Two Isometric Viruses in Poinsettias

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ABSTRACT

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Two isometric viruses were found to be common in commercially grown poinsettias: Fulton's poinsettia mosaic virus (PoiMV) and a new virus tentatively named poinsettia cryptic virus (PoiCV). We were unable to transmit the two viruses mechanically to common indicator plants, and they did not react with each other's antisera or antisera to 52 other isometric viruses. PoiMV sedimented as two components with sedimentation velocities (S_{rel}) of about 53 and 115. After negative staining with uranyl acetate, the two components had average particle diameters of 25 and 31 nm. PoiCV sedimented as one component with S_{rel} of about 120 and average particle diameter of 28 nm. The two viruses also differed in electrophoretic mobility. PoiMV was detected more reliably in infected plants with enzyme-linked immunosorbent assay or the Derrick method of immunoelectron microscopy than with the agar gel double diffusion test. The latter test usually failed with PoiCV.

Poinsettia plants (*Euphorbia pulcherrima* Willd.) showing leaf mosaic (Fig. 1) and containing isometric virus particles were received repeatedly in 1978 and 1979 from commercial growers. Isometric virus particles in poinsettias have been described by Fulton et al (3) and Gardner et al (4). We present evidence that two different isometric viruses are common in commercial poinsettias.

MATERIALS AND METHODS

Purified virus preparations were obtained by homogenizing 100 g of poinsettia leaves in 200 ml of 0.25 M phosphate buffer, pH 7.5, containing 0.2% ascorbic acid and 0.2% sodium sulfite. The supernatant obtained after low-speed centrifugation was stirred for 30 min with one-half volume of chloroform and kept at 4 C overnight. After low-speed centrifugation, the virus was sedimented from the aqueous phase by high-speed centrifugation. The sediments were resuspended in 0.05 M phosphate buffer, pH 7.5. For further purification, the material was centrifuged at 8 C in a Beckman SW 27 rotor for 4 hr at 25,000 rpm or in a Beckman SW 39 rotor for 90 min at 35,000 rpm on 10–40% linear sucrose density gradients (SDG) made up in 0.05 M phosphate buffer, pH 7.0. The gradients were fractionated on an Isco fractionator. Sedimentation velocities (S_{rel}) relative to that of top component (53S) and bottom component (113S) of belladonna mottle virus (6) and tobacco mosaic virus (194S) (7) were estimated by SDG centrifugation of purified virus.

Rabbits were immunized by two 2-ml injections spaced 1 wk apart of virus suspensions (top I, bottom I, preparation II) emulsified in an equal volume of Freund's adjuvant complete and incomplete, respectively. Bleedings were taken at 2-wk intervals. Agar gel double diffusion tests were done with 0.85% Difco Noble agar containing 0.85% sodium chloride, 0.25% sodium azide, and 0.01 M Tris-HCl buffer, pH 8.0. The reactant wells, 4 mm in diameter, were spaced 2 mm apart. For immunoelectrophoresis, 1% agarose in 0.025 M phosphate buffer, pH 7.0, was used. Enzyme-linked immunosorbent assay (ELISA) was done as described by Clark and Adams (1). The Derrick method (2) of immunoelectron microscopy was done essentially as described by Milne and Lesemann (5). Carbon-Formvar coated grids were floated for 5 min on serum diluted 1:1,000 in 0.1 M phosphate buffer, pH 7.0; washed with 20 drops of buffer; floated for 15 min on the virus sample; and washed with 40 drops of distilled water followed by 5–7 drops of 2% aqueous uranyl acetate, which was carefully drained off to leave a thin film of negative stain on the grid. Particle counts were made at a magnification of 40,000 from 50 random viewing fields of the binocular on each of two duplicate grids.

RESULTS

Crude extracts from poinsettias with mosaic symptoms (received from German growers) gave a strong precipitin line in the agar gel double diffusion test with an antiserum prepared by R. Fulton against isometric virus particles from poinsettias in the United States. Fulton (*personal communication*) proposes the name poinsettia mosaic virus (PoiMV) for his virus. Purified preparations from these German poinsettias with mosaic (preparations I) in SDG centrifugation

yielded two types of virus particles sedimenting at 53 and 115 S_{rel}, respectively. Both components reacted with Fulton's antiserum to PoiMV without spur formation. After negative staining with uranyl acetate, the particle diameters of the top component ranged from 22 to 27 nm with a mean of 25 ± 0.5 nm. The diameters of the bottom component ranged from 28 to 34 nm with a mean of 31 ± 0.8 nm. Antisera to both components were prepared, and a survey was made with about 300 leaf samples received from poinsettia growers in different parts of Germany (R. Koenig, D.-E. Lesemann, and H. Kühne, *unpublished*). With the agar gel double diffusion test, we detected PoiMV in about 85% of these leaves, although not all showed symptoms. With ELISA, 95% of the samples proved to be infected, indicating that PoiMV is sometimes missed with the agar gel double diffusion test. ELISA was done with the antiserum to the top component because this antiserum gave less nonspecific background in preliminary trials.

In further tests, the reliability of ELISA was compared with that of the Derrick method of immunoelectron microscopy. We used the antiserum to the bottom component because the nonspecific reactions did not interfere with this method. Thirty-three plants were tested six times from May until October. With ELISA, 13 plants reacted positively in all six tests, and five plants reacted positively in all but one or two tests. The Derrick method confirmed these positive reactions. All 18 plants eventually developed mosaic symptoms. The 15 plants that consistently gave negative results with ELISA did not develop mosaic symptoms. Nevertheless, we always detected small numbers of isometric virus particles in these plants with the Derrick method.

Purified preparations from the 15 plants that gave negative results with ELISA (preparations II) contained virus particles that sedimented in SDG centrifugation at 120 S_{rel}. After negative staining with uranyl acetate, these particles had diameters from 24 to 31 nm with a mean of 28 ± 1.7 nm. Two types of particles with diameters of about 26 and 31 nm, respectively, may have been present.

Preparations I and II that had not been subjected to SDG centrifugation were compared in the agar gel double diffusion test (Figs. 2 and 3) and by the Derrick method (Table 1). In the latter method, we also used the separated top component

of preparation I.

Fulton's antiserum to PoiMV gave a single precipitin line with preparation I and no line with preparation II (Fig. 2). Similarly, by the Derrick method we saw large numbers of particles in preparation I and small numbers of particles resembling the control values in preparation II (Table 1).

Antiserum to preparation II gave a single precipitin line with both preparations. This line crossed the line given with Fulton's antiserum and preparation I (Fig. 2). By the Derrick method, the antiserum to preparation II trapped many particles from the unfractionated preparations I and II, but not from the top component of preparation I.

Antiserum to the bottom component of preparation I gave one precipitin line with preparation II and two precipitin lines with preparation I (Fig. 3). One of these lines fused with that given by Fulton's antiserum; the other fused with that given by the antiserum to preparation II (Fig. 3). In immunoelectrophoresis, the material reacting with Fulton's antiserum moved more rapidly toward the anode than the material reacting with the antiserum to preparation II (Fig. 4). With the Derrick method, the antiserum to the bottom component of preparation I trapped large numbers of particles from both preparations I (including its top component) and II.

Undiluted antiserum to the top component of preparation I in the agar gel double diffusion test behaved essentially like antiserum to the bottom component, although the one line given with preparation II and the second line given with preparation I were much weaker. With antiserum diluted 1:32 in saline, these weak lines did not develop; the reaction patterns were then identical to those of Fulton's antiserum to PoiMV.

Preparation I, containing the two types of virus particles, did not react with antisera to 52 isometric viruses including como-, cucumo-, nepo-, tombus-, tymo-, and unclassified viruses. We were unable to transmit the two viruses mechanically from poinsettia to *Antirrhinum majus* L., *Chenopodium amaranticolor* Coste & Reyn, *C. murale* L., *C. quinoa* Willd., *Gomphrena globosa* L., *Nicotiana clevelandii* Gray, *N. tabacum* L. 'White Burley,' or *Petunia hybrida* Vilm.

DISCUSSION

Two isometric viruses appear to be common in commercially grown poinsettias. Our preparations II from plants that at no time showed mosaic symptoms contained virus particles that did not react with Fulton's antiserum to PoiMV and which in SDG centrifugation sedimented at about 120 S. This virus is tentatively named poinsettia cryptic virus. Our preparations I from poinsettias that eventually did develop mosaic symptoms

contained a mixture of PoiCV and PoiMV. In SDG centrifugation, PoiMV sedimented as two components at about 53 and 115 S. At 254 nm, the peak of the bottom component was about twice as high as that of the top component and apparently overlapped that of PoiCV, which was also present, though in much

Table 1. Number of virus particles[a] trapped from different virus preparations from poinsettias on antiserum-coated electron microscope grids
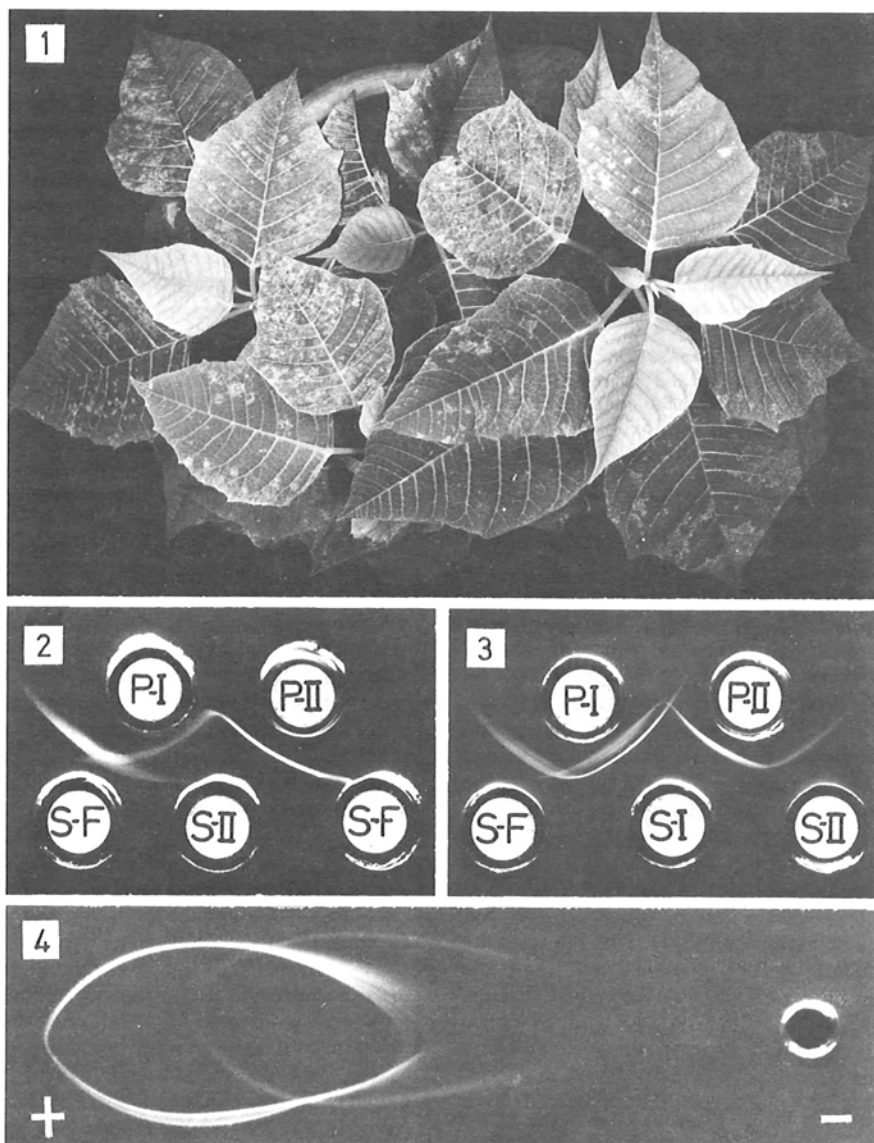
	Virus preparation		
Coating antiserum to:	I (top and bottom component)[b]	I (top component)	II[c]
Poinsettia mosaic virus	1,560	3,058	31
Preparation II	438	3	1,380
Bottom component of preparation I	3,575	1,177	2,075
Control[d]	20	0	32

[a] Means, from two duplicate grids, of particle counts per 50 random viewing fields of the binocular at a magnification of 40,000.
[b] From mosaic-affected plants.
[c] From symptomless plants free of poinsettia mosaic virus.
[d] Antiserum to potato virus Y.

Figs. 1–4. (1) Poinsettia plant with mosaic symptoms. Such plants yielded preparations I containing poinsettia mosaic virus (PoiMV) and another virus tentatively named poinsettia cryptic virus (PoiCV). **(2 and 3)** Agar gel double diffusion tests of preparations I and II (P-I and P-II) from mosaic-affected and symptomless poinsettias, respectively. P-II contains only PoiCV. S-F is Fulton's antiserum to PoiMV, S-II is our antiserum to P-II, and S-I is our antiserum to the bottom component of P-I. **(4)** Immunoelectrophoretic separation of PoiMV and PoiCV in P-I. Antiserum was to the bottom component of P-I. PoiMV moves more rapidly toward the anode.

lower concentration. The antiserum to the bottom component of preparation I contained antibodies to PoiMV and PoiCV. From the results of the Derrick method, the top component seemed to contain only PoiMV. However, the antiserum to this top component also contained small amounts of antibodies to PoiCV. Thus, the separation in SDG centrifugation was possibly incomplete. With ELISA, the antiserum to the top component detected only PoiMV.

PoiCV and PoiMV can also be distinguished on the basis of electrophoretic mobility and particle diameter. The two components of PoiMV,

surprisingly, also differ in particle diameter. PoiCV may be seed-transmitted, since several newly bred cultivars contained PoiCV but not PoiMV.

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