (data not included), in addition to the fine filaments and tubular helical structures associated with MYSV, indicating a double infection with both pathogens.

DISCUSSION

The following results from our study suggest that maize yellow stripe disease is caused by or at least associated with a virus: 1) Purified preparations with typical nucleoprotein spectra were obtained from diseased leaves; 2) these preparations contained masses of fine filaments similar to those associated with tenuiviruses (14); and 3) no mycoplasma, spiroplasma, bacteria, or rickettsialike structures were observed in thin sections from diseased leaves. Thus, a viral etiology is indicated, and maize yellow stripe virus (MYSV) is suggested as the name for the disease agent.

Symptoms associated with MYSV resemble some of those induced by the pathogens assayed by PAS- and $F(ab')_2$-ELISA; for example, the fine- and coarse-stripe symptoms are similar to those of MStV and MMV, respectively (8). However, antisera to these two pathogens detected neither of them. Only MCDV and MDMV-A and -B antisera reacted with extracts from some diseased leaves but only weakly and only in one of two assays; in the case of MDMV, a double infection was indicated by electron microscopy. MDMV and MSV are the only viruses previously reported on maize in Egypt (2). With the exception of MSV and CSS (19), none of the pathogens assayed is transmitted by *Cicadulina* spp., providing further evidence that MYSV is unrelated to any of the previously identified viruses of maize. Finally, no serological reactions were detected between MYSV extracts and antisera to MStV (a tenuivirus), or between the putative nonviral protein of MYSV and antisera to the noncapsid protein of MStV.

Despite this lack of serological relatedness to MStV, MYSV appears to share some properties with tenuiviruses, that is, the rice stripe virus group (14). Morphologically, the fine filaments found in MYSV-purified preparations are similar to those associated with several tenuiviruses (14,21). MYSV also has an associated protein, but it is smaller than the noncapsid proteins associated with MStV and other tenuiviruses (14,15). On the other hand, MYSV is transmitted by a cicadellid leafhopper (5,6), whereas all known tenuiviruses have delphacid planthopper vectors (14). However, members of the Rhabdoviridae and Reoviridae are transmitted either by leafhoppers or planthoppers (20); thus there are precedents for members of a virus group being transmitted by the two Auchenorrhyncha vector groups.

The cytopathology of MYSV-infected maize leaves seems different from that of MStV-infected ones (7), and the tubular helical structures described here for MYSV infections have not been reported for the tenuiviruses (14) or any other plant virus group (12,13). However, these structures are reminiscent of, but appear considerably longer than, the helical nucleocapsids of rhabdoviruses and myxoviruses (Figs. 2 and 3 in reference 17). It is surprising that the tubular helical structures, which were abundantly found in infected leaf cells (Figs. 3–9) were not detected in MYSV-purified preparations, although similar but shorter structures (Fig. 11) were found in clarified extracts of infected tissue. It is possible that these structures break down or alter morphologically during ultracentrifugation or other purification steps. Thus, the relationship between the tenuiviruslike filaments found in MYSV extracts and the tubular helical structures and fine fibrils found in MYSV-infected leaf cells requires further investigation, using immunocytochemical and other techniques. Also, the relationship between any of these structures and infectivity needs to be established.

The cytopathology and ultrastructure of MYSV-infected leaves showing fine-stripe, coarse-stripe, or chlorotic-stunt symptom types generally were similar. These symptoms usually are produced on different leaves of the same plant, fed upon by a single, infective leafhopper (5). It appears likely that these symptoms represent different stages of MYSV infection in maize plants rather than different pathogens. However, the possibility that these symptoms may be induced by different MYSV strains cannot be ruled out. In view of its unique ultrastructural features and its potential impact on maize production in Egypt (4,5) and perhaps other African or Middle East countries, MYSV merits further investigation.

LITERATURE CITED

1. Aboul-Ata, A. E., and Ammar, E. D. 1985. Occurrence of some maize virus diseases on eight cultivars of late sown maize in Giza, Egypt. (Abstr.) Int. Conf. Impact Viral Dis. Dev. Afr. Middle East Countries, 4th, Rabat, Morocco.
2. Ammar, E. D. 1983. Virus diseases of sugarcane and maize in Egypt. Pages 122-126. in: Proc. Int. Maize Virus Dis. Colloq. Workshop. D. T. Gordon, J. K. Knoke, L. R. Nault, and R. M. Ritter, eds. Ohio State Univ., Ohio Agric. Res. Dev. Cent., Wooster. 266 pp.
3. Ammar, E. D. 1987. Fine, helical, filamentous structures associated with maize yellow stripe, a leafhopper-borne disease agent from Egypt. (Abstr.) Phytopathology 77:1732.
4. Ammar, E. D., Aboul-Ata, A. E., El-Sheikh, M. A., and Sewify, G. H. 1987. Incidence of virus and viruslike disease syndromes on maize and sugarcane in Middle and Lower Egypt. Egypt. J. Phytopathol. 19:97-107.
5. Ammar, E. D., Elnagar, S., Aboul-Ata, A. E., and Sewify, G. H. 1989. Vector and host-plant relationships of the leafhopper-borne maize yellow stripe virus. J. Phytopathol. 126:246-252.
6. Ammar, E. D., Elnagar, S., Tolba, A., and Aboul-Ata, A. E. 1984. Three maize diseases in Egypt, associated with leafhoppers (Cicadellidae, Homoptera). (Abstr.) Congr. Mediterr. Phytopathol. Union, 6th, Cairo, Egypt.
7. Ammar, E. D., Gingery, R. E., and Nault, L. R. 1985. Two types of inclusions in maize plants infected with maize stripe virus. Phytopathology 75:84-89.
8. Ammar, E. D., Gingery, R. E., and Nault, L. R. 1987. Interactions between maize mosaic and maize stripe viruses in their insect vector, *Peregrinus maidis,* and in maize. Phytopathology 77:1051-1056.
9. Barbara, D. J., and Clark, M. F. 1982. A simple indirect ELISA using $F(ab')_2$ fragments of immunoglobulin. J. Gen. Virol. 58:315-322.
10. Edwards, M. L., and Cooper, J. I. 1985. Plant virus detection using a form of indirect ELISA. J. Virol. Methods 11:309-319.
11. Evert, R. F., Eschrich, W., and Heyser, W. 1977. Distribution and structure of the plasmodesmata in mesophyll and bundle-sheath cells of *Zea mays* L. Planta 136:77-89.
12. Francki, R. I. B., Milne, R. G., and Hatta, T. 1985. Atlas of Plant Viruses. Vol. I. CRC Press, Boca Raton, FL. 222 pp.
13. Francki, R. I. B., Milne, R. G., and Hatta, T. 1985. Atlas of Plant Viruses. Vol. II. CRC Press, Boca Raton, FL. 284 pp.
14. Gingery, R. E. 1988. The rice stripe virus group. Pages 297-329 in: The Plant Viruses. Vol. IV. The Filamentous Plant Viruses. R. G. Milne, ed. Plenum Publishing Corp., New York.
15. Gingery, R. E., Nault, L. R., and Bradfute, O. E. 1981. Maize stripe virus: Characteristics of a member of a new virus class. Virology 112:99-108.
16. Gingery, R. E., Nault, L. R., Tsai, J. H., and Lastra, R. J. 1979. Occurrence of maize stripe virus in the United States and Venezuela. Plant Dis. Rep. 63:341-343.
17. Joklik, W. K. 1985. The nature, isolation, and measurement of animal viruses. Pages 3-14 in: Virology. 2nd ed. W. K. Joklik, ed. Appleton-Century-Crofts, East Norwalk, CT.

18. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680-685.
19. Markham, P. G., and Alivizatos, A. S. 1983. The transmission of corn stunt spiroplasma by natural and experimental vectors. Pages 51-61 in: Proc. Int. Maize Virus Dis. Colloq. Workshop. D. T. Gordon, J. K. Knoke, L. R. Nault, and R. M. Ritter, eds. Ohio State Univ., Ohio Agric. Res. Dev. Cent., Wooster. 266 pp.
20. Nault, L. R., and Ammar, E. D. 1989. Leafhopper and planthopper transmission of plant viruses. Annu. Rev. Entomol. 34:503-529.
21. Toriyama, S. 1983. Rice stripe virus. No. 245 in: Descriptions of Plant Viruses. Commonw. Mycol. Inst., Assoc. Appl. Biol., Kew, Surrey, England.