Two Flexuous-Rod Viruses in Dioscorea floribunda: Symptoms, Identification, and Ultrastructure

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We wish to thank F. W. Zettler for pepper mottle and dasheen mosaic viruses and their antisera, and F. W. Martin for *Dioscorea* spp. seeds.

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Accepted for publication 30 December 1977.

ABSTRACT

HEARON, S. S., M. K. CORBETT, R. H. LAWSON, A. G. GILLASPIE, Jr., and H. E. WATERWORTH. 1978. Two flexuous-rod viruses in Dioscorea floribunda: symptoms, identification, and ultrastructure. Phytopathology 68:1137-1146.

A naturally infected *Dioscorea floribunda* plant with greenbanding and mosaic symptoms contained two viruses. One, which was mechanically transmissible and designated dioscorea latent virus (DLV), produced no macroscopic symptoms in yams, but formed inclusions that resembled those formed by several potexviruses. The second virus produced greenbanding and mosaic symptoms in yams and was transmitted mechanically and by aphids. This latter virus, which was designated dioscorea greenbanding virus (DGBV), induced the formation of pinwheel and nuclear associated inclusions. Additional hosts for DGBV were not found. The modal length of DGBV varied from 600-720 nm depending on the method of extraction and preparation of particles for electron microscopy. No relationship was found between DGBV and dasheen mosaic, pepper mottle, or sugarcane mosaic viruses. In systemically infected leaves of plants infected with DLV and DGBV, DGBV was found primarily in yellow tissue. These yellow sectors had no differentiated palisade cell layer. Both the yellow and the green tissue contained DLV. Virions of DLV and DGBV were found between the arms of pinwheel inclusions in doubly infected cells. Particles of DLV were 7-9 nm in diameter, whereas DGBV particles were 9-11 nm in diameter.

Additional key words: *Philodendron selloum*, ultrastructural cytology.

The genus *Dioscorea* contains all the edible yams such as *D. alata* L., *D. cayenensis* Lam., *D. rotundata* Poir., and *D. trifida* L.; and as well, the medicinal yams, *D. floribunda* Mart. & Gal. and *D. composita* Hemsl., which are sources of steroid precursors for progesterone, testosterone, and cortisone synthesis (19). Two common diseases of *Dioscorea* spp. are attributed to viral infections: internal brown spotting of tubers has been associated with infection by a bacilliform virus (12, 21) and flexuous rods have been found in plants with mosaic, mottle, and greenbanding symptoms (12, 13, 18, 20, 21, 22, 30) in the Caribbean Islands and in Africa.

We previously reported two flexuous-rod viruses in naturally infected *D. floribunda* from Puerto Rico (18). A short (400-450 nm) flexuous-rod virus which was mechanically transmitted produced no symptoms in *D. floribunda* and *D. composita*. The virus has been purified and designated dioscorea latent virus (DLV) (31). A longer flexuous-rod virus, dioscorea greenbanding virus (DGBV), induced greenbanding and mosaic symptoms and pinwheel inclusions when mechanically or aphid-transmitted to *D. floribunda* and *D. composita* (13, 18). In this report, results from ultrastructural studies of yams infected with DLV and/or DGBV are presented, along with the results of attempts to transmit, purify, and establish a normal length for DGBV and to relate DGBV to other plant viruses.

00032-949X/78/000202$03.00/0

MATERIALS AND METHODS

Source material.—Tuberous roots of a naturally infected *D. floribunda* from Mayaguez, Puerto Rico, were grown in quarantine at the Plant Introduction Station, Glenn Dale, MD and then at U.S. Department of Agriculture facilities in Beltsville, MD.

Green peach aphids, *Myzus persicae* Sulz. transmitted DGBV from the naturally infected *D. floribunda* to *D. composita* or *D. floribunda* seedlings. The virus then was maintained in yams by mechanical inoculations. A purified preparation of DLV (31) was mechanically transmitted to and maintained in *D. floribunda* and *D. composita* seedlings.

Host range of dioscorea greenbanding virus.—Leaves from DGBV-infected yam plants were ground in distilled water or 0.01 M phosphate buffer, pH 7.1, and rubbed on Carborundum-dusted seedlings of *Nicotiana glutinosa* L., *N. tabacum* L. 'Kentucky 35', *Chenopodium quinoa* Willd., *Cassia occidentalis* L., *Datura stramonium* L.,

Phytolacca americana L., *Vigna unguiculata* (L.) Walp., *Phaseolus vulgaris* L. 'Pinto', *Lycopersicon esculentum* Mill. 'Marglobe', *Petunia hybrida* Vilm. 'Pink Cascade', *Crotalaria juncea* L., *C. striata* DC., *C. spectabilis* Roth, and *Capsicum frutescens* L. 'Greenleaf Tabasco' and 'Tabasco'. Inoculations were repeated several times and at different times of the year to test for seasonal influences on transmission results. *Saccharum* sp. interspecific hybrid CP 44-101 and *Sorghum bicolor* (L.) Moench 'Rio' and 'Atlas' were inoculated (by means of an artist's air brush) with crude sap from DGBV-infected yam leaves in distilled water containing Carborundum.

Seedlings of *Philodendron selloum* C. Koch, an indicator host for dasheen mosaic virus (DMV) (34), were inoculated with crude sap from the naturally infected *D. floribunda*. Dasheen mosaic virus from a naturally infected *Aglaonema* sp. was maintained in *P. selloum* and inoculated to *D. composita* seedlings.

Preparation of Dioscorea species for observations of ultrastructure.—Beginning 2-3 mo after DLV and/or DGBV were established in *D. composita* and *D. floribunda* plants by mechanical inoculation, the plants were sampled periodically during the year for ultrastructure observations. Leaf pieces from healthy plants and from the naturally infected D. *floribunda* plant also were fixed and embedded to compare ultrastructural cytology with that of the mechanically inoculated plants. When pieces of DGBV+DLV-or DGBV-infected leaves were selected for embedding, samples were separated into yellow, green, or half-green and half-yellow tissues. The leaf pieces were fixed in a glutaraldehyde-acrolein mixture, postfixed in osmium tetroxide and embedded in Epon 812 (14).

Light microscopy of epidermal strips and thick sections of Epon-embedded Dioscorea leaves.—Epidermal strips of healthy and virus-infected *Dioscorea* leaves were stained with calcomine orange 2 RS and "Luxol" brilliant green BL in Cellosolve (4). Thick sections of Epon-embedded leaf pieces were mounted on glass slides with heat, treated with hydrogen peroxide to remove osmium tetroxide, and stained with lactofuchsin (14).

Purification of dioscorea greenbanding virus.—Leaves (90-170 g) from DGBV-infected *Dioscorea* spp. plants were extracted by the ascorbic acid method (8) and clarified in chloroform. Virus was concentrated by centrifugation at 31,000 *g* for 1.5 hr and resuspended in 0.1 M boric acid-KCl-NaOH buffer, pH 8.0. A sample was removed for measuring virus particle lengths. The remaining sample was centrifuged in linear 10-40% sucrose gradients at 81,000 *g* for 1.75 hr. One gradient was fractionated on an ISCO Model D fractionator with UA-2 UV analyzer and the virus-containing fractions were concentrated to 1.5-2.0 ml in 0.01 M borate buffer with an XM 100A filter on an Amicon Model 52 ultrafiltration unit (Amicon Corp., Lexington, MA 02173) for particle measurements. The opaque band (3 cm below meniscus) was collected by needle puncture from the other two gradients, combined, diluted, and concentrated to 2 ml by ultrafiltration. Then this preparation was centrifuged on 10-40% linear sucrose gradients and fractionated as previously described. The ultraviolet-absorbing fractions were centrifuged at 80,000 *g* for 2 hr and pellets were resuspended in 0.5 ml of 0.01 M borate buffer for particle length measurements. *Dioscorea* sp. seedlings were inoculated also with tenfold dilutions of the final preparations in 0.01 M borate buffer to assay for infectivity.

Dioscorea greenbanding virus modal length determination.—Leaf preparations were made by chopping leaf pieces in 2% potassium phosphotungstate (KPTA), pH 7.0, dropping the sap-stain mixture on Formvar-coated grids and diluting with KPTA. Preparations for measuring particle lengths in the clarified extracts that received one high-speed centrifugation were made by mixing a drop of the resuspended pellet with a drop of KPTA on Formvar-coated grids. Half of each preparation from ultrafilter-concentrated virus from the ultraviolet-absorbing fractions of the first density gradient was diluted 1:1 (v/v) in distilled water and a sample was shadowed with platinum-paladium or uranium oxide or negatively stained with KPTA or 3% ammonium molybdate, pH 7.0. The remaining half of each preparation was fixed with one volume of 4% glutaraldehyde (adjusted to pH 6.8 with KOH) and dialyzed against 0.01 M borate buffer. Samples of the fixed fractions were shadowed or negatively stained in the same manner as the unfixed samples. The particles in the final purified preparation from high-speed centrifugation of the gradient fractions were measured after shadowing the particles or making negative stains in KPTA.

RESULTS

Symptomatology of dioscorea greenbanding virus.—*Dioscorea composita* and *D. floribunda* seedlings inoculated mechanically or by aphids with DGBV developed faint vein yellowing and chlorosis on the inoculated leaves and on the leaves immediately above the inoculated leaves. The time required for expression of symptoms varied from 2 wk to 3 mo. New leaves subsequently showed greenbanding or mosaic symptoms resembling those of the naturally infected *D. floribunda* (Fig. 1). Systemically infected leaves also were reduced in size, distorted, and undulated along the lamina. Symptomless leaves were sometimes produced for a period of time on plants that had symptomatic leaves. No plants, other than *Dioscorea* spp., developed symptoms after inoculation with DGBV. Virus particles were not detected in leaf-dip preparations from DGBV-inoculated sugarcane, pepper, *Crotalaria*, *N. glutinosa*, *N. tabacum*, sweet sorghum, and *P. selloum*. If inoculated plants showed no symptoms and virus concentration was too low to be detected by electron microscopy, further testing for infectivity, as by back inoculations, was considered impractical because of the long, indefinite incubation needed for symptom development in *Dioscorea* spp. Inoculations of DGBV to other *Dioscorea* species was limited by the availability of seed.

Symptomatology of dioscorea latent virus.—Plants of *D. composita* and *D. floribunda* inoculated with DLV developed no symptoms, but virus particles were observed in leaf dips and ultrathin sections of the plants. Inoculated leaves of *P. selloum* developed faint vein yellowing and mottling 3 wk after inoculation with crude sap from the naturally infected *D. floribunda*. Flexuous rods, apparently DLV, were found in leaf dips prepared from the inoculated leaves and ultrathin sections of these

leaves contained a few cells with bundles of filamentous viruslike particles but no pinwheel inclusions. Particles were not detected in leaves above the inoculated leaves, and no systemic symptoms developed.

Light microscopy.—Epidermal strips of the naturally infected *D. floribunda* and symptomless plants infected with DLV contained green-stained cytoplasmic inclusions that sometimes appeared banded, as well as the amorphous, vacuolated, inclusions described for some potexviruses (5). Sticklike inclusions described for potyviruses (5, 6) and densely stained greenish-black inclusions, usually appressed to the nuclei, were found in the epidermal strips of the naturally infected *D. floribunda* and plants infected with DGBV. The inclusions appressed to the nuclei stained bright red with lactofuchsin in thick sections of Epon-embedded tissue.

Yellow and green leaf areas of DGBV-infected leaves were distinctly different anatomically. The green areas (Fig. 2) contained a palisade layer and an orderly, compact arrangement of cells within the mesophyll area. These cells had many chloroplasts. The leaf anatomy was similar to that of immature healthy leaves. The yellow tissue (Fig. 3) contained larger cells with more intercellular space between the mesophyll cells. These cells had few chloroplasts. There was no differentiated palisade layer in the yellow tissue. The cells in this layer

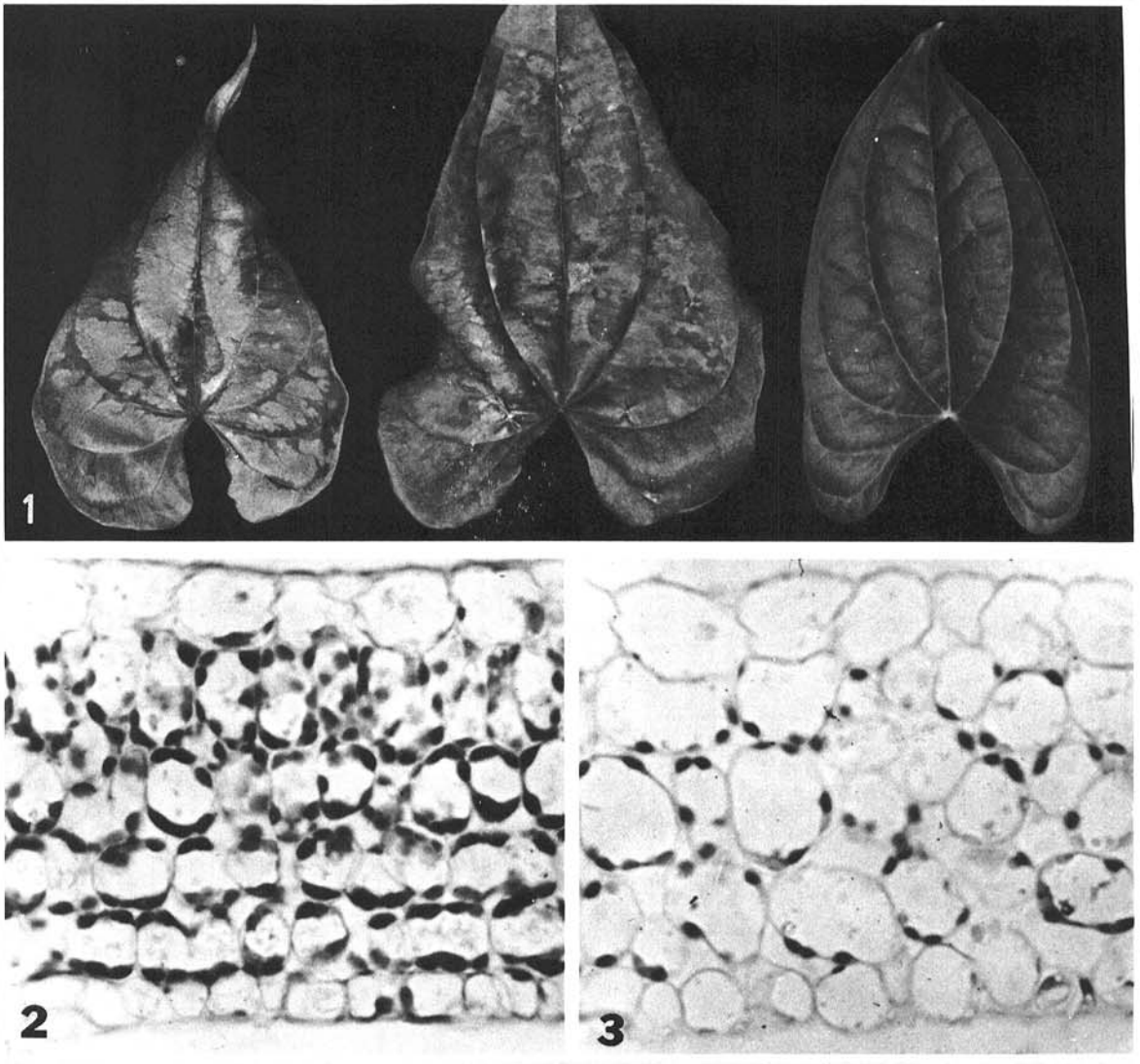

Fig. 1-3. Leaves from healthy and diseased *Dioscorea floribunda* plants and sections of a green sector and a yellow sector of a dioscorea greenbanding virus-infected *D. composita* leaf. **1)** Leaves from a healthy seedling (right) and from the naturally infected *D. floribunda* from Puerto Rico. One leaf has green tissue along the large vascular bundles of a yellow leaf (left); the other shows a mottle and mosaic pattern on a green leaf (center). **2)** A thick (4-μm) section of Epon-embedded leaf tissue from *D. composita* infected with dioscorea greenbanding virus. The photomicrograph was taken of the green half of the leaf piece and showed differentiated palisade and mesophyll cells and numerous chloroplasts (×500). **3)** Photomicrograph of the yellow half of the embedded leaf piece in Fig. 2. There was no palisade cell layer and only a few small chloroplasts in the cells (×500).

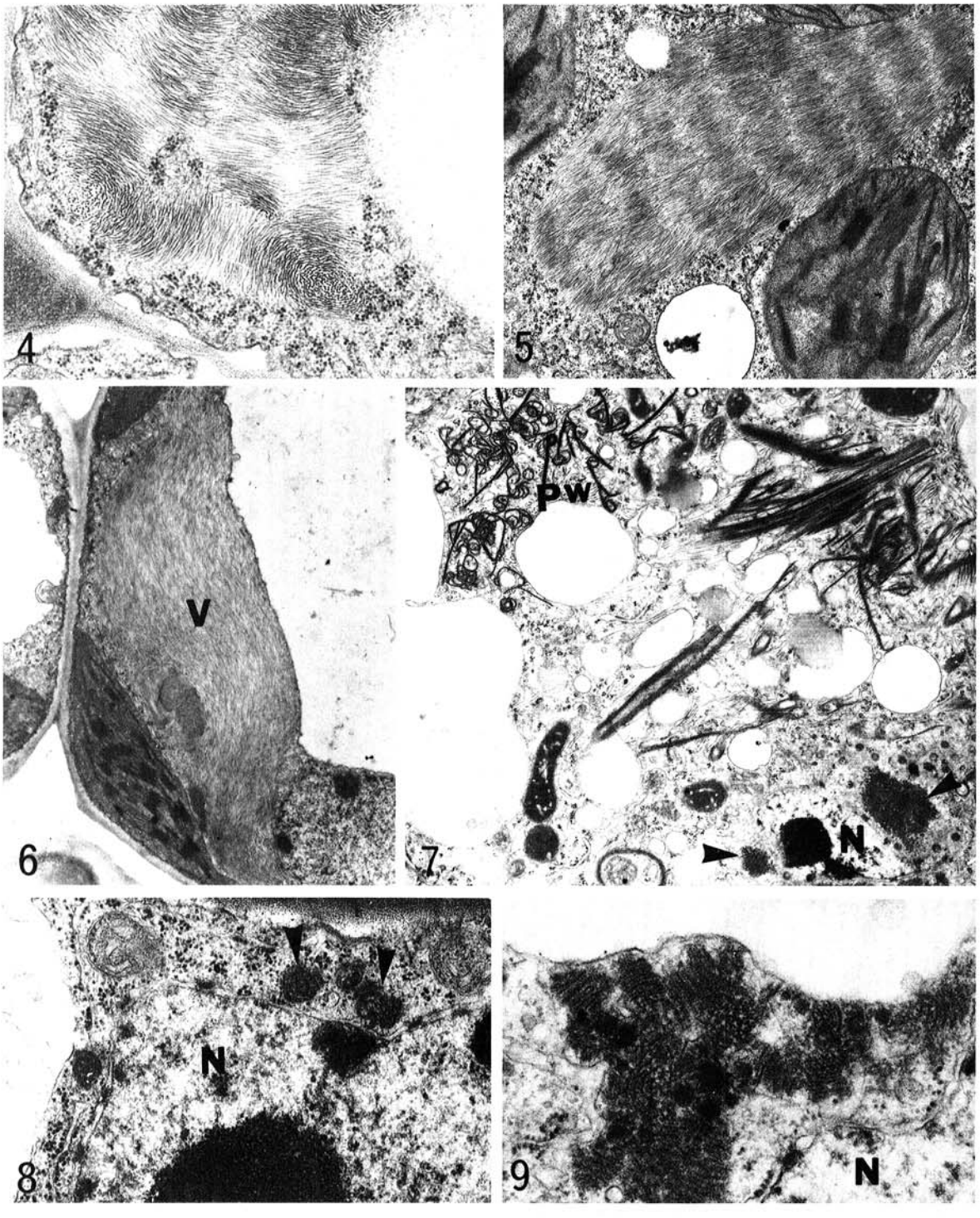

Fig. 4-9. Electron micrographs of ultrathin sections of yam leaf tissue infected with dioscorea latent virus and/or dioscorea greenbanding virus. **4)** Swirls of fingerprint inclusions of viruslike particles in a symptomless leaf from a *Dioscorea composita* seedling inoculated with dioscorea latent virus (×23,000). **5)** A tiered inclusion of viruslike particles in an immature, symptomless leaf from a *D. composita* seedling inoculated with dioscorea latent virus (×11,000). **6)** A large cytoplasmic aggregate (V) of viruslike particles in a mature symptomless leaf from a *D. composita* seedling inoculated with dioscorea latent virus. Note the lack of tiers or rows in the particle packing or aggregation (×8,600). **7)** Pinwheel inclusions (pw), viruslike particles, and nuclear associated inclusion (►) appressed to the nucleus (N) in an epidermal cell of the yellow tissue of a leaf from a *D. composita* seedling that was aphid inoculated with dioscorea greenbanding virus (×9,500). **8)** Small nuclear associated inclusions (►) at the periphery of the nucleus (N) in a leaf cell of the naturally infected *D. floribunda* (×26,700). **9)** A larger nuclear associated inclusion adjacent to the nucleus (N) and containing dense globules, a moderately dense-staining matrix and dense striations or threads (×37,000).

generally were isodiametric and lacked the elongated cell shape of normal palisade cells; they resembled the cells in the mesophyll area. Anatomical differences between the yellow and green tissues were more pronounced as the color differences intensified.

Ultrastructure of healthy and infected Dioscorea leaves.—Symptomless yam seedlings mechanically inoculated with DLV contained aggregates of filamentous particles. Often the particles were arranged in fingerprint aggregates (11) (Fig. 4) or in single or tiered rows that measured 440-460 nm wide (Fig. 5). Tiered inclusions were more common in immature leaves than in older leaves. These structures may correspond to the cytoplasmic banded inclusions in the epidermal strips. Frequently, non-tiered masses of particles formed large cytoplasmic inclusions in mature leaves of systemically infected plants (Fig. 6). Viruslike particles also were scattered throughout the cytoplasm of most cells.

Dioscorea greenbanding virus induced the formation of pinwheel inclusions in the two *Dioscorea* spp. that were examined (Fig. 7). However, the pinwheel inclusions and virus particles rarely were found in the green areas of infected leaves. Virus particles and inclusions usually were associated with cells of the vascular tissue and the yellow areas, but the amount of virus and the number of inclusions in the yellow tissue varied greatly among experiments.

In addition to the pinwheels and laminations, cells also contained one or more electron-dense masses of varying size with darker, parallel, and curved striations (Fig. 7, 8, 9). The striations were circular in cross section and appeared to be thread- or rod-shaped. The masses were adjacent to the nucleus in the majority of the cells, but occasionally the masses were free in the cytoplasm. In some nuclear associated inclusions (NAI) the denser bands were not observed; in others, dense globules also

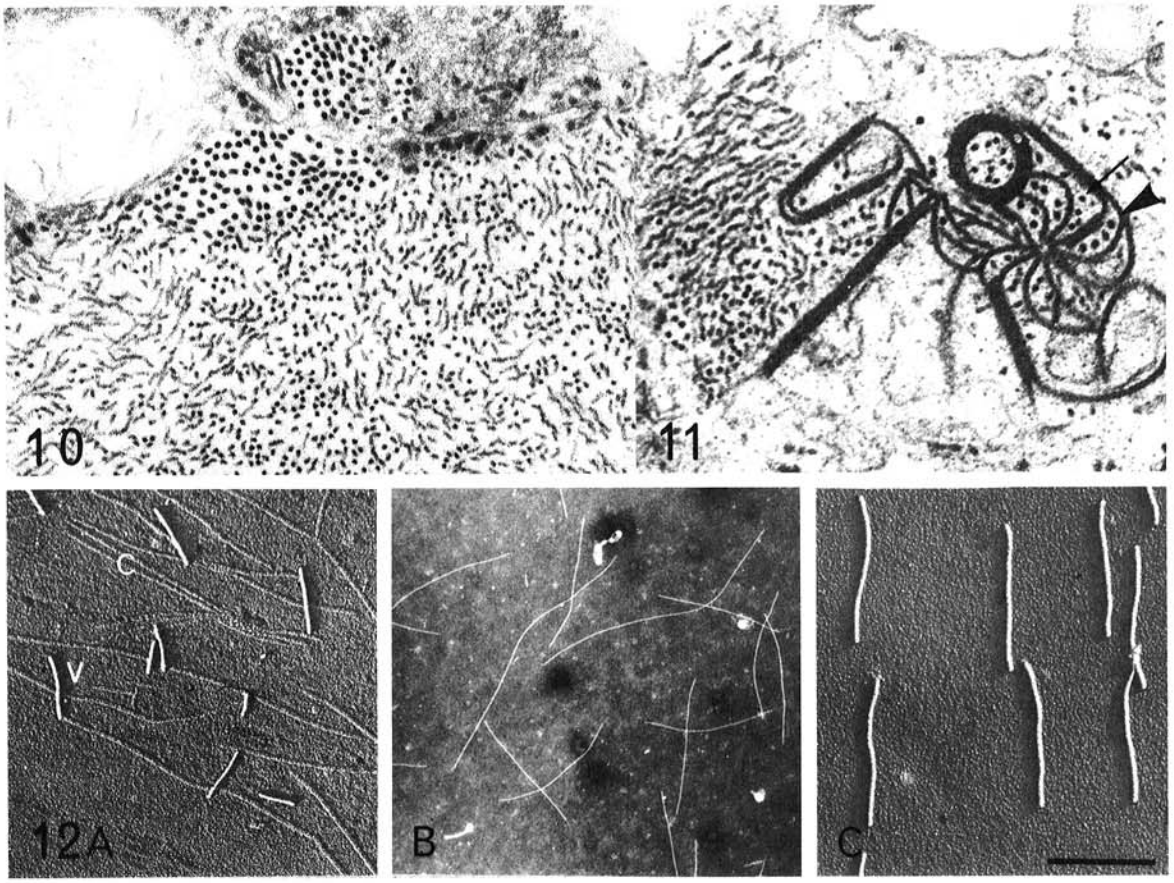

Fig. 10-12-(A to C). Ultrathin sections of yam leaf tissue infected with dioscorea latent virus and dioscorea greenbanding virus and electron micrographs of partially purified dioscorea greenbanding virus in shadowed 2% potassium phosphotungstate (KPTA) preparations. **10)** A portion of a large inclusion in a naturally infected *Dioscorea floribunda* leaf cell showing the viruslike particles in cross section. Two particle sizes are evident. The particles with the smaller diameter were similar to particles observed in plants infected with only dioscorea latent virus. The particles with the larger diameter were similar to particles in plants infected with only dioscorea greenbanding virus. **11)** Pinwheel inclusions with viruslike particles of a larger diameter size (►) and a smaller diameter size (→) between the arm plates in a leaf cell of naturally infected *D. floribunda* (×87,500). **12-A)** Electron micrograph of the short particles (v) and fibrous or tubular contaminating plant material (c) in the upper virus-containing fractions of a sucrose density gradient. Preparation was shadowed with uranium oxide. (×20,000). **12-B)** Potassium phosphotungstate (2%) negative stain and **C)** uranium oxide-shadowed preparation of the particles in the lower virus-containing fraction of a sucrose density gradient. Bar represents 500 nm. (×28,000).

were present (Fig. 9). The inclusions appeared to correspond to the bright red inclusions associated with the nucleus in thick Epon sections stained with lactofuchsin and the dark green-black inclusions in the epidermal strips.

In plants infected with DLV + DGBV, some cells in the yellow areas of the leaf were infected by both viruses as shown by the presence of large DLV cytoplasmic masses of virus and DGBV-induced pinwheels. In addition, virus particles of two different diameters could be recognized. Inclusions consisted of one or both types (Fig. 10) of particles and both particle sizes were associated with the pinwheels (Fig. 11). Dioscorea latent virus had a diameter of 7 to 9 nm and DGBV had a diameter of 9 to 11 nm in both singly and doubly infected plants. Dioscorea greenbanding virus occurred predominantly in the yellow leaf tissue; DLV was abundant in both the yellow and the green tissues. All inclusions observed in the naturally infected *D. floribunda* were found in the plants mechanically inoculated with DLV + DGBV.

Purification of dioscorea greenbanding virus.—Purification of DGBV was hampered by excessive latex, green pigment, and other unidentified plant compounds. Some of the virus-containing fractions were contaminated with a long, thin, tubular, or rodlike particle (Fig. 12-A) that also was observed in healthy and diseased leaf chop preparations. The amount of virus recovered varied among experiments. The particles from one purification are shown in negative stain in Fig. 12-B and shadowed in Fig. 12-C. The number of ultraviolet-absorbing zones found in the sucrose density gradients and the optical density of the zones varied among experiments. The ISCO profiles from the first and second sucrose density gradient centrifugations of one experiment are shown in Fig. 13-A. No detectable virus was obtained from the purification experiments done in the summer. Seasonal influences on the virus concentration may account for some of the inconsistencies; other factors have not been identified. The ultraviolet-absorbing fractions from the second density gradients were infectious at a dilution of 10^{-1} but not at 10^{-2}. Antiserum was not prepared to DGBV because of the variability of virus recovery, the low concentration of any virus recovered, and the presence of contaminating materials. Other workers have experienced similar problems with purification of the 750-nm rod virus(es) in yams (20, 30); so antisera to these viruses are not available.

Dioscorea greenbanding virus modal length determinations.—The length of DGBV particles in crude sap and sucrose density gradient fractions was compared in shadowed and negatively stained preparations of fixed and nonfixed virions (Table 1). Since the virus particles varied considerably in length, the modal length (ML, the most frequent length) rather than the normal length (NL, the average particle length for the major mode) was used for comparisons (10). In KPTA chop preparations, the ML was 640 nm with 83% of the particles measuring 610-700 nm (Fig. 13-B). The number of particles in most preparations was low and usually only one to three particles occurred in each electron micrograph. After the clarified sap extract received one high-speed centrifugation, the ML of the virus particles was 720 nm in KPTA (Fig. 13-C) and 36% were 710-750 nm long. However, preliminary tests using 8% *n*-butanol for clarification, rather than chloroform, resulted in extensive aggregation and fragmentation.

The lengths of the particles in the ultraviolet-absorbing gradient fractions are shown in Table 1 and Fig. 13-D to F. Similar results were obtained in other experiments when the effects of purification, fixing, shadowing, and negative staining on particle lengths were compared. Electron microscopic observations of the gradient fractions showed that the rods sedimented in the gradient according to particle length. Fraction F1-1 contained a high proportion of short rods under 400 nm, but of varying lengths, and a tubular contaminating material (Fig. 12-A). The very short rods were assumed to be particle fragments and were not shown in Table 1 or the histograms. The F1-2 and F2-2 contained a high proportion of extremely long particles that were assumed to be particle dimers and trimers. These also were not shown because a large proportion of such particles ended on grid bars or were not contained within one micrograph and, therefore, could not be measured accurately.

The ML's of the populations in F1-1 and F1-2 from the first sucrose density gradient were 600 nm in KPTA. With glutaraldehyde fixation and KPTA staining in F1-2, a ML of 680 nm was found. In the shadowed F1-2 preparation the ML was 720 nm.

The ML of the particles (in F2-1) negatively stained with KPTA was 600 nm but in the shadowed F2-1 preparations the ML was 640 nm. In F2-2 there were more particles over 660 nm than in F2-1. When shadowed, the particles in F2-2 had a ML of 680 nm (Fig. 13-F). This was shorter than the ML of 720 nm for the shadowed F1-2.

DISCUSSION

Symptoms of DGBV-infected *Dioscorea* spp. resembled the mosaic and greenbanding symptoms observed by others (1, 12, 20, 21, 22, 28, 30) in diseased yams; but we were unable to transmit DGBV to *Crotalaria*, cucumber, tobacco, or pepper (1, 28) though it infected *D. bulbifera*. Harrison and Roberts (12) also were unable to transmit the 750-nm rod virus in *D. alata* to cucumber, *N. clevelandii*, *N. tabacum*, *P. vulgaris*, or *P. selloum*. A 750- to 800-nm flexuous rod virus in *D. cayenensis* from the Ivory Coast infected some species of yams but could not be transmitted to *D. floribunda*, *D. composita*, or *D. bulbifera* (30). The relationship of the yam diseases and viruses to the long flexuous-rod virus we observed, and to each other, remains undetermined.

Host range studies indicated that DGBV was not related to SCMV, DMV (22), or PMV. Although homologous antiserum was not available, an attempt was made to relate DGBV to other potyviruses. A modified sodium dodecyl sulfate (SDS) plate immunodiffusion method (26) was used to test DGBV in SDS crude sap extracts and in purified preparations against antisera to PMV, DMV, tobacco etch virus [American Type Culture Collection (ATCC) PV 69], bean common mosaic virus (Bercks), bean yellow mosaic virus (Bercks), soybean mosaic virus (ATCC PV 94), potato virus Y, datura Columbian virus, and sugarcane mosaic virus. Within 2 days PMV, DMV, and SCMV produced a single precipitin line with the homologous antisera. Dioscorea

greenbanding virus did not react with any of the antisera tested.

Virions in crude sap from DGBV-infected yams had a ML of 640 nm, indicative of a carlavirus. However, leaf cells contained pinwheel inclusions, which are indicative of a potyvirus (6). The ML of DGBV from purified preparations varied during the purification procedure and with the method used to prepare the sample for electron microscopy. Similar difficulties in the determination of particle length for pea seed borne mosaic virus (PSbMV) (10) and SCMV (16) have been reported. The ML for isolates of these viruses ranged from 500-660 nm in KPTA dip preparation. However, ML's of 700-800 nm were obtained for PSbMV by

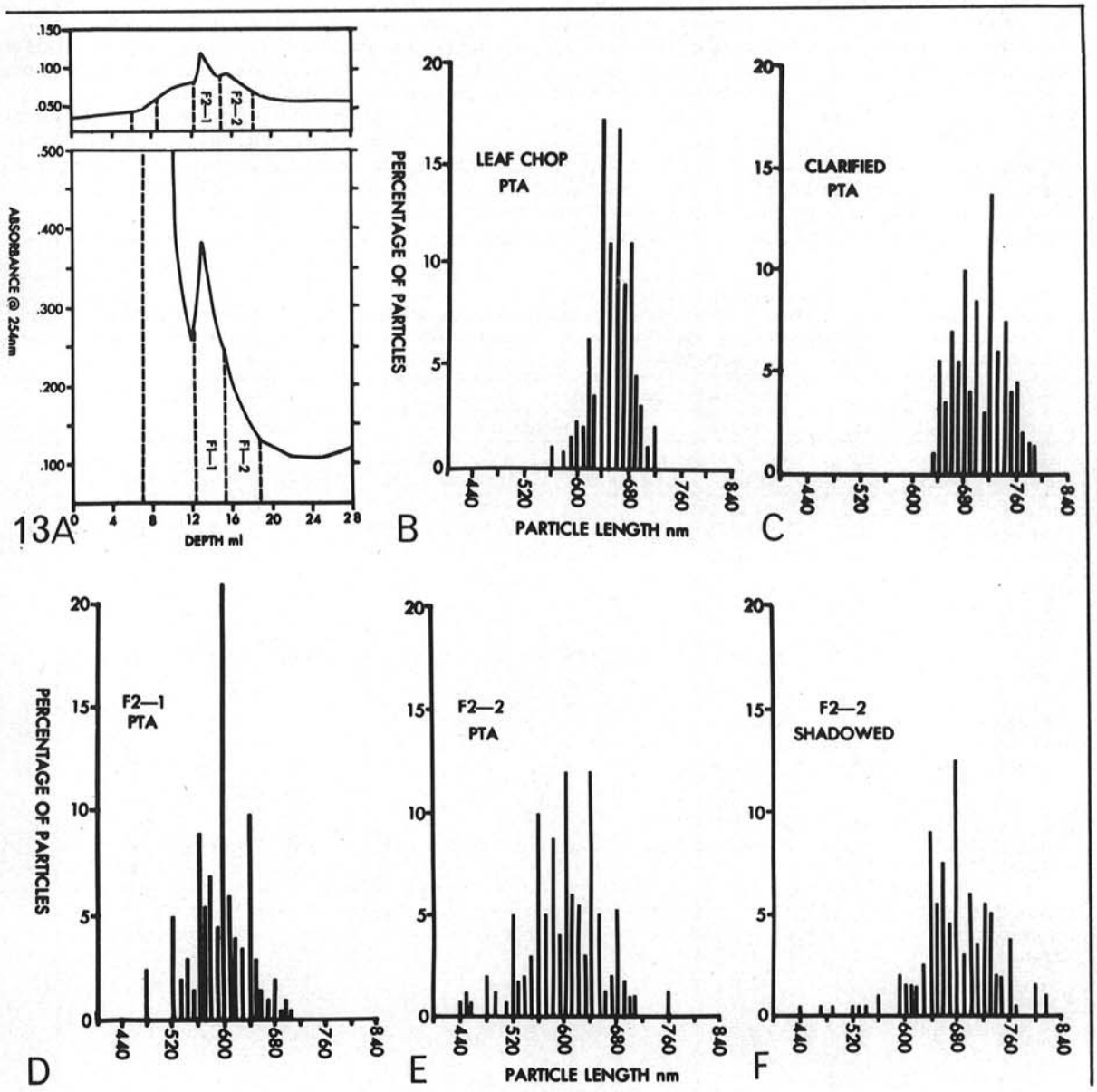

Fig. 13-(A to F). Ultraviolet absorbance profiles of sucrose density gradients containing dioscorea greenbanding virus and histograms of the particle lengths in leaf chops, in a clarified extract, and in the virus containing fractions from sucrose density gradients. **A)** Ultraviolet absorbance profiles of a gradient from the first (bottom) and second (top) sucrose density gradient centrifugations (80,000 *g* for 2 hr) of dioscorea greenbanding virus preparations. The abbreviations F1-1, F1-2, F2-1, and F2-2 designate the fractions collected and used for virus particle-length measurements. (B-F) Histogram of the particle lengths measured **B)** in 2% potassium phosphotungstate (KPTA) negatively stained leaf chops. Histogram represents 136 particles; 6% of the particles were longer than 840 nm. **C)** In a KPTA-stained, partially purified preparation that was clarified with chloroform, centrifuged at 31,000 *g* for 1.5 hr and the pellet resuspended in 0.1 M borate. Of 328 particles, 293 were 400-840 nm long and 14% were longer than 840 nm. **D)** From F2-1 stained with KPTA. Essentially all of the 183 measured particles were 400-840 nm long. **E)** From F2-2 stained with KPTA. Of 361 particles, 10% were longer than 840 nm. **F)** From F2-2 shadowed. Of 206 particles, 11% were longer than 840 nm.

varying purification techniques and fixing with glutaraldehyde before negative staining (10). Particles of SCMV measured 720-780 nm when shadowed and 660 nm in KPTA (16). We consistently found that the ML of shadowed particles was 30-40 nm longer than particles in KPTA preparations. Short, presumably fragmented particles, were fewer in the shadowed preparation and in the fixed-KPTA preparations than in corresponding nonfixed KPTA preparation. We found that the ML of the particles decreased as purification progressed, probably as a result of fragmentation with handling of the preparation. Since the fragmented particles were of different lengths, they failed to sediment uniformly in the sucrose gradients and multiple ultraviolet-absorbing regions resulted. Measurements from the clarified extracts stained with KPTA and from the F1-2 shadowed preparations indicated that DGBV had a particle length of 720-730 nm. Others have reported NL's of 750-770 nm for the virus particles in leaf dips of naturally infected *Dioscorea* spp. (12, 20, 21, 30). The differences in reported particle lengths may result from differences in viruses or strains, environmental conditions for growing plants, the use of NL instead of ML for reporting results, and/or differences in handling procedures. Leaf chops in KPTA were not reliable for detecting double infections in *Dioscorea* spp. plants because bimodal histograms were not obtained. The number od DGBV particles in the leaf chops varied, but were often low compared to the number of DLV particles with a ML of 400-450 nm (31). The ML of DGBV was about 650 nm instead of the expected length of 750 nm. Dimers of DLV measuring 800-900 nm were also common in the leaf chop preparations.

The number of pinwheel inclusions and NAI's varied and affected the ease of detecting DGBV infection in epidermal strips and ultrathin sections. The number of inclusions appeared to be the greatest and virus recovery the most successful when the daylight hours were short. Conversely, symptom expression after mechanical

TABLE 1. Comparison of the range of particle lengths and the modal lengths for dioscorea greenbanding virus in leaf chop, clarified sap, and sucrose density gradient fractions prepared for electron microscopic examination by shadowing or negatively staining glutaraldehyde-fixed or nonfixed preparations

Sample	Leaf dip	Clarified sap	Density gradient one[a]					Density gradient two[a]			
			Fraction 1	Fraction 2				Fraction 1		Fraction 2	
Treatment	KPTA[b]	KPTA	KPTA	KPTA	Glut-KPTA[c]	Am Molb[d]	Shad[e]	KPTA	Shad	KPTA	Shad
Particles measured (no.)	145	328	330	200	221	224	95	196	190	361	206
Major range of particle lengths (nm)	610-710	650-760	510-760	570-710	610-750	610-750	610-750	570-660	610-750	530-710	610-750
Particles within the range (%)	83	83	79	76	68	67	79	76	75	87	79
Modal length (nm)	645	720	600	600	680	645	720	600	645	600 & 645	680
Particles at modal length	17	14	11	10	15	17	18	22	12	11, 11	14
Particles 700-750 nm (%)	4	36	15	0	10	10	28	0	14	0	20
Particles over 840 nm (%)	6	14	6	12	8	16	17	0	5	10	11

[a]Sucrose density gradient fractions as designated in Fig. 13A.
[b]Samples were negatively stained with 2% potassium phosphotungstate, pH 7.
[c]Samples were fixed with 2% glutaraldehyde, pH 6.8, dialyzed against 0.01 M borate buffer, and negatively stained with 2% potassium phosphotungstate, pH 7.
[d]Samples were negatively stained with 3% ammonium molybdate, pH 7.
[e]Samples were shadowed at 14-16 degrees with 80-20% platinum-paladium.

inoculation of plants often required more than 2 mo to develop during the winter, but only 2-4 wk in the summer. Controlled-environment studies are needed to substantiate these observations and to establish optimum conditions for DGBV replication and/or symptom expression.

Data on the ultrastructure of virus-infected *Dioscorea* spp. are scarce (20, 21). Based on examination of ultrathin sections of naturally infected yams, Mohamed (21) proposed that two flexuous-rod viruses occurred in *Dioscorea* spp. Bundles of filamentous particles and pinwheels were found in most infected plants; however, only bundles of particles were found in *D. alata* 'Ashmore' and *D. cayenensis* and only pinwheels were found in *D. bulbifera*. Mohamed suggested that one virus caused slight or no symptoms and induced pinwheel inclusions and another caused severe symptoms and formed large bundles of filamentous particles. He did not detect a 400- to 450-nm rod in leaf dips; however, the fingerprint virus inclusion in an ultrathin section of *D. trifida* appears to be similar to the fingerprint inclusions (Fig. 7) formed by DLV.

Based on examination of both the naturally infected *Dioscorea floribunda* plant and mechanically inoculated yams, we concluded that DLV was present in symptomless *Dioscorea* spp. and that the virus might be a potexvirus, even though the 400-450 nm particle length (31) is shorter than the proposed 500 nm length for potexviruses. Sheet laminations associated with PVX infection (2, 5), but not with other potexviruses, were absent in DLV-infected *Dioscorea* spp. Large masses of loosely packed virus particles, short bands or tiered aggregates, and swirls or fingerprints (11) of virus that have been described in ultrastructure studies of some potexviruses (5) as papaya mosaic (33), cymbidium mosaic (11, 17), clover yellow mosaic (25), and cassava mosaic viruses (15) also were observed in DLV-infected *Dioscorea* spp. Tiered structures were typical of immature or maturing cells or early stages of the infection process, whereas the large masses of loosely packed particles were common in older tissue or advanced stages of infection in systemically infected plants. This association has not been made for other potexviruses but crystalline inclusions were found in symptomless areas of cymbidium mosaic virus-infected *Cattleya* sp. sepals and petals, while noncrystalline aggregates of virus were found in and adjacent to necrotic areas (11).

Pinwheel formation, aphid transmission, and the 720-730 nm particle length characterized DGBV as a potyvirus. The presence of the unique NAI associated with DGBV infection aids in diagnosing the virus in the light microscope. The significance of the association of DLV and DGBV with DGBV-induced pinwheels in doubly infected cells cannot be assessed until the function of the pinwheel has been established.

Dioscorea greenbanding virus was found predominantly in the yellow sectors of leaves from singly or doubly infected plants. Similar observations have been reported for other viruses. Inclusions and infectivity were highest in the light-green sectors of *Crotalaria* sp. leaves infected with a PVY-like virus (32). Pinwheels also were found predominantly in the yellow tissue of maize infected with SCMV; however, they were equally common in yellow and green leaf tissues of sorghum (24). The concentration of virus was higher in yellow tissue than in green tissue of tobacco infected with tobacco mosaic virus (TMV) (3, 7, 9, 23, 29) and in Chinese cabbage infected with turnip yellow mosaic virus (27). The modification in leaf tissue structure within yellow areas of infected yams also was similar to the alterations in yellow tissue of tobacco infected with TMV (9). The reasons for low concentrations of virus in green tissue are undetermined, but may be related to an immune or inhibitor mechanism in the cells of the green tissue (9, 23).

Information present in this report can be useful in detecting and diagnosing two viruses in *Dioscorea* spp. using the light and the electron microscope. However, an expanded host range, better purification methods, and more serological tests are needed to characterize and to determine relationships among the viruses in yams.

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