Ultrastructure of *Datura stramonium* Infected with an Euphorbia Virus Suggestive of a Whitefly-Transmitted Geminivirus

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ABSTRACT

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Inoculation of *Datura stramonium* with an Euphorbia virus in raw leaf sap from wild *Euphorbia heterophylla* (which occurs widely in southern Florida), caused chlorotic lesions on inoculated primary leaves and mosaic or mottling on newly developed leaves. Thin-section electron microscopy of infected leaves revealed that the ultrastructural changes resembling those caused by whitefly-transmitted geminiviruses were greatly enhanced. These included the segregation of nucleolar components into discrete fibrillar and granular regions and the occurrence of fibrillar bodies in the nucleoplasm associated with isometric viruslike particles 15–18 nm in diameter. In addition, certain cytopathic effects not common to other whitefly-transmitted geminiviruses were observed: the infection was not limited to phloem cells, and the occurrence of cytoplasmic inclusions surrounded by rough endoplasmic reticulum and Golgi bodies. In leaf dip preparations, viruslike particles appeared predominantly in pairs, which is typical of other geminiviruses. It is suggested that the Euphorbia virus belongs to the group of whitefly-transmitted geminiviruses.

Additional key words: cytopathology, virus-induced inclusions.

The foliar mosaic and mottling symptoms common in wild *Euphorbia* spp. in Florida were brought to our attention by the late R. A. Conover when the junior author was investigating poinsettia mosaic virus (12). Preliminary studies revealed that the agent (hereafter called Euphorbia virus) was not related to poinsettia mosaic virus (*unpublished*), and that it was readily transmitted to *Datura stramonium* by crude sap from the mosaic-affected leaves of *Euphorbia* spp. The natural occurrence in *Euphorbia* spp. and the symptoms caused in *D. stramonium* suggested that the Euphorbia virus could be similar to whitefly-borne Euphorbia mosaic virus described by Bird et al (1), Costa and Bennett (5), and Kim and Flores (16).

Since this virus was not previously reported in the continental United States, we undertook this study to investigate some of its properties. As one approach, an ultrastructural study was carried out to determine if the Euphorbia virus is indeed a whitefly-transmitted geminivirus with geminate particles of characteristic morphology and if cytopathic effects distinct from those caused by other groups of plant viruses are induced (15–21).

MATERIALS AND METHODS

Wild *Euphorbia heterophylla* L. and *Poinsettia pinetorum* Small showing viruslike symptoms were collected in the Homestead, FL,

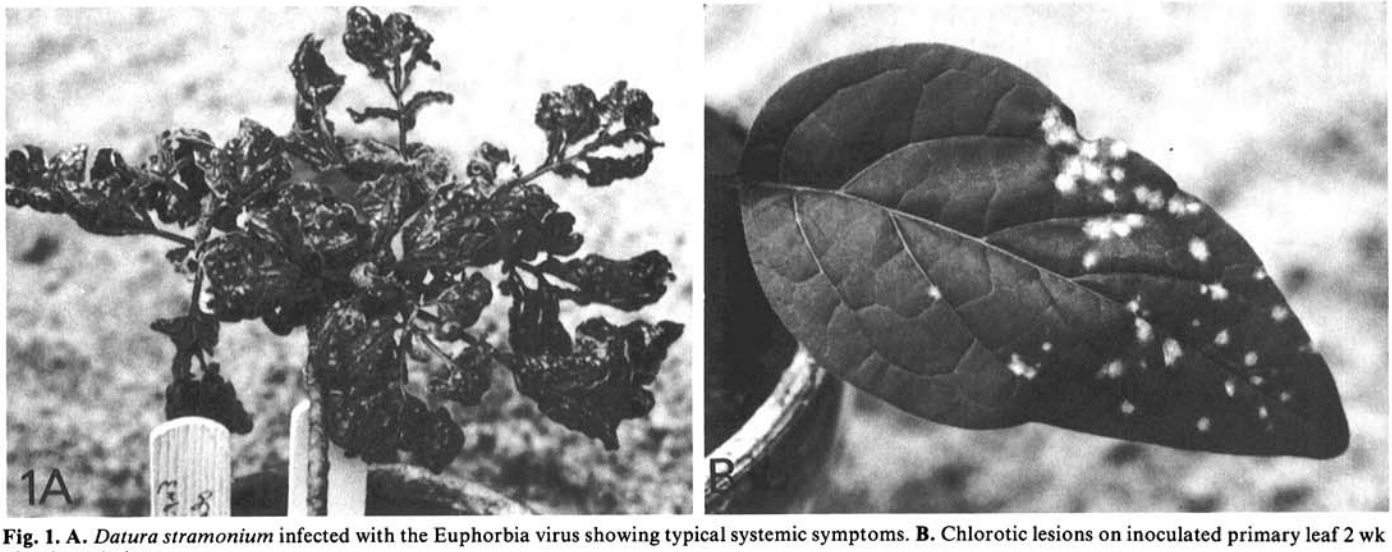

Fig. 1. A. *Datura stramonium* infected with the Euphorbia virus showing typical systemic symptoms. **B.** Chlorotic lesions on inoculated primary leaf 2 wk after inoculation.

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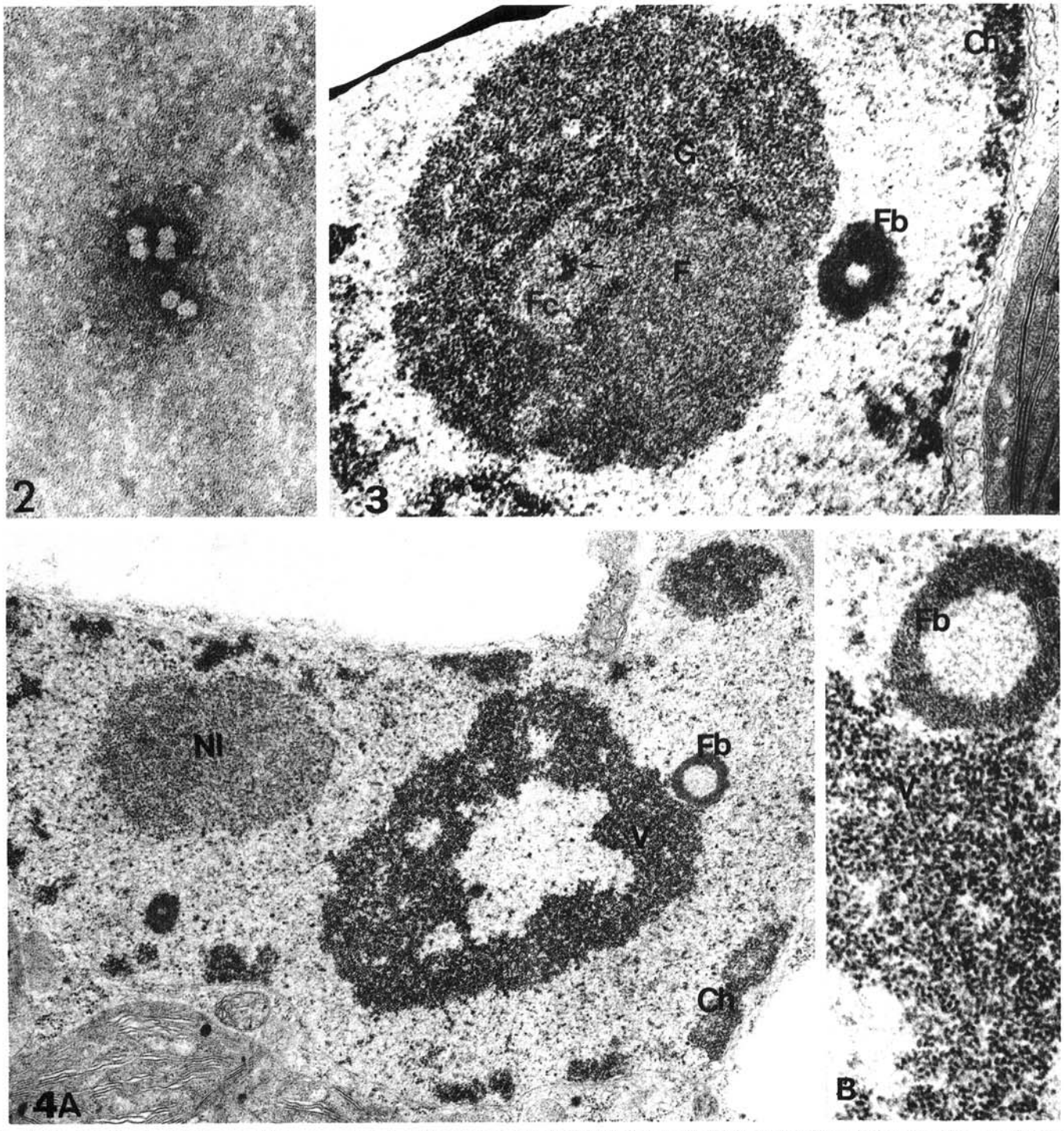
area by the late R. A. Conover. A virus was transmitted from an extract of young infected leaves prepared in 0.01–0.03 M PO_4 buffer, pH 7.0 or 8.0, and wiped on Carborundum-dusted leaves. *D. stramonium* readily became infected and developed distinctive symptoms (Fig. 1A and B).

For ultrastructural studies, seedlings of *D. stramonium* with two true leaves were inoculated. Circular, mildly chlorotic lesions, 1–2 mm in diameter, were evident 6 days later. Lesions increased in size and became more severely chlorotic after 2 wk. Systemic symptoms of severe mosaic, distortion, and stunting became evident in newly developing leaves (Fig. 1A and B). Leaf pieces, 1–2 mm^2, were sampled from inoculated leaves showing the earliest visible lesions and periodically for the next 2 wk as lesions became progressively more chlorotic. Leaf specimens were also taken periodically from the first leaves developed after the inoculation showing systemic symptoms. Leaves of uninoculated plants were sampled at similar developmental stages for controls.

Leaf samples were placed in modified Karnovsky's fixative (14)



Figs. 2–4. Isolated Euphorbia virus particles and nucleopathic effects in leaf cells of *Datura* infected with the Euphorbia virus. **2,** Typical geminate particles of the Euphorbia virus prepared by negative staining of leaf dip (×200,000). **3,** A segregated nucleolus at an early stage of infection by the Euphorbia virus exhibiting granular (G) and fibrillar (F) components which are separated into discrete regions. Between these regions is a fibrillar center (Fc) containing dense chromatin (arrows) in its center. A ring-shaped fibrillar body (Fb) composed of electron-dense fibrils that are finer and more compact than those of the fibrillar regions of the nucleolus is also shown. Ch = chromatin (×35,000). **4A,** A general view of a nucleus at a late stage of infection containing an extremely large aggregate of virus particles (V) associated with a ring-shaped fibrillar body (Fb). The nucleolus (Nl) is smaller than the virus aggregate. Ch = chromatin (×18,000). **4B,** A higher magnification of the portion of virus aggregate (V), and fibrillar ring (Fb) in Fig. 4A showing the details of these structures (×60,000).

consisting of 2% glutaraldehyde and 2% paraformaldehyde in 0.05 M cacodylate buffer, pH 7.2, for 2 hr at room temperature under vacuum. After being rinsed several times with the same buffer, the tissues were postfixed in 1% OsO_4 for 2 hr, then prestained in bulk overnight in 0.5% aqueous uranyl acetate at 4 C. The tissues were dehydrated in an ethanol series, embedded in Spurr's medium and thin-sectioned with a diamond knife. Sections were double stained in 2% aqueous uranyl acetate for 5 min and lead citrate for 2 min before examination under a JEOL 100 CX electron microscope. Leaf dips were made by stripping the epidermis from the leaf surface and removing small pieces of mesophyll tissue from chlorotic lesions. They were stained in a drop of 2% sodium phosphotungstate, pH 5.5, on a specimen grid covered with Formvar film for 30–60 sec. Excess stain was removed and the specimen was air-dried at room temperature. The grid was examined for the presence of paired geminate particles.

RESULTS

Paired particles were consistently found in leaf dip preparations from Euphorbia virus-infected plants of *D. stramonium* (Fig. 2). The individual particles measured 15–18 nm in diameter. They were structurally indistinguishable from those of other geminiviruses (13).

Cytological changes induced by infection with the Euphorbia virus in primary lesions and in systemically infected leaves were identical. However, more infected cells with characteristic ultrastructural changes were found in the chlorotic lesions on inoculated leaves than in tissues from the systemically infected leaves.

The consistent cytopathic effects associated with infection by the Euphorbia virus occurred both in the nucleus and cytoplasm. Nuclear changes included the segregation of nucleolar components and the occurrence of fibrillar bodies and isometric viruslike particles, 15–18 nm in diameter, in the nucleoplasm which resembled those associated with infection of many whitefly-transmitted geminiviruses (13,15). In many nuclei, the granular and fibrillar components of the nucleolus were sharply segregated into two discrete regions (Fig. 3). The fibrillar centers (3), which occur randomly in uninfected nucleoli, were often located between the two regions of segregated nucleolus (Fig. 3). In young, primary, chlorotic lesions segregated nucleoli were more frequently observed than in older chlorotic lesions or in tissues from systemically infected leaves. Nuclei containing segregated nucleolus also contained fibrillar bodies which appeared more electron-dense than other nuclear components such as chromatin and the nucleolus (Fig. 3). The fibrillar bodies consisted of fine, densely packed fibrils and appeared either as ring-shaped or solid spheres (Figs. 3, 7, and 10). Many nuclei containing segregated nucleolus and fibrillar bodies were without apparent virus particles (Figs. 3 and 10). Nucleolar segregation and the occurrence of fibrillar bodies are apparently indicative of early stages of infection.

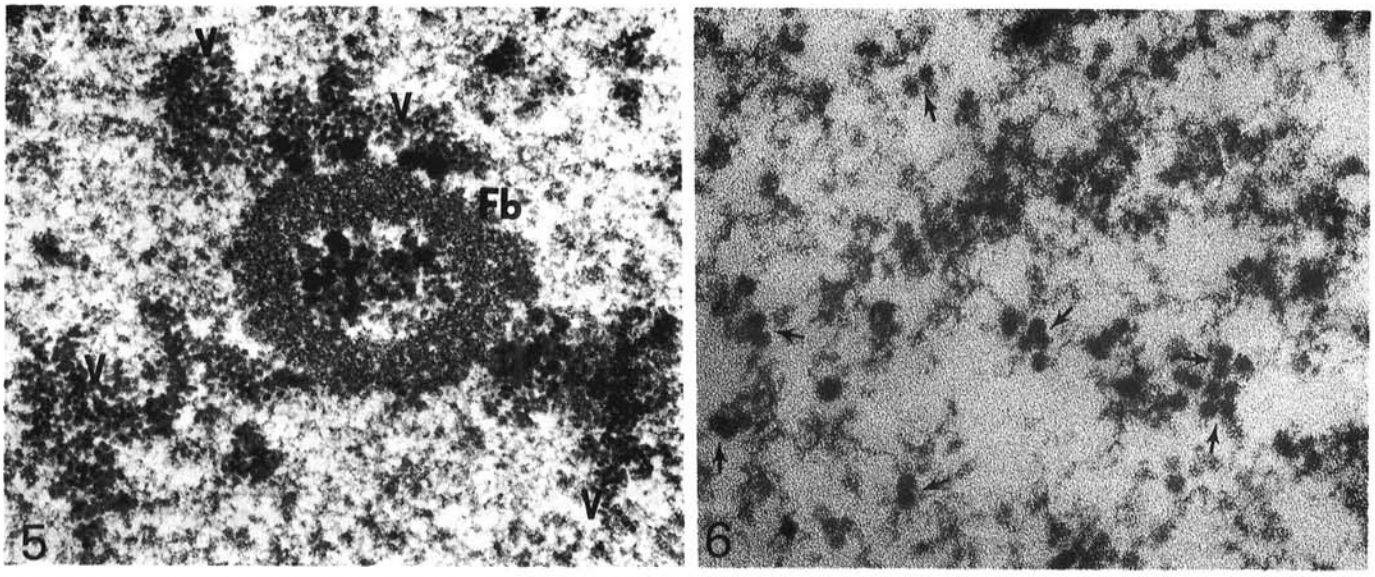
In tissue from primary lesions showing advanced chlorosis and from systemically infected leaves, many cells contained clusters of virus particles associated with ring-shaped fibrillar bodies (Fig. 4A and B). Virus particles occurred only in the nucleus and appeared either as numerous clumps of variously sized aggregates (Fig. 5) or as one extremely large aggregate which was often larger than the nucleolus (Fig. 4A and B). No paracrystalline arrangements of virus particles were observed. However, cells in very late stages of infection, as suggested by depletion of chromatin and degenerating cell organelles, contained randomly dispersed virus particles which were often arranged in pairs (Fig. 6).

Cells containing nuclear changes also contained cytoplasmic inclusions composed of central, electron-dense aggregates surrounded by segments of rough endoplasmic reticulum (Fig. 7). Between the aggregates and the rough endoplasmic reticulum there was a wide electron-lucent zone filled with fine fibrils and ribosomes (Fig. 7). Golgi bodies were also abundant around the inclusions (Figs. 7 and 8). The central aggregates were formed by accumulation of electron-dense, spherical granules (Fig. 7). Inclusions often appeared to be formed by assembly of small, somewhat circular lobes. Each lobe was separated by cisternae of rough endoplasmic reticulum and a large amount of ribosomes and Golgi bodies (Fig. 8). The center of many of the lobes contained electron dense material (Fig. 8). No virus particles were observed in any of these inclusions.

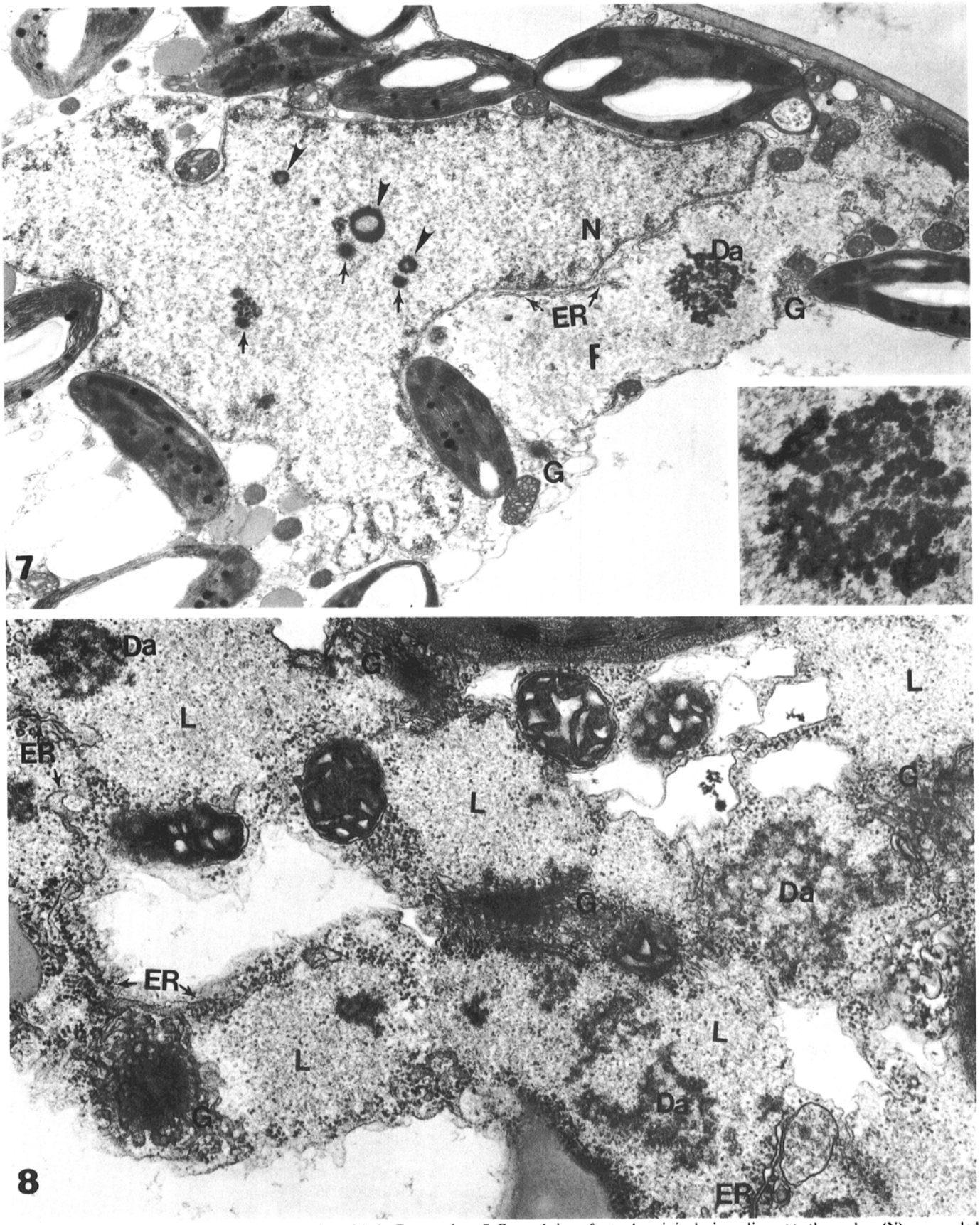
Unlike other whitefly-transmitted geminiviruses, the ultrastructural changes described above were not limited to vascular regions, but occurred in most cell types including epidermal (Fig. 9) and mesophyll cells (Fig. 10). Often the majority of the cells, especially those from advanced chlorotic regions of inoculated leaves, displayed either segregated nucleoli, fibrillar bodies, virus particles or all of them (Fig. 10).

DISCUSSION

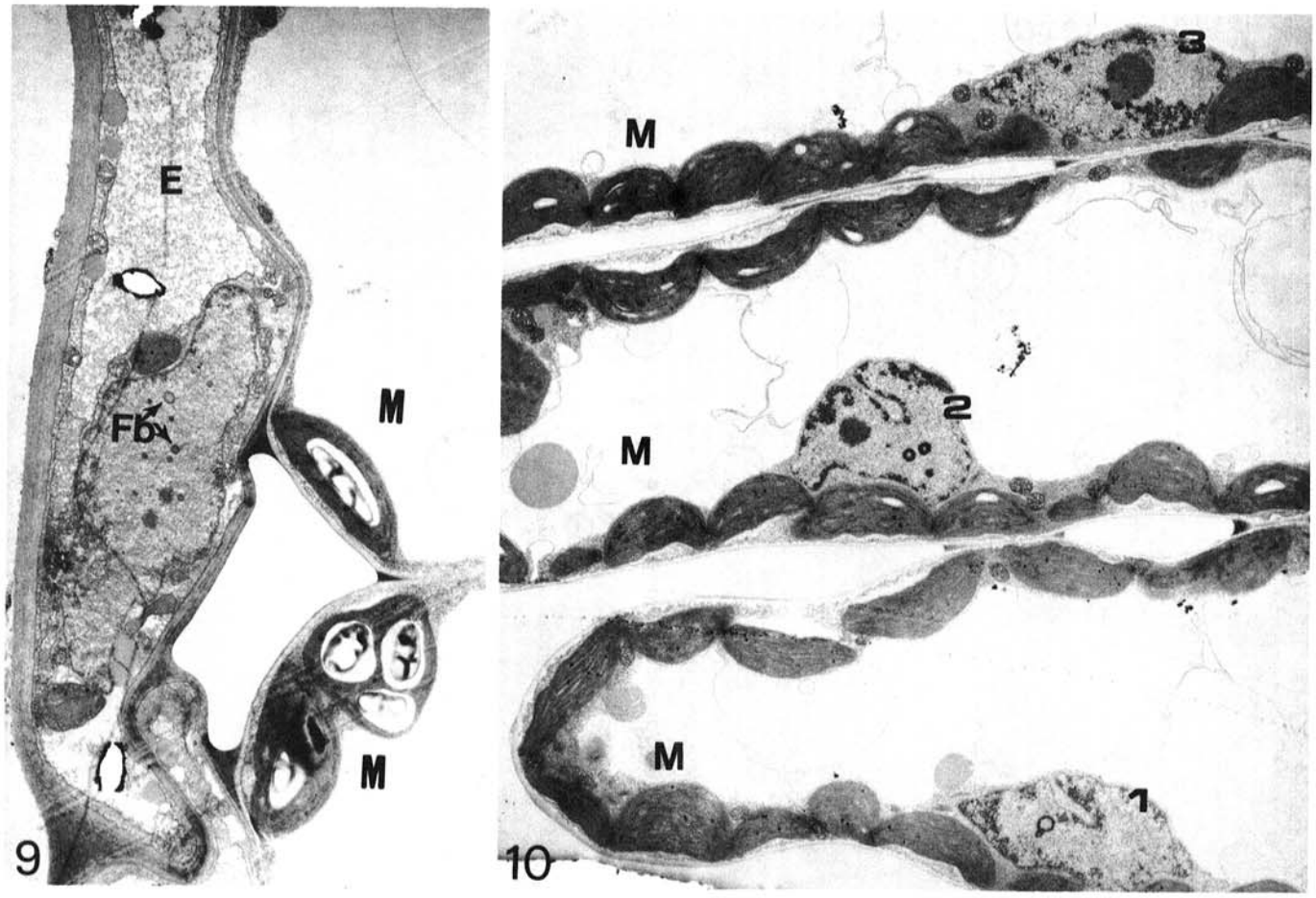
The nuclear changes induced by the Euphorbia virus described in this paper were nearly identical to those induced by bean golden mosaic virus and other whitefly-transmitted geminiviruses (15–21).



Figs. 5–6. Different distribution of Euphorbia virus particles in nuclei at the late stages of infection. **5,** Clumps of virus particles (V) associated with a ring-shaped fibrillar body (Fb) at a late stage of infection (×58,000). **6,** Randomly dispersed virus particles in the nucleoplasm at a late stage of infection in which the particles were often paired (arrows) (×150,000).



Figs. 7–8. Cytoplasmic inclusions induced by infection with the Datura virus. **7,** General view of cytoplasmic inclusion adjacent to the nucleus (N) surrounded by rough endoplasmic reticulum (ER) and Golgi bodies (G). An electron-dense aggregate (Da) is located in the center of inclusion. Fine fibrils (F) fill the gap between the aggregate and ER. The nucleus contains several fibrillar bodies which are either ring-shaped (arrowheads) or solid spheres (arrows) (×10,000). Inset: A higher magnification of the electron-dense aggregate in Fig. 6 showing the granular substructures (×30,000). **8,** Cytoplasmic inclusions appeared as an assembly of small circular lobes (L) consisting of fibrils, ribosomes, and electron-dense aggregates (Da). Each lobe is separated from each other by rough endoplasmic reticulum (ER) and Golgi bodies (G) (×30,000).



Figs. 9–10. Nucleopathic effects in epidermal and mesophyll cells infected with the Datura virus. **9,** An epidermal cell (E) with the nucleus containing several fibrillar bodies (Fb). M = mesophyll cells (×5,000). **10,** Three palisade mesophyll cells (M) and their nuclei. Nuclei 1 and 2 contain ring-shaped fibrillar bodies (Fb) whereas nucleus 3 contains segregated nucleolus (×4,000).

The ultrastructural studies in situ on the leafhopper-transmitted geminiviruses, chloris striated mosaic (11), maize streak (2) and curly top (8–10) viruses revealed that the virus particles and other cytopathic changes similar to whitefly-transmitted geminiviruses occur in the nuclei of infected cells. Fibrillar bodies associated with whitefly-transmitted geminiviruses have, however, not been reported in the leafhopper-transmitted geminiviruses. Therefore, the presence or absence of fibrillar bodies may distinguish between these two groups of geminiviruses. Accordingly, the presence of fibrillar bodies in infected cell nuclei and the finding of geminate particles in leaf dip preparations strongly suggest that the Euphorbia virus is a whitefly-transmitted geminivirus. In this regard, the Euphorbia virus is important because it was found in the continental United States where the virus has not previously been reported. Although a vector study was not carried out, a species of whitefly, *Bemicia tabaci*, the most common geminivirus vector, has been reported to be widespread in the southern United States, including Florida, California, and Georgia (4). In fact, whitefly-transmitted, gemini- and rod-shaped viruses attacking cucurbits (6) and lettuce (7), respectively, have recently been reported to occur in California, although their cytopathic effects have not been described.

The cytoplasmic inclusion that occurs in cells infected with the Euphorbia virus is noteworthy because no cytoplasmic inclusions have been reported to be associated with other whitefly-transmitted geminiviruses, including an Euphorbia mosaic virus from Costa Rica (16).

The nature and functional significance of the cytoplasmic inclusions in Euphorbia virus-infected cells are not known, but it is assumed that the central electron-dense bodies in the inclusions are proteins since the inclusions are consistently associated with rough endoplasmic reticulum and Golgi bodies. It is of interest to determine if these dense granules are antigenically related to the viral protein. If they are viral coat protein, then this protein must migrate into the nucleus to be assembled as virions, since no virus particles were located in the cytoplasm.

The fact that the infection of plants of *Datura* with the Euphorbia virus is not restricted to the phloem cells is another cytopathic feature that is not common to other whitefly-transmitted geminiviruses. Nuclear and cytoplasmic inclusions characteristic of tissue infected with the Euphorbia virus occurred in all cell types of infected *Datura* cells. Whether other susceptible hosts would respond similarly is unknown.

LITERATURE CITED

1. Bird, J., Sanchez, J., Rodriquez, R. L., and Julia, F. J. 1975. Rugaceous (whitefly-transmitted) viruses in Puerto Rico. Pages 3-25 in: Tropical Diseases of Legumes. J. Bird and K. Maramorsch, eds. Academic Press, New York. 171 pp.
2. Bock, K. R., Guthrie, E. J., and Woods, R. D. 1974. Purification of maize streak virus and its relationship to viruses associated with streak diseases of sugarcane and *Panicum maximum*. Ann. Appl. Biol. 77:289-296.
3. Bouteille, M., and Dupuy-Coin, A. M. 1979. Localization of nuclear functions at the cellular and molecular level. Pages 1-33 in: Effects of Drugs on the Cell Nucleus. H. Busch, S. T. Crooke, and Y. Daskal, eds. Academic Press, New York. 567 pp.
4. Costa, A. S. 1976. Whitefly-transmitted diseases. Annu. Rev. Phytopathol. 14:429-449.
5. Costa, A. S., and Bennett, C. W. 1950. Whitefly-transmitted mosaic of *Euphorbia prunifolia*. Phytopathology 40:266-283.
6. Dodds, A. J., Nameth, S. T., Lee, J. G., and Laemmlen, F. F. 1982. Aphid and whitefly-transmitted cucurbit viruses in Imperial County, California. (Abstr.) Phytopathology 72:963.

7. Duffus, J. E., Mayhew, D. E., and Flack, R. A. 1982. Lettuce infectious yellows—A new whitefly-transmitted virus of the desert southwest. (Abstr.) Phytopathology 72:963.
8. Esau, K. 1977. Virus-like particles in nuclei of phloem cells in spinach leaves infected with the curly top virus. J. Ultrastruct. Res. 61:78-88.
9. Esau, K., and Hoefert, L. L. 1973. Particles and associated inclusions in sugarbeet infected with the curly top virus. Virology 56:454-464.
10. Esau, K., and Magyarosy, A. C. 1979. Nuclear abnormalities and cytoplasmic inclusion in *Amsinckia* infected with the curly top virus. J. Ultrastruct. Res. 66:11-21.
11. Francki, R. I. B., Hatta, T., Grylls, N. E., and Grivell, C. J. 1979. The particle morphology and some other properties of chloris striate mosaic virus. Ann. Appl. Biol. 91:51-59.
12. Fulton, R. W., and Fulton, J. L. 1980. Characterization of a tymolike virus common in poinsettia. Phytopathology 70:321-324.
13. Goodman, R. M. 1981. Geminiviruses: the single-stranded DNA viruses of plants. Pages 879-910 in: Handbook of Plant Virus Infection. E. Kurstak, ed. Amsterdam:North Holland/Elsevier. 943 pp.
14. Karnovsky, M. J. 1965. A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. J. Cell. Biol. 27:137a.
15. Kim, K. S., Shock, T. L., and Goodman, R. M. 1978. Infection of *Phaseolus vulgaris* by bean golden mosaic virus: ultrastructural aspects. Virology 89:22-23.
16. Kim, K. S., and Flores, E. M. 1979. Nuclear changes associated with Euphorbia mosaic virus transmitted by the whitefly. Phytopathology 69:980-984.
17. Lastra, R., and Gil, F. 1981. Ultrastructural host cell changes associated with tomato yellow mosaic. Phytopathology 71:524-528.
18. Osaki, T., and Inoue, T. 1978. Resemblance in morphology and intranuclear appearance of viruses isolated from yellow dwarf diseased tomato and leaf curl diseased tobacco. Ann. Phytopathol. Soc. Jpn. 44:167-178.
19. Osaki, T., Kobatake, H., and Inoue, T. 1979. Yellow vein mosaic of honeysuckle (*Lonicera japonica* Tunb.), a disease caused by tobacco leaf curl virus in Japan. Ann. Phytopathol. Soc. Jpn. 45:62-69.
20. Russo, M., Cohen, S., and Martelli, G. P. 1980. Virus-like particles in tomato plants affected by the yellow leaf curl disease. J. Gen. Virol. 49:209-213.
21. Thongmeearkom, P., Honda, Y., Saito, Y., and Syamananda, R. 1981. Nuclear ultrastructural changes and aggregates of viruslike particles in mungbean cells infected with mungbean yellow mosaic disease. Phytopathology 71:41-44.