Ultrastructural Studies of *Jatropha gossypifolia* Infected with Jatropha Mosaic Virus, a Whitefly-Transmitted Geminivirus

K. S. Kim, J. Bird, R. L. Rodriguez, E. M. Martin, and J. Escudero.

First and fourth authors, Department of Plant Pathology, University of Arkansas, Fayetteville 72701; second, third, and fifth authors, Agricultural Experiment Station, University of Puerto Rico, Rio Piedras.
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ABSTRACT

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Plants of *Jatropha gossypifolia* of various ages infected with Jatropha mosaic virus via the whitefly, *Bemisia tabaci*, were studied ultrastructurally. Leaves with typical symptoms exhibited the nucleopathic effects that are characteristic of known whitefly-transmitted geminiviruses. Electron-dense fibrillar bodies occurred at an early stage of infection and were closely associated with viruslike particles at a late stage of infection. Many fibrillar bodies were ring-shaped and consisted of orderly aligned spherical "beads" of high electron density embedded in a less electron-dense fibrillar matrix. Cytochemical studies indicated that the fibrillar bodies are DNA-containing structures. Cytoplasmic inclusions (membrane-bound oval bodies containing granular and/or fibrillar material) also were observed. The central cavity of the ring-shaped fibrillar bodies was often filled with material similar to those of the cytoplasmic inclusions. Viruslike particles, 15–18 nm in diameter, occurred only in the nuclei and mature sieve elements. The infection appeared to be limited to phloem cells. It is suggested that Jatropha mosaic disease is caused by a whitefly-transmitted geminivirus.

Additional key words: cytopathology, electron microscopy, single-stranded DNA virus.

Jatropha gossypifolia is an euphorbiaceous weed prevalent throughout the West Indies and is usually found affected by a mosaic disease (3). The disease was first described in Puerto Rico (7). The Jatropha mosaic disease agent also occurs naturally on *J. multifida* and *Croton lobatus* (4,5) and can be transmitted by grafting to those species as well as to *J. podagrica*. Various aspects of the disease, including agent-vector relations, were studied in some detail in Puerto Rico (3) where it was found that a distinct race of the whitefly, *Bemisia tabaci*, transmits the disease (5). The Jatropha mosaic disease has been presumed to be caused by a virus based merely on its virus-like foliar symptoms and transmissibility by grafting and via insects (3,5). The type of symptoms, geographical locations of prevalent occurrence, and the association of the disease with a whitefly vector suggested that it could be caused by a member of the geminiviruses. However, no direct morphological evidence has been presented that characterizes the disease agent as a virus.

Recently, a whitefly-transmitted mosaic of *J. multifida* was reported from Kenya (6). The causal agent, cassava latent virus strain C (CLV-C) is a strain of cassava latent virus and, like the Jatropha mosaic agent in Puerto Rico, is capable of infecting some species belonging to the Euphorbiaceae, Convolvulaceae, and Solanaceae (5,6). The foliar symptoms induced by the causal agent of Jatropha mosaic in Puerto Rico on *J. multifida* are almost identical to those incited on the same host by CLV-C. At least outwardly, causal agents of the African and the Puerto Rican Jatropha mosaics seem to be related.

Following the report that bean golden mosaic virus (BGMV), a well characterized, whitefly-transmitted geminivirus (9), induced cytopathic effects in situ that were distinct from those induced by other groups of known plant viruses (15), numerous other whitefly-transmitted viruses occurring in different countries have been studied ultrastructurally and were found to induce cytopathic changes similar to those induced by BGMV (1,10,12,13,15,16,18,21). Thus, an ultrastructural study of the Jatropha mosaic disease occurring in Puerto Rico was carried out to determine whether the agent induced the cytopathic effects characteristic of other known whitefly-transmitted geminiviruses.

This paper reports the occurrence of such characteristic cytopathic effects in *J. gossypifolia* leaf cells infected by the Jatropha mosaic agent and also describes novel structural features which are apparently involved in virus assembly.

MATERIALS AND METHODS

Plants of *J. gossypifolia* of various ages infected with the Jatropha mosaic agent via *B. tabaci* (3) have been kept in insectaries at the Rio Piedras Research Center of the Agricultural Experiment Station for many years. Some of these plants support colonies of the whitefly vector. Pieces of leaves with typical Jatropha mosaic symptoms (5) from three infected plants of different ages as well as from presumably healthy plants were sampled.

Young healthy seedlings of *J. gossypifolia*, germinated in 30-cm-diameter pots, were inoculated via *B. tabaci* obtained from the aforementioned stock culture (viruliferous) to verify once again the whitefly transmissibility of the disease agent. Typical symptoms started to develop 2 wk after inoculation. Additional specimens from plants thus infected and from healthy controls were also secured for ultrastructural studies.

Specimens were fixed for 2 hr at room temperature in a modified Karnovsky's fixative (11) consisting of 2% glutaraldehyde and 2% paraformaldehyde in 0.05 M sodium cacodylate buffer, pH 7.2. After several rinses with the same buffer solution, the tissues were postfixed in 1% OsO_4 for 2 hr, prestained in bulk overnight in 0.5% aqueous uranyl acetate at 4C, and dehydrated in an ethanol series before they were embedded in Spurr's medium. Thin sections were stained with 2% aqueous uranyl acetate followed by lead citrate before examination with an electron microscope.

For the uranyl-EDTA-lead staining method (2,15) additional specimens were fixed in Karnovsky's fixative without postfixation in OsO_4 and processed further for sectioning as described above. This method was employed to determine whether the intranuclear fibrillar bodies and their associated structures induced by the Jatropha mosaic virus were composed of ribonucleoprotein (RNP) or deoxyribonucleoprotein (DNP). Sections were stained in 5%

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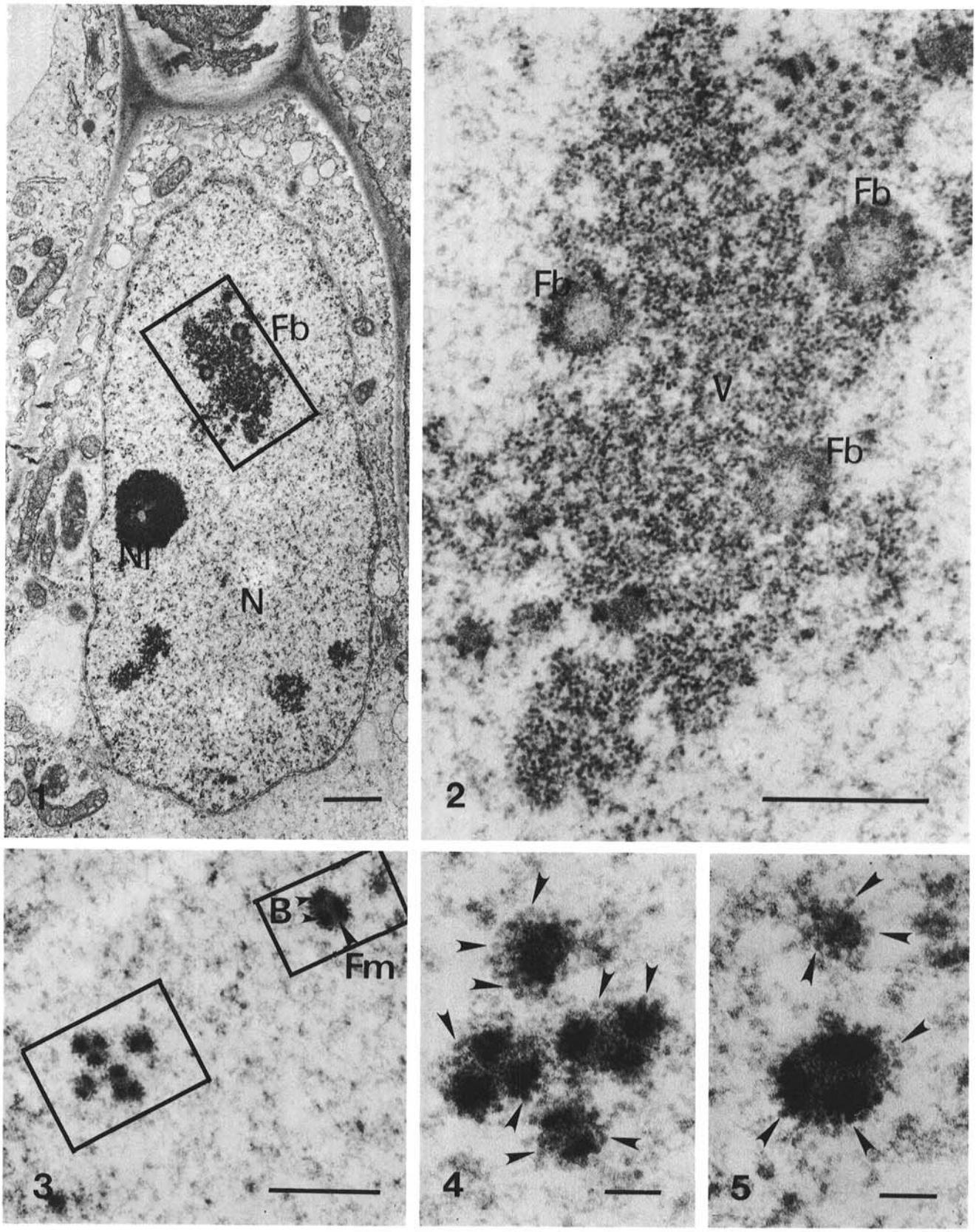
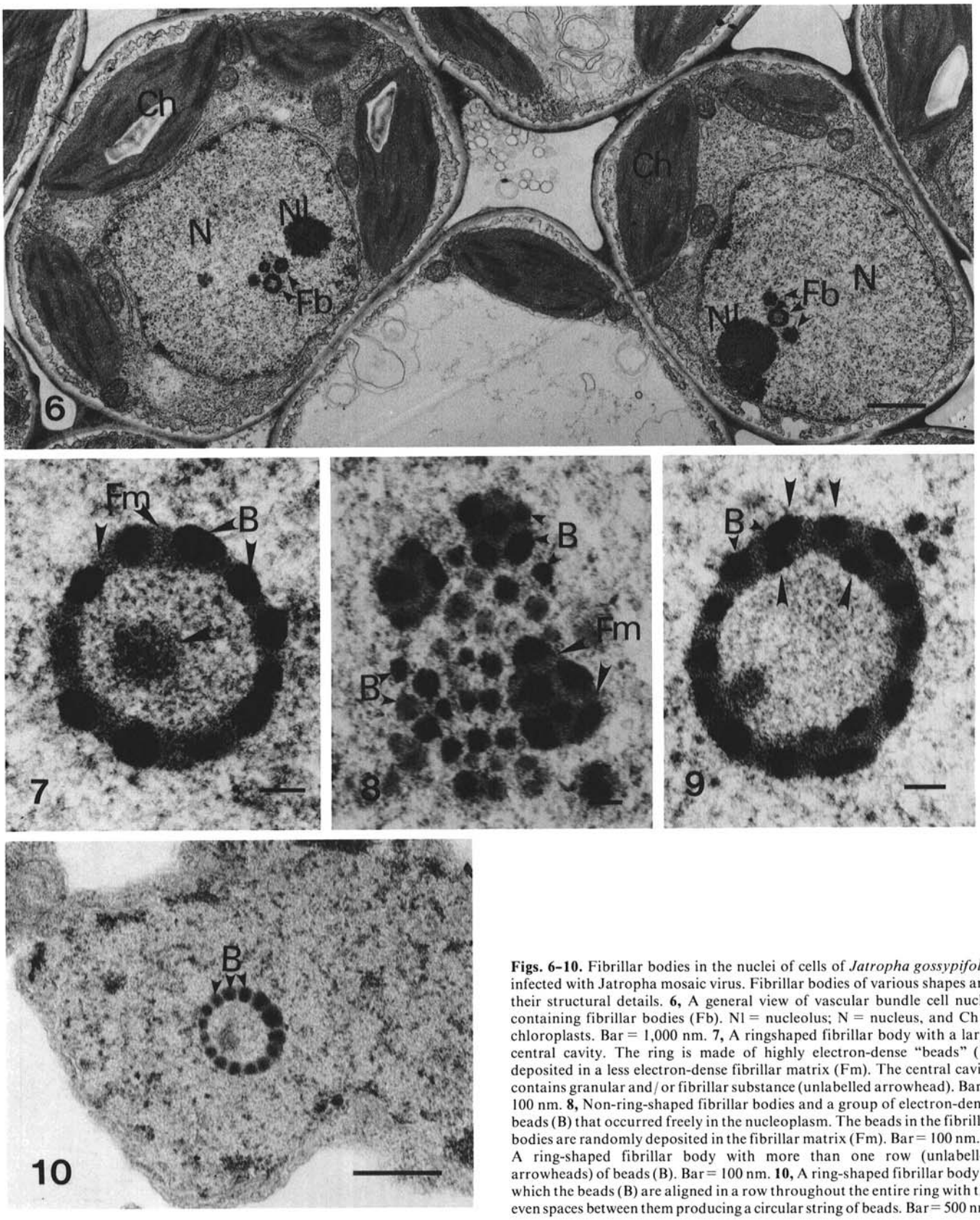


Fig. 1–5. Virus particles associated with fibrillar bodies in the nuclei of cells of *Jatropha gossypifolia* infected with Jatropha mosaic virus. **1,** A low magnification of a vascular bundle parenchyma cell nucleus containing aggregates of virus particles associated with ring-shaped fibrillar bodies (Fb). N = nucleus and Nl = nucleolus. Bar = 1,000 nm. **2,** A higher magnification of the boxed area in Fig. 1 showing the details of three ring-shaped fibrillar bodies (Fb) associated with a large aggregate of virus particles (V). The fibrillar bodies appear diffuse. Bar = 500 nm. **3,** Several small, non-ring-shaped fibrillar bodies in a nucleus, each of which consists of electron-dense beads (B) embedded in a less-dense fibrillar matrix (Fm). Bar = 500 nm. **4,** A higher magnification of the left inset rectangle in Fig. 3 showing four fibrillar bodies, each of which is decorated by virus particles (arrowheads). Bar = 100 nm. **5,** A higher magnification of the right inset rectangle in Fig. 3 showing a fibrillar body surrounded by virus particles (arrowheads). Bar = 100 nm.

aqueous uranyl acetate for 15 min. After they were washed with distilled water, some sections were floated on 0.5 M EDTA solution at pH 7.2 for 20–30 min at room temperature, again washed with distilled water, and stained with lead citrate for 3–5 min. For the controls, EDTA treatment was omitted.

RESULTS

Cytopathic structures such as intranuclear fibrillar bodies and associated viruslike particles which were similar to those characteristic of whitefly-transmitted geminiviruses (1,10,12,13, 15,16,18–21) occurred consistently in cells of all specimens taken



Figs. 6–10. Fibrillar bodies in the nuclei of cells of *Jatropha gossypifolia* infected with Jatropha mosaic virus. Fibrillar bodies of various shapes and their structural details. **6,** A general view of vascular bundle cell nuclei containing fibrillar bodies (Fb). Nl = nucleolus; N = nucleus, and Ch = chloroplasts. Bar = 1,000 nm. **7,** A ringshaped fibrillar body with a large central cavity. The ring is made of highly electron-dense "beads" (B) deposited in a less electron-dense fibrillar matrix (Fm). The central cavity contains granular and/or fibrillar substance (unlabelled arrowhead). Bar = 100 nm. **8,** Non-ring-shaped fibrillar bodies and a group of electron-dense beads (B) that occurred freely in the nucleoplasm. The beads in the fibrillar bodies are randomly deposited in the fibrillar matrix (Fm). Bar = 100 nm. **9,** A ring-shaped fibrillar body with more than one row (unlabelled arrowheads) of beads (B). Bar = 100 nm. **10,** A ring-shaped fibrillar body in which the beads (B) are aligned in a row throughout the entire ring with the even spaces between them producing a circular string of beads. Bar = 500 nm.

from infected *J. gossypifolia* regardless of age, but not from specimens taken from uninfected control plants. These structures were primarily found in sieve elements and associated phloem parenchyma cells of vascular bundles.

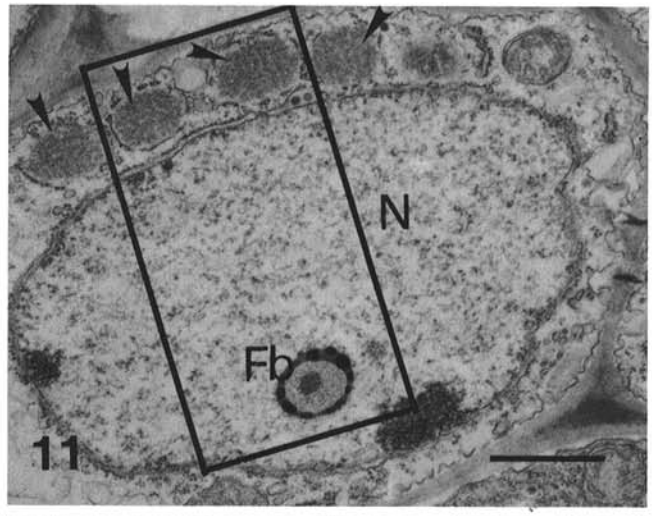
Particles measuring approximately 15–20 nm in diameter (individual particles), which were assumed to be the virus and which will be referred to as virus particles, occurred either in loosely clustered aggregates (Figs. 1 and 2) or in closely packed large bodies which often occupied more than three-quarters of the nuclear volume. Virus particles were usually associated with the fibrillar bodies (Figs. 3 to 5). In mature sieve elements where the nuclei had degenerated and disappeared, no fibrillar bodies were found, but virus particles occurred as aggregated masses.

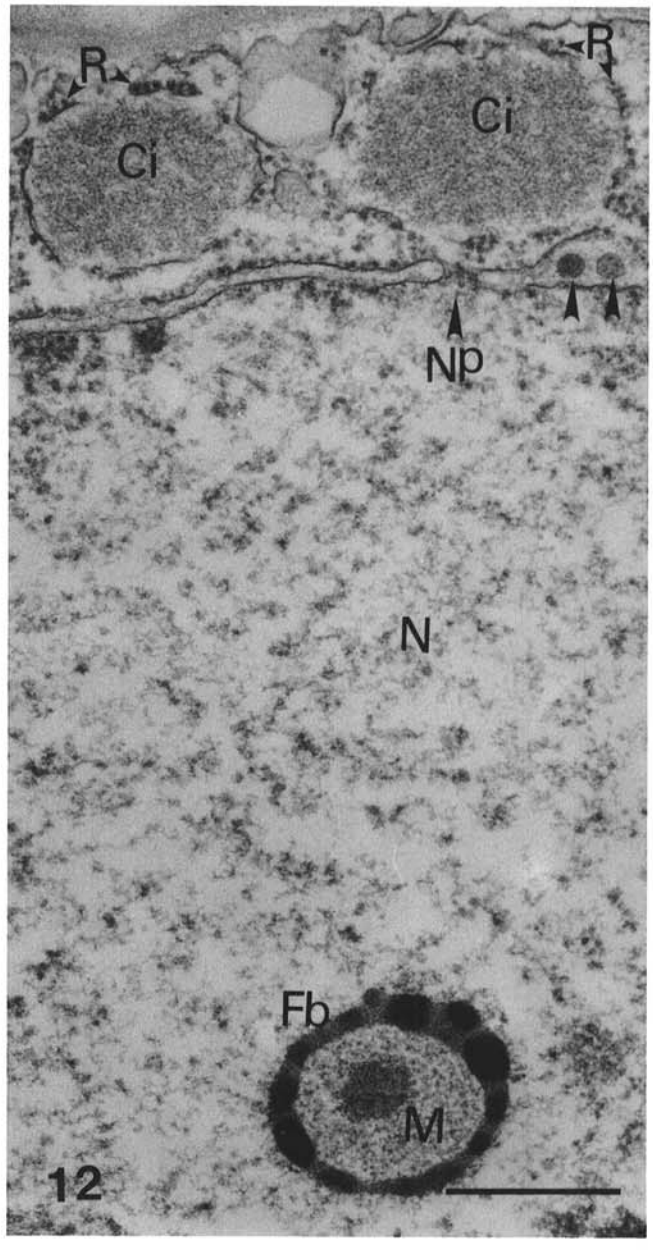
Fibrillar bodies varied in size but were generally circular in profile (Figs. 6 to 10). Many of them were ring-shaped with a large central cavity containing a substance that is different from the nucleoplasm (Figs. 7, 11, and 12). In serial sections, these ring-shaped fibrillar bodies were seen having various diameters depending on the depth of sectioning. When the bodies were sectioned at their periphery, they appeared as non-ring-shaped bodies as shown in Fig. 8 suggesting that they were hollow spheres in a three dimensional view. These fibrillar bodies, unlike those induced by other whitefly-transmitted geminiviruses, were, in many cases, made of two types of material with different electron densities; one with highly electron-dense spherical "beads" embedded in the other, a less electron-dense matrix of finely fibrillar material (Figs. 6, 7, 9, 10, and 11). In some ring-shaped fibrillar bodies, the electron-dense beads were aligned in a row throughout the entire ring with even spaces between them producing a structure resembling a circular string of beads (Fig. 10). When the beads were smaller in their diameter than the width of the ring of a fibrillar body, more than one row of beads occurred in the fibrillar body (Fig. 9).

Non-ring-shaped fibrillar bodies were usually smaller than those of ring-shaped ones and were produced by tangential sectioning through the periphery of the hollow spheres (Fig. 8). The electron-dense beads in these bodies appeared to be deposited randomly in the matrix of fibrillar material (Fig. 8). The exterior of these smaller fibrillar bodies was often decorated by virus particles in direct contact with the bodies (Figs. 3, 4, and 5). The electron-dense beads also occurred freely in groups in the ground nucleoplasm near the fibrillar bodies (Fig. 8). Virus particles were, however, not associated with these free beads. Fibrillar bodies, especially those ring-shaped ones associated with large aggregates of virus particles, appeared somewhat diffuse as though they had lost some of their constituents (Figs. 1 and 2). Also, the distinctiveness of the two structural components of fibrillar bodies, the beads and fibrillar matrix, was not as pronounced as in those fibrillar bodies not associated with virus particles (Figs. 1 and 2).

Some cells containing fibrillar bodies and/or virus particles in the nucleus also contained cytoplasmic inclusions (Figs. 11 and 12). These were membrane-bound bodies with circular profiles containing granular and/or fibrillar material of moderate electron density. The external surface of the binding membranes of these inclusions was studded with ribosomes, similar to membranes of rough endoplasmic reticulum (Figs. 11 and 12). In cells with cytoplasmic inclusions, the central cavity of the ring-shaped fibrillar bodies in the nucleus was often filled with material similar in texture and electron density to those of the cytoplasmic inclusions (Figs. 11 and 12). The perinuclear space in these cells also had small vesicles containing material similar to those of the cytoplasmic inclusions (Figs. 11 and 12).

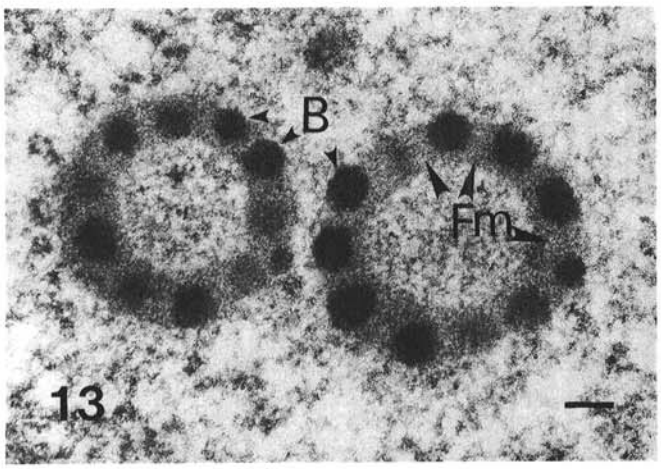
After infected tissues were fixed with aldehyde (Karnovsky's)

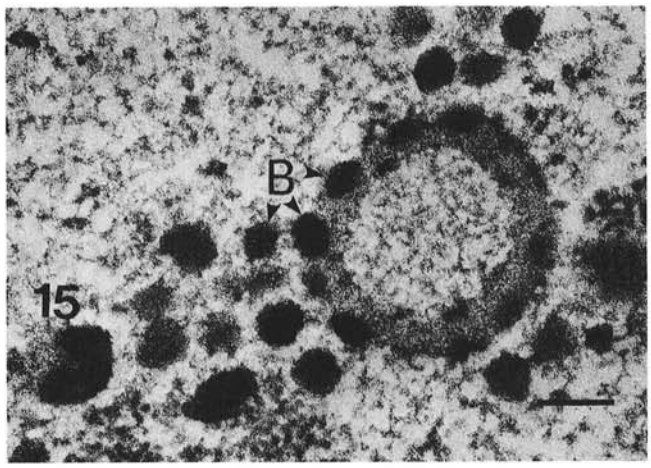


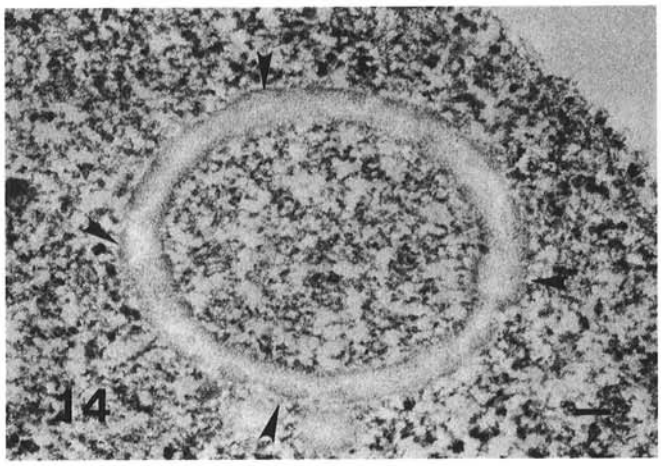


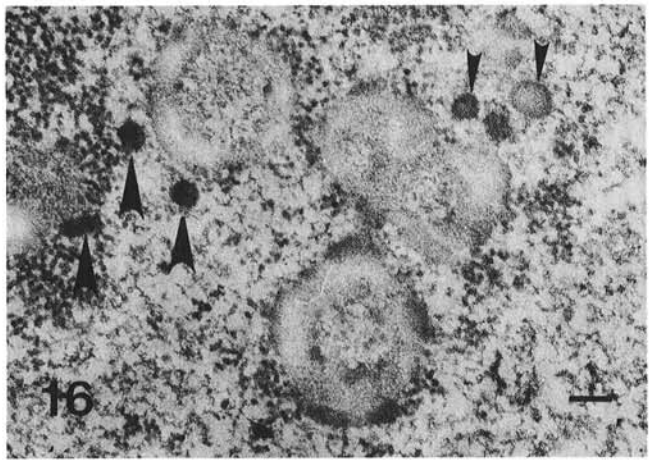
Figs. 11–12. A vascular parenchyma cell containing cytoplasmic inclusions and a fibrillar body. **11,** Four oval inclusion bodies (arrowheads) each of which is surrounded by a membrane are shown in the cytoplasm. A ring-shaped fibrillar body (Fb) is in the nucleus (N). Bar = 1,000 nm. **12,** A higher magnification of the rectangle in Fig. 11 showing the details of cytoplasmic inclusions and fibrillar body. The cytoplasmic inclusions (Ci) consist of granular and/or fibrillar material of moderate electron density surrounded by a ribosome (R)-studded membrane. The fibrillar body (Fb) in the nucleus (N) contains material (M) in its central vacuole similar to those of the cytoplasmic inclusions. Two small circular vesicles are also shown in the perinuclear space (unlabeled arrowheads). Np = Nuclear pore. Bar = 500 nm.

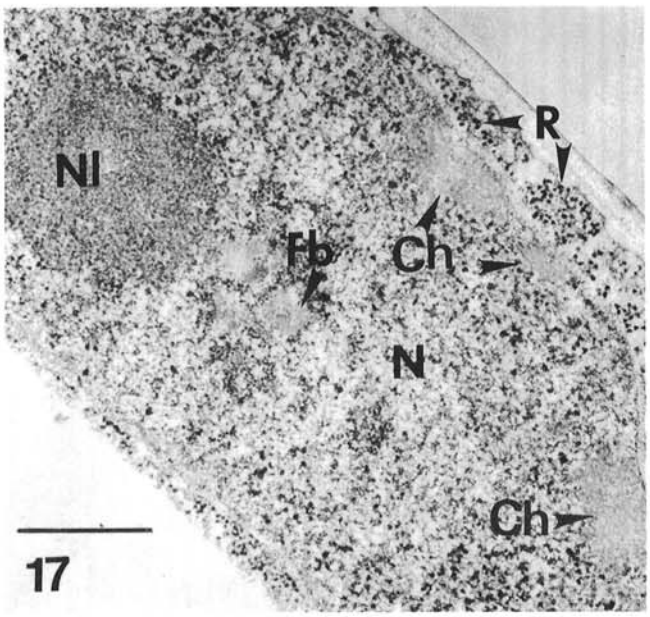
alone and sections were stained with 5% uranyl acetate followed by lead citrate, the fibrillar bodies were similar in appearance to those prepared by conventional aldehyde-OsO_4 fixation. Their two structural components, the electron-dense beads and fibrillar matrix were distinctive (Figs. 13 and 15). When sections were treated with EDTA for 30 min at room temperature, however, the fibrillar bodies (Figs. 14, 16, and 17) and the chromatin (Fig. 17) were strikingly destained leaving vestiges of the structures, thus suggesting that they are DNA-containing structures. On the other hand, the electron density of RNA-containing organelles such as the nucleolus and ribosomes (Fig. 17) was not affected by the EDTA treatment confirming their RNA composition. Many ring-shaped fibrillar bodies were homogeneously destained following EDTA treatment; therefore, it was difficult to distinguish the two structural components of the bodies except that the areas of the beads appeared somewhat bumpy (Fig. 14). The electron-dense beads in some fibrillar bodies and those occurring freely in the nucleoplasm were, however, often less destained by the EDTA treatment than was the matrix material of fibrillar bodies or not affected at all (Fig. 16).











Figs. 13–17. The effects of the uranyl-EDTA-lead staining technique on fibrillar bodies in the nuclei of cells of *Jatropha gossypifolia* infected with Jatropha mosaic virus. **13,** Fibrillar bodies after fixation with aldehyde (Karnovsky's) alone and staining with 5% uranyl acetate and lead citrate. The bodies appear similar to those prepared with aldehyde-OsO_4 fixation, exhibiting distinct beads (B) and a fibrillar matrix (Fm). Bar = 100 nm. **14,** A fibrillar body treated with EDTA between uranyl acetate and lead citrate stains. The body is destained homogeneously and the two structural components were difficult to distinguish except that the areas of the beads were somewhat bumpy (arrowheads). Bar = 100 nm. **15,** A ring-shaped fibrillar body and a group of free electron-dense beads (B) in the nucleoplasm before treatment with EDTA. **16,** Structures similar to those in Fig. 15 after treatment with EDTA. The ring-shaped fibrillar bodies are all destained, but some beads occurring freely in the nucleoplasm are less destained (small arrowheads) or not affected (large arrowheads). Bar = 100 nm. **17,** A low magnification of a portion of a nucleus (N) and cytoplasm after EDTA treatment. The fibrillar bodies (Fb) and chromatin (Ch) along the inner nuclear membrane are clearly destained while RNA-containing organelles such as the nucleolus (Nl) and ribosomes (R) retain their electron density.

DISCUSSION

The major cytopathic effects found in cells affected by the Jatropha mosaic disease, namely, the fibrillar bodies and associated virus-like particles in the nuclei of phloem-associated paranchyma cells and sieve elements, are very similar to those induced by other whitefly-transmitted geminiviruses (1,10,12, 13,15,16,18,21). As in the cases of BGMV (15) and Euphorbia mosaic virus (14), the results of Bernhard's EDTA-staining method (2) indicate that the fibrillar bodies found in the present study are also composed of DNA, the type of nucleic acid found in geminiviruses. It is suggested, therefore, that the agent of Jatropha mosaic disease, which is transmitted by the whitefly, *B. tabaci*, is a virus that belongs to the geminivirus group (17).

Two ultrastructural features which are thus far undescribed in cells infected with other whitefly-transmitted geminiviruses were consistently found in Jatropha mosaic virus-infected cells. These were: fibrillar bodies consisting of two structural components with different electron densities, the highly electron-dense beads and less electron-dense fibrillar matrix; and the central cavity of ring-shaped fibrillar bodies containing material similar to those of the cytoplasmic inclusions. The fibrillar bodies induced by other whitefly-transmitted geminiviruses are usually composed of tightly packed fine fibrils with homogeneous electron density (1,10,12,15,20,21).

Fibrillar bodies are the most common intranuclear structures induced by other whitefly-transmitted geminiviruses (see above references). Since fibrillar bodies precede the appearance of virus particles (13,15) and are associated with virus particles when they appear, it was suggested that fibrillar bodies are the site of viral DNA synthesis and virus assembly (15,16). This suggestion was strengthened by the fact that the fibrillar bodies contain DNA which coincides with the type of nucleic acid in geminiviruses (14,15). Observation of this study that the external surface of the smaller fibrillar bodies is decorated with virus particles and that the fibrillar bodies appear diffuse when associated with large aggregates of viral particles, further substantiates the role of fibrillar bodies in viral synthesis and/or assembly.

Very little information is available on the cytological aspects of the protein(s) of geminiviruses, where they are synthesized, and how they are brought into the nucleus where they are assembled into virions. Virions of all known whitefly-transmitted geminiviruses have been found to occur only in the nucleus. In this regard, the occurrence of cytoplasmic inclusions in Jatropha mosaic virus-infected cells is noteworthy since no other whitefly-transmitted geminiviruses have been reported to induce inclusions in the cytoplasm of infected cells except for the Euphorbia virus reported from Florida (13). The cytoplasmic inclusions induced by the Euphorbia virus were morphologically different from those of Jatropha mosaic virus. A close association with rough endoplasmic reticulum was, however, a common feature in both inclusions suggesting that they are proteinaceous (13). In fact, the cytoplasmic inclusions of Jatropha mosaic virus, which consist of amorphous dense material surrounded by ribosome-studded membrane, are indistinguishable from distended cisternae of rough endoplasmic reticulum containing its synthesized protein products seen commonly in some specialized mammalian cells such as plasma cells (8).

Presently, the nature and role of the electron-dense beads incorporated in the matrix of fibrillar bodies and the dense material in the central cavity of the ring-shaped fibrillar bodies are not known. However, since these two structures are actual components of the fibrillar bodies and the fibrillar bodies are apparently involved in virus replication, as discussed above, it is assumed that they have some role in virus replication. It should also be mentioned that the dense material in the central cavity of fibrillar bodies as shown in Fig. 2 is very similar in both morphology and electron density to the content of cytoplasmic inclusions. It is not known whether these materials in the cytoplasmic inclusions and in the central cavity of fibrillar bodies are antigenically related to the viral protein. If they are viral coat proteins and synthesized in the ribosome-studded cytoplasmic inclusions, then the material in the central cavity of fibrillar bodies in the nuclei must have been transported from the cytoplasm to be assembled as virions, since virus particles occur only in the nucleus.

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