Characterization of Dogwood Mosaic Nepovirus from *Cornus florida*

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ABSTRACT

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A virus serologically related to Arabis mosaic virus was isolated from dogwood (*Cornus florida*) growing wild in South Carolina in two areas about 0.4 km apart. The virus infected plants in 10 families but caused only mild symptoms on some diagnostic hosts. The particles had a diameter of 27 nm, and purified preparations sedimented as three components in sucrose density gradients. Density gradient electrophoresis of freshly purified virus produced only one band, whereas virus treated with ethylenediaminetetraacetic acid or stored virus had two bands with electrophoretic mobilities of 5.57 and 6.17 $cm^2 sec^{-1} V^{-1}$. Negative stain penetrated the slower band but not the faster band. Coat protein was predominantly in one band with an estimated molecular mass of 54,100 daltons. After electrophoresis in denaturing gels, RNA separated into two bands: an RNA 1 of 2.9×10^6 daltons and an RNA 2 of 1.4×10^6 daltons. The virus reacted with antisera to four Arabis mosaic virus strains in gel double-diffusion tests and formed spurs with three strains of Arabis mosaic virus and with grape fanleaf virus. Based on epitope similarity indices, this virus was different from Arabis mosaic virus; therefore it was designated dogwood mosaic virus, a new member of the Arabis mosaic virus subgroup of the nepoviruses.

Dogwood, *Cornus florida* L., is native to the United States and is used extensively as an ornamental tree. Several viruses infect dogwood: tobacco ringspot virus (40), cherry leafroll virus (39), tomato ringspot virus (29), cucumber mosaic virus (CMV) (33), and broad bean wilt virus (BBWV) (33). A faint yellow mosaic was observed in the early 1970s on a dogwood tree growing wild near Clemson, SC. The virus isolated from this tree was characterized, found related to but different from Arabis mosaic virus (ArMV), and named dogwood mosaic virus (DMV). This virus is in the ArMV subgroup of the nepoviruses (25).

MATERIALS AND METHODS

The residential area where the initial infection was found was surveyed for virus-infected dogwood trees by inspecting leaves for virus symptoms. Seeds were collected in the fall from dogwood trees with and without symptoms and vernalized in sphagnum moss. Resulting seedlings in the one- to two-leaf stage were indexed to *Chenopodium quinoa* Willd. Seedlings from trees with symptoms were indexed again after further growth, whereas seedlings from trees without symptoms were inoculated with homogenates of DMV-infected tissue of *Nicotiana clevelandii* Gray.

Inoculations were made by rubbing infected tissue (about 1 g/5 ml) ground in 0.03 M sodium phosphate buffer, pH 8, with 0.03 M 2-mercaptoethanol over corundum-dusted leaves. Tissue from dogwood was ground in 2% nicotine (10). Inoculum for host range studies was made from tissue of *N. clevelandii* infected for 12–16 days; four or more plants of each species were inoculated. After a minimum of 3 wk, inoculated and uninoculated leaves were tested for DMV by inoculating *C. quinoa*. Persistence of infectivity in expressed sap was determined as described previously (3) by using tissue of *N. clevelandii*. Each test was replicated three times.

Virions were purified (by modifications of methods in references 18 and 35) from systemically infected leaves and roots of *N. clevelandii* (1 g in 1–1.5 ml) homogenized in 0.05 M sodium phosphate buffer, pH 7, with 0.002 M tetrasodium ethylenediaminetetraacetate (EDTA), 1% 2-mercaptoethanol and antifoam A (Dow Corning, Midland, MI), followed by butanol/chloroform (1/1) clarification, precipitation with 1% NaCl and 10% PEG-8000 (Union Carbide Corporation, Institute, WV), resuspension in 0.05 M sodium phosphate, pH 7, + 0.002 M EDTA, addition of $MgCl_2$ to 0.005 M, differential centrifugation (230,000 *g*, 45 min; 2,200 *g*, 10 min), and separation of components on 10–40% (w/v) sucrose gradients, 81,000 *g* for 5 hr or 43,000 *g* for 16 hr at 4 C (Beckman SW27 rotor). Fractions of the gradients with virions were collected and virions were concentrated by centrifugation. An extinction coefficient of 10 $(mg/ml)^{-1}cm^{-1}$ at 260 nm was used to estimate virion concentrations (26).

DMV (processed through the sucrose gradient step) was dialyzed into 0.02% formaldehyde, then into distilled water before emulsification in Freund's complete adjuvant. A rabbit was given two intramuscular injections, 3 wk apart, followed in 3 wk by weekly bleedings. Antiserum collected in successive weeks had homologous titers of 1:1,024, 1:8,192, and 1:2,048, measured by Ouchterlony gel diffusion tests using 0.5% agarose and 0.05% sodium azide in 0.03 M sodium phosphate buffer, pH 7.

Antisera made against Arabis mosaic virus strains were obtained as follows: type (AB-10 [homologous titer 1:512], Scottish Crop Research Institute [SCRI], Dundee, Scotland), rhubarb (serologically close to type, R. Stace-Smith, Agriculture Canada, Vancouver), hop (RSP 247, M. F. Clark, East Malling Research Institute, Maidstone, England), and ivy (M. F. Clark). Purified, formaldehyde-fixed Arabis mosaic virus strains also were obtained: rhubarb (R. Stace-Smith), hop (RSP 247, M. F. Clark) and red currant (H. L. Paul, Biol. Bundesanstalt, Braunschweig). An isolate of grapevine fanleaf virus (GFV) in a grapevine was obtained from California (A. C. Goheen, Davis) and GFV antiserum was from G. I. Mink (Prosser, WA).

For electron microscopy, partially purified virions were diluted with an equal volume of 0.001% bovine serum albumin, applied to a carbon-coated grid, and stained with 2% ammonium molybdate, pH 7. A carbon grating (21,600 lines/cm) was used for magnification calibration. Virus particles (200) were measured from prints at a final magnification of 163,000.

Density gradient electrophoresis was done in an ISCO Model 211 electrophoresis apparatus (area of column = 0.798 cm^2) with a Model 430 programmed electrophoresis pump. The sucrose gradient (5–10%) was made in 0.03 M sodium phosphate buffer, pH 7 (ionic strength, 0.062); the same buffer was between the sucrose gradient and the anode and in the 25% sucrose solution between the sucrose gradient and the cathode. Sodium phosphate buffers of the same ionic strength but at pH 6 or pH 8 also were used. Conductivity of the pH 6, 7, and 8 buffers was 2.03, 1.76, and 1.67×10^3 mhos, respectively, when measured in ice at 0 C with platinum electrodes. Electrophoresis of the virus was for successive periods of 30 min at 4 mA. The rate of virus movement was estimated by least squares.

Molecular weight of the coat protein was determined by adding virus samples to a dissociation buffer (0.01 M sodium phosphate buffer, pH 7, 1% 2-mercaptoethanol, 1% sodium dodecyl sulfate [SDS]) and heating them for 2 min at 100 C. The samples were placed on 6, 8, or 10% polyacrylamide gels and run at 8 mA per tube until the bromophenol blue neared the tube end (41). The molecular weight standards used were as follows: phosphorylase B (94,000 daltons), bovine serum albumin (67,000 daltons), ovalbumin (43,000 daltons), glyceraldehyde-3-phosphate dehydrogenase (36,000 daltons) or carbonic anhydrase (30,000 daltons), soybean trypsin inhibitor (20,100 daltons), and alpha-lactalbumin (14,400 daltons) or lysozyme (14,300 daltons). Gels were stained with amido schwartz and the bands were measured relative to bromophenol blue.

RNA was extracted either by a modification of the hot SDS-phenol method of Bruening et al (9) or with proteinase K. The phenol method used phenol:*m*-cresol (9:1, v/v) saturated with 0.1 M Tris-Cl, pH 9, and heated at 60 C for 5 min. Alcohol precipitations were with isopropanol rather than ethanol. For proteinase K extraction, virus pellets were resuspended in 50 mM Tris-Cl, pH 7.8, with 0.5% SDS, 0.1% NaN_3 and 40 μg/ml of proteinase K (Sigma Chemical Co.) and incubated at 24 C for 16–20 hr. The RNA either was used immediately, ethanol precipitated and stored at −18 C under ethanol, or freeze-dried.

Nondenaturing, cylindrical gels (8 × 90 mm) contained 0.5% agarose and 1.8% acrylamide (Bio-Rad) (9). After 1 hr of pre-electrophoresis, electrophoresis of the RNA was at 5 mA per gel until the bromophenol blue marker had migrated approximately 85 mm. Gels were immediately scanned at 260 nm. For electrophoresis of RNA under denaturing conditions, freeze-dried samples were resuspended in a denaturing mixture of glyoxal and dimethyl sulfoxide for 1 hr at 50 C (27). Samples were layered immediately on 0.9% agarose gels in 0.01 M sodium phosphate, pH 7, which had been pre-electrophoresed for 15 min at 2 mA/gel. Electrophoresis was for 15 min at 0.5 mA/gel, then at 1–2 mA/gel until the bromophenol blue marker reached the bottom; gels were scanned at 260 nm. Tobacco mosaic virus (TMV) RNA (2.19×10^6 daltons) and *Escherichia coli* ribosomal RNAs (1.009 and 0.534×10^6 daltons) were used as molecular weight standards. TMV RNA was extracted from whole virus (9), and *E. coli* RNA was purchased from Miles Laboratories.

RESULTS

DMV occurrence and symptoms in dogwood. Wild dogwood trees growing on a roadside in a residential area near Clemson, SC, displayed viruslike symptoms. Symptoms varied from a mildly yellowed leaf tip to a light-green/dark-green or faint yellow-green mosaic in the spring (Fig. 1, also see color plate in reference 30) to a severe white/light-green mosaic in the summer (Fig. 2). The first tree on which symptoms were observed was infected with three viruses: DMV, CMV, and BBWV. Other trees from which only DMV was recovered displayed the same symptoms or were symptomless. The original tree had few flowers and produced few seed. Several small trees in the vicinity showed the same symptoms when initially observed in 1972, but these trees all seemed to have originated as adventitious buds from the roots of the larger tree. In 1977, a visual survey of dogwood trees in the residential area was made. A second area with DMV-infected trees was found 0.4 km uphill from the initial area. Twenty dogwood trees in these two areas exhibited leaf symptoms. Ten symptomatic and 10 symptomless dogwood trees were assayed; virus was transmitted from five symptomatic and three symptomless trees. Serology confirmed that these isolates were DMV. Symptomatic dogwood trees and all dogwood trees within a 10-m radius of symptomatic trees were eradicated after the survey. All three viruses in the original tree were graft transmitted to a young dogwood tree that was maintained in the greenhouse to serve as a virus source. DMV also was maintained in dried tissue of *N. clevelandii* and in a living periwinkle (*Catharantus roseus* (L.) Don. = *Vinca rosea* L.) plant.

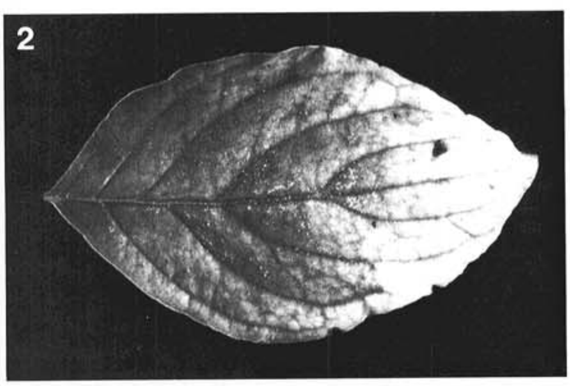
Virus was mechanically transmitted from dogwood to *C. quinoa* during spring and early summer but not in late summer or fall. During winter, the virus could be transmitted from leaves forced from dormant branches.

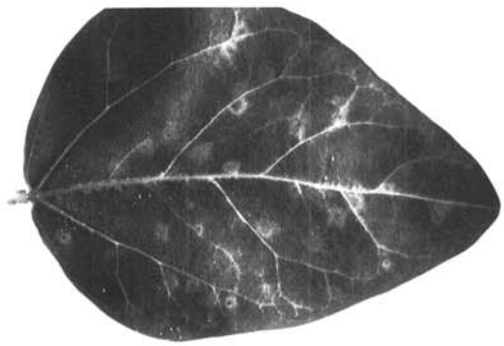
Germination of seed collected from the first dogwood tree found infected with DMV was lower than germination of seed from healthy trees. Plants from the few seed that germinated were weak and died soon after emergence.

Young dogwood seedlings inoculated with DMV were indexed for infection; five of 36 seedlings were infected 2 mo after inoculation. Symptoms were not detected on these five seedlings because they were malformed, presumably due to insufficient chilling hours for the seed.

Host range and symptomatology. Symptoms due to DMV infection developed on the following species after mechanical inoculation ($^+$ = virus not detected by assay of locally or systemically infected tissue as indicated). AIZOACEAE—*Tetragonia expansa* Murr., systemic mosaic. AMARANTHACEAE—*Amaranthus caudatus* L., systemic necrotic streaks. *Celosia argentea* L. var. *cristata* Kuntze., chlorotic local lesions; systemic mosaic$^+$. *Gomphrena globosa* L., local necrotic lesions and necrotic ringspots; systemic mosaic, line patterns, and leaf malformation. APOCYNACEAE—*Catharantus roseus*, systemic mosaic. ASTERACEAE—*Lactuca sativa* L. 'Buttercrunch', systemic mosaic. *Verbesina encelioides* (Cav.) Benth. & Hook. ex Gray, local chlorotic lesions; not systemic^{+}. *Zinnia elegans* Jacq. 'Wild Cherry', systemic chlorotic spots. CHENOPODIACEAE—*Atriplex hortensis* L., systemic mosaic. *Beta patellaris* Moq., systemic mosaic. *B. vulgaris* L. 'Detroit Dark Red', local red ringspots; not systemic^{+}. *Chenopodium amaranticolor* Costa & Reyn., local chlorotic lesions; systemic chlorotic spots, line patterns, mosaic, and malformation of shoot tip (Fig. 3). *C. amaranticolor* seed from Corvallis, OR, and SCRI, Scotland, gave similar symptoms. *C. botrys* L., local necrotic lesions; systemic mosaic and leaf malformation. *C. foetidum* Schrad., local chlorotic lesions; systemic mosaic and necrotic line pattern. *C. murale* L., local chlorotic and necrotic lesions; systemic necrosis and malformation. *C. quinoa*, local chlorotic lesions that turn necrotic; systemic mosaic, shoot tip necrosis and malformation. *Spinacia oleracea* L. 'Bloomsdale', local necrotic lesions; systemic mosaic or latent. CUCURBITACEAE—*Cucumis sativus* L. 'Chicago Pickling' and 'Model', local chlorotic lesions; not systemic^{+}. *C. sativus* 'Lemon' and 'Marketer', local chlorotic lesions^{+}; usually not systemic but occasionally chlorotic vein banding developed along a few veins^{+}. *C. sativus* 'Ashley', local chlorotic and necrotic lesions^{+}; not systemic^{+}. *C. sativus* 'Pixie', local necrotic lesions; not systemic^{+}. FABACEAE—*Dolichos lablab* L., locally latent; systemic mosaic^{+}. *Glycine max* Merr. 'Bragg' and 'Davis', systemic mosaic. *Lupinus albus* L., systemic mosaic and chlorotic ringspots. *L. hirsutus* L., systemic mosaic. *L. luteus* L., systemic mosaic. *Phaseolus vulgaris* L. 'Black Turtle Soup', locally latent; systemic chlorotic spots^{+}. *P. vulgaris* 'Bountiful', local chlorotic lesions; systemic chlorotic spots^{+}. *Pisum sativum* L. 'Alaska', local chlorotic lesions; systemic mosaic. *P. sativum* 'Bonneville', 'Little Marvel', 'Perfected Wales', and 'Ranger', systemic mosaic. *P. sativum* 'Dark Skin Perfection', systemic mosaic, chlorosis, and stunting. *Vicia faba* L. 'Bell Bean' and 'Long Pod', locally latent; systemic mosaic^{+}. *Vigna unguiculata* (L.) Walp. subsp. *unguiculata* 'California Blackeye', local chlorotic ringspots (Fig. 4); systemic mosaic. *V. unguiculata* subsp. *cylindrica* (L.) van Eseltine, systemic mosaic. SCROPHULARIACEAE—*Torenia fournieri* Lind., systemic chlorosis and veinal necrosis. SOLANACEAE—*Datura stramonium* L., local chlorotic lesions; systemic chlorotic spots and mosaic^{+}. *Nicotiana clevelandii*, local chlorotic lesions and ringspots, sometimes with necrotic line patterns; systemic mosaic, ringspots, and necrotic line patterns (Fig. 5). *N. longiflora* Cav., locally latent; systemic mosaic^{+}. *N. occidentalis* Wheeler, local lesions; systemic mosaic. *N. sylvestris* Speg. & Comes, local ringspots; systemic ringspots^{+}. *N. tabacum* L. 'Kentucky-16', local chlorotic lesions; systemic ringspots, necrotic line pattern, and chlorosis. *N. tabacum* 'North Carolina-95' and 'Burley-21', local chlorotic lesions; not systemic^{+}. *N. tabacum* 'Xanthi nc', local necrotic ringspots; not systemic^{+}. *Petunia* × *hybrida* Vilm. 'Bonanza', local necrotic ringspots; systemic necrotic ringspots^{+}.

The following species became infected with DMV by mechanical inoculation without showing symptoms (the species followed by an asterisk became infected only in inoculated leaves): *Antirrhinum majus* L., *Celosia argentea* L. 'Cockscomb', *Lycopersicon esculentum* Mill. 'Marglobe'*, *Nicandra physalodes* (L.)





Figs. 1–5. Symptoms caused by dogwood mosaic virus. **1,** Yellow green/light-green mosaic on leaves of dogwood in spring and early summer. **2,** In summer the yellow areas of the dogwood leaves gradually turn white. **3,** Chlorotic local lesions on lower right leaf of *Chenopodium amaranticolor*. Upper leaves display chlorotic spots, puckering, and leaf malformation; the shoot tip also is malformed. **4,** Primary leaf of *Vigna unguiculata* subsp. *unguiculata* with local chlorotic ringspots. **5,** Leaves from *Nicotiana clevelandii*. Left leaf has local chlorotic ringspots that have changed into necrotic etched ringspots. Leaves on right show systemic mosaic, necrotic line patterns, and ringspots.

Gaertn.*, *Nicotiana megalosiphon* Heurch & Muell., *N. palmeri* Gray*, *Phlox drummondii* Hook., *Physalis alkekengi* L.*, and *Trifolium pratense* L. 'Kenstar'*.

The following species were not infected with DMV by mechanical inoculation: *Anthriscus cerefolium* Hoffm., *Apium graveolens* L. var. *dulce* Pers., *Arabis alpina* L. 'Rockcress' and 'Springcharm', *Brassica juncea* Coss. 'Giant Southern Curled', *B. nigra* Koch, *B. pekinensis* Rupr., *Capsicum annuum* L. 'Carolina Hot' and 'Sweet Banana', *Chenopodium album* L., *Citrullus lunatus* (Thunb.) Matsum. & Nukai var. *lanatus* 'Sugar Baby', *Cucurbita pepo* L. 'Small Sugar', *Desmodium tortuosum* (Sw.) D.C., *Dolichos bifloris* L., *Fragaria vesca* L. 'Baron Solemached' and 'Bush Type Alexandrica', *Lagenaria siceraria* Standl., *Medicago lupulina* L., *M. sativa* L. 'Appalachee' and 'Team', *Mirabilis jalapa* L. 'Petticoat Rose', *Momordica balsamina* L., *Nicotiana tabacum* L. 'X-73', *Phaseolus lunatus* L., *P. vulgaris* L. 'Kentucky Wonder' and 'Pinto UI 111', *Pueraria lobata* (Willd.) Ohwi, *Quamoclit sloteri* Nieuwl (*Ipomoea sloteri* House), *Trifolium pratense* L. 'Kenland', *Zea mays* L. 'Golden Cross Bantam', and *Zinnia elegans* Jacq. 'Redman' and 'Emperor.'

Persistence of infectivity in expressed sap. Crude extracts of DMV were infective for 8, but not 10, days when stored at greenhouse temperature (about 27 C). Virus was infective after being heated for 10 min at 50, but not 60, C and after diluting 10^{-4}, but not after dilution to 10^{-5}.

Purification. *N. benthamiana*, *C. quinoa*, and *G. globosa*, as well as *N. clevelandii*, were tested as propagation hosts. *N. clevelandii* gave the most virions and the least host contaminants with yields of 3–4 mg/100 g of tissue. More virions could be obtained from tissue of *N. clevelandii* 13 days after inoculation than after 7, 10, or 16 days (Fig. 6). Three major density gradient components, top (T), middle (M), and bottom (B), were routinely obtained (Fig. 6). The B component was usually the largest ultraviolet absorbing peak. The T component was usually wide with a suggestion of a double peak. Two peaks below the B component (B_1 and B_2) as well as a trailing shoulder on the M component also occurred routinely. Absorbance profiles from the components are shown in Figure 7; 260/280 and maximum/minimum ratios were 0.88 and 1.25, 1.50 and 1.28, and 1.63 and 1.45 for the T, M, and B components, respectively. Addition of $MgCl_2$ for the first high-speed centrifugation was critical for storage at 4 C. A white pellet resulted after the next low-speed centrifugation, and virus preparations were much more stable than preparations without the magnesium treatment.

Electron microscopy. Most negatively stained virus particles were hexagonal in outline (Fig. 8) with some particles penetrated by stain and others not penetrated. The particles had an average diameter of 27 nm.

Density gradient electrophoresis. Freshly purified virus preparations electrophoresed at pH 7 (Fig. 9) gave a single slowly migrating component (3.34–5.71 $cm^2 sec^{-1} V^{-1}$). After EDTA

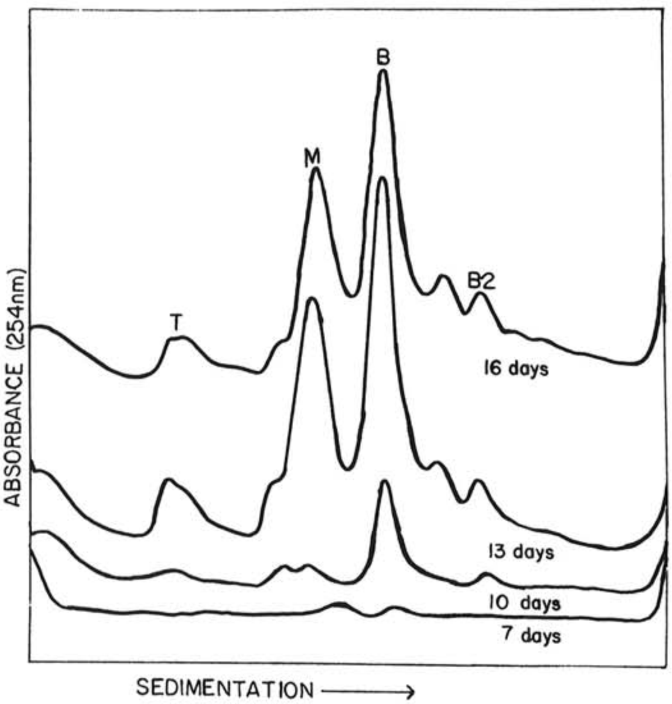


Fig. 6. Absorbance profiles in 10–40% sucrose gradients of dogwood mosaic virus preparations harvested 7, 10, 13, and 16 days after inoculation. Maximum yield was from the 13-day tissue. Components top (T), middle (M), and bottom (B), and heavier components (B2).

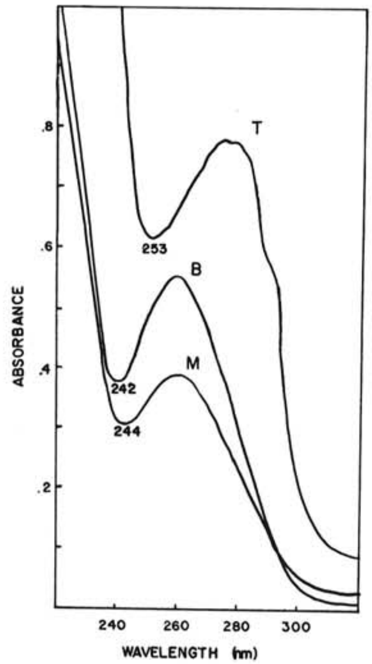


Fig. 7. Absorbance of top (T), middle (M), and bottom (B) components after separation by density gradient centrifugation. Absorption minimum denoted by wavelength value.

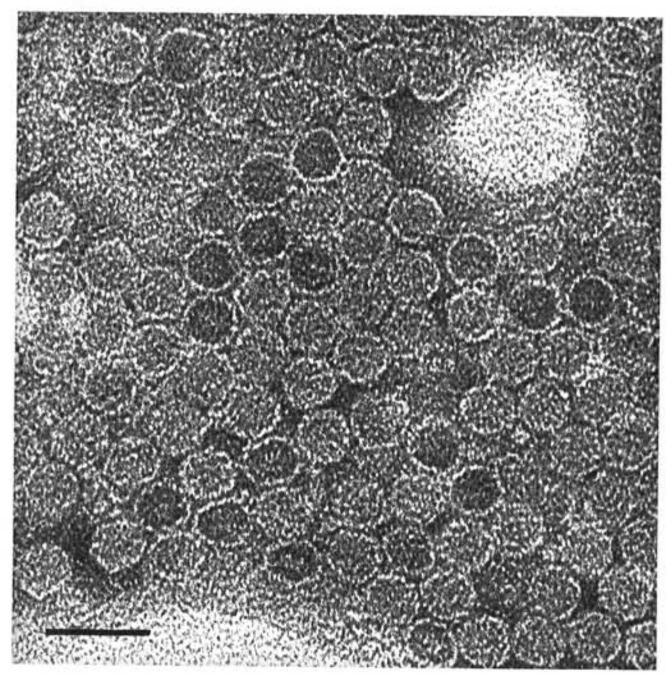


Fig. 8. Unfractionated dogwood mosaic virus particles stained with 2% ammonium molybdate, pH 7. Line represents 50 nm.

treatment or storage at 4 C for 3–4 wk, two electrophoretic components were observed (5.57 and 6.17 $cm^2 sec^{-1} V^{-1}$). Most particles in the slower moving component (5.57 $cm^2 sec^{-1} V^{-1}$) were penetrated by negative stain (uranyl acetate), whereas most particles in the 6.17 $cm^2 sec^{-1} V^{-1}$ component were not. Both components also occurred after electrophoresis at pH 6 or 8. A fast migrating component (electrophoretic mobility = 10.76 to 14.53 $cm^2 sec^{-1} V^{-1}$) was present in many preparations (bottom profile of Fig. 9); this component was composed of either host material or virus degradation products.

Protein molecular weight. Based on 13 estimations from five different virus preparations, all on 10% polyacrylamide gels, molecular mass (Mr) of the coat protein was estimated to be 52,400 daltons ($\log_e Mr = 10.867$; standard error (SE) = ± 0.013). One or two faint bands of lower molecular mass also were seen in some gels. All protein standards except phosphorylase B converged at the same relative mobility ($\log_e\% R_f = 5.03–5.08$; SE = ± 0.11 each) at zero gel concentration (Fig. 10). Phosphorylase B converged at $\log_e\% R_f = 5.53$ (SE = ± 0.11), which was larger than the other standards. DMV coat protein extrapolated to a point ($\log_e\% R_f = 5.17$; SE = ± 0.11) that was not detectably different from any of the standards. With the phosphorylase B standard omitted and the results from all gel percents combined, the estimated Mr for DMV coat protein was 54,100 daltons (SE = ± 1,850), adjusted to zero gel percent.

RNA composition. Virions purified from DMV isolates derived from single chlorotic lesions on cucumber contained RNA molecules of two sizes. Middle virion component had RNA 2 of 1.3×10^6 daltons in nondenaturing gels and 1.38×10^6 daltons in glyoxal denaturing gels. Bottom virion component had two RNA molecules: RNA 1 of 2.4×10^6 in nondenaturing gels and 2.9×10^6 daltons in denaturing gels, and RNA 2 of 1.3×10^6 daltons in nondenaturing gels and 1.38×10^6 in denaturing gels. RNA 2 from middle and bottom components gave a single peak when electrophoresed together in the same nondenaturing gel.

Serology. DMV reacted with ArMV antiserum (type, AB-10) but did not react with antisera to alfalfa mosaic; cucumber mosaic (H. Scott, University of Arkansas); southern bean mosaic; tobacco ringspot; tomato ringspot (J. P. Fulton, University of Arkansas); broad bean wilt I or II (J. K. Uyemoto, Davis); tobacco streak, rose mosaic, and prunus necrotic ringspot-G (R. W. Fulton, University of Wisconsin); peanut stunt-T (H. E. Waterworth, Beltsville); cowpea chlorotic mottle (C. W. Kuhn, University of Georgia); cowpea mosaic-Sb strain; raspberry bushy dwarf, sowbane mosaic, strawberry latent ringspot, and raspberry ringspot (SCRI); cherry leafroll-dogwood isolate (H. E. Waterworth); and artichoke Italian latent and grapevine chrome mosaic (G. P. Martelli, Bari, Italy) viruses.

Microprecipitin titers of six antisera with four virus isolates are given in Table 1. These titers were in agreement with the analysis of Ouchterlony gel diffusion results partially illustrated in Figure 11 and tabulated in Table 2. Presence of a spur in an Ouchterlony gel means that the antiserum contains paratopes against an epitope that is present on one but not on the other member of a pair of antigens in adjacent wells (37). This allows assignment of the presence or absence of epitopes to virus isolates (Table 3) by logically determining minimum numbers of epitopes

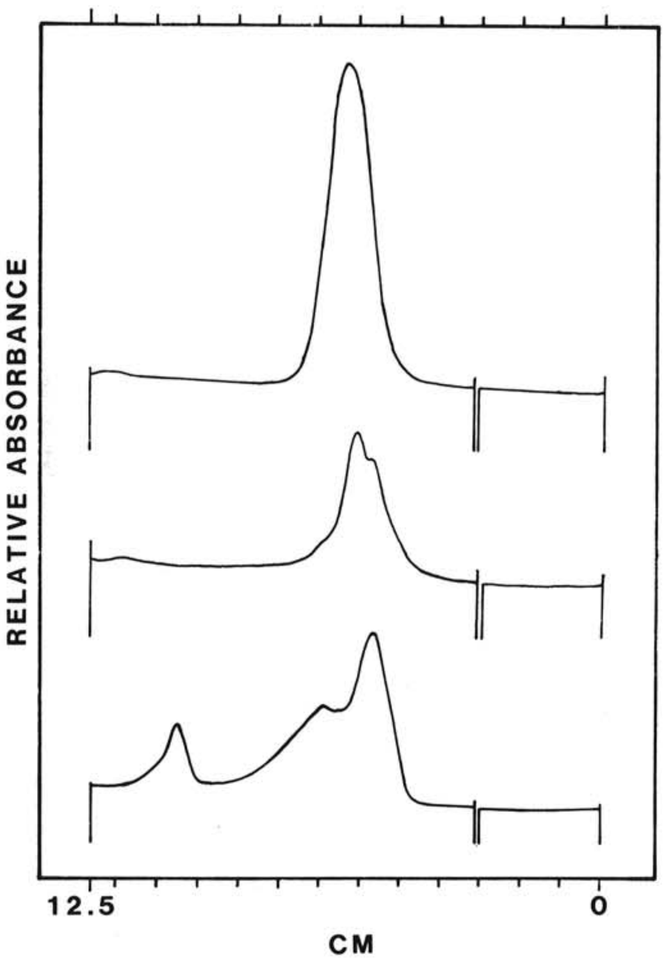


Fig. 9. Absorbance profiles after sucrose density gradient electrophoresis at pH 7, top to bottom: fresh preparation of dogwood mosaic virus (6.5 hr electrophoresis); after treatment in 0.001 M ethylenediaminetetraacetic acid (6 hr); and after storage at 4 C for 4.5 wk (6.5 hr).

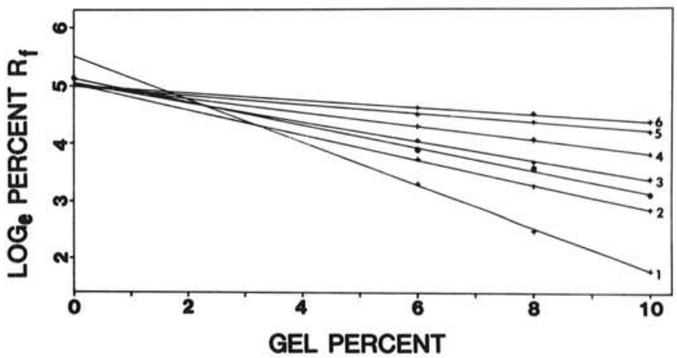


Fig. 10. Plot of $\log_e$ percent relative mobility (R_f) vs. gel percent after polyacrylamide gel electrophoresis of dogwood mosaic virus capsid protein (●) and standards (+) on 6, 8, and 10% gels. 1 = phosphorylase B (94,000 daltons), 2 = bovine serum albumin (67,000 daltons), 3 = ovalbumin (43,000 daltons), 4 = carbonic anhydrase (30,000 daltons), 5 = soybean trypsin inhibitor (20,100 daltons), 6 = alpha-lactalbumin (14,400 daltons).

TABLE 1. Arabis mosaic virus subgroup antisera titers against four virus isolates

Antisera[a] against virus isolate	Virus isolates			
	Dogwood	Rhubarb	Hop-247	Fanleaf
Dogwood	1/1024[b]	1/8	1/8	NR[c]
Rhubarb	1/128	1/256	1/128	1/8
Hop-247	1/4	1/32	1/128	1/2
Fanleaf	NR	NR	NR	1/64
Type	1/64	1/128	1/256	1/4
Ivy	1/16	1/256	1/256	1/8

[a]Reactions were carried out on microscope slides in 0.5% agarose in 0.03 M phosphate, pH 7, buffer with 0.05% NaN_3. Two concentrations of rhubarb (undiluted and 1:5) and dogwood (undiluted [3 mg/ml] and 1:5) isolates were tested against a twofold dilution series of each serum; only one concentration of fanleaf (1.3 mg/ml) and hop-247 was used. Reactions were read after 24 hr and the greatest dilution to give a reaction was recorded. Because rhubarb and hop-247 isolates were obtained in very small volumes, their concentrations were not measured. Because concentrations of the virus strains are not the same, comparison of serum titer between viruses may not be valid.
[b]Greatest dilution that gave visible reaction.
[c]NR = no reaction.

that satisfy spur patterns (presence or absence of single or double spurs) for each serum. Lack of double spurs was used in several instances when information was otherwise not available to determine if an epitope was present on an antigen in a particular serum-antigen combination. Similar epitope patterns in different antisera were assumed to be reflective of the same epitope and were combined to give the minimal epitope content for each virus. Information was not available to determine if epitope 'g' occurred in the hop isolate; however, when the paratope compositions of the antisera were combined (Table 3), it was found that hop antiserum did not contain paratopes against the 'g' epitopes. From this it was inferred that epitope 'g' was not present in the hop isolate. From the data in Table 2, we could not determine if DMV antiserum contains 'B' or 'D' paratopes or both. Counts of epitopes present in each isolate and of epitopes common to each pair of isolates were used to calculate percent similarities for each pair of isolates (Table 4), which allows hierarchical clustering (4) with formation of the corresponding dendrogram (Fig. 12). Although all five of these ArMV subgroup isolates are serologically related, analysis of identifiable epitopes revealed that the DMV isolate entered the hierarchical cluster further from the ArMV isolates (rh, rc, hp) than GFV.

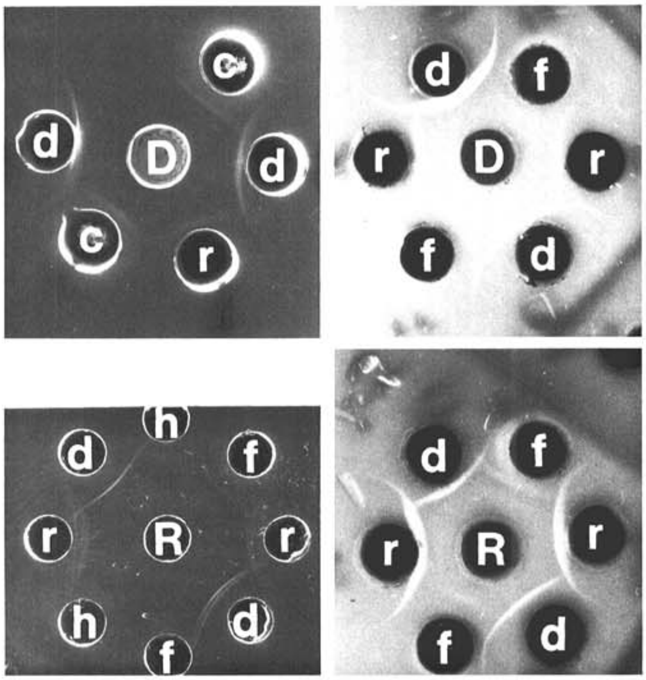


Fig. 11. Gel double diffusion serology. Antiserum in center wells of upper patterns: D = dogwood mosaic virus: left undiluted, right diluted 1:2; R = rhubarb strain of Arabis mosaic virus: left undiluted, right diluted 1:2. Virus antigens in outside wells: r = rhubarb isolate, c = red currant isolate, h = hop isolate of Arabis mosaic virus, d = dogwood mosaic virus, and f = grapevine fanleaf virus.

TABLE 2. Serological reactions among Arabis mosaic virus subgroup isolates in Ouchterlony tests[a]

Pairs of virus isolates[b]	Antisera to virus isolate[c] -Dogwood	-Rhubarb	-Hop	-Type	-Ivy
dw:rh	ov	un	un	un	un
dw:hp	•	ds	un	ov	un
dw:fl	ov	ov	ov	ov	un
dw:rc	ov	un	un	un	un
rh:hp	•	ov	ns	ov	ns
rh:fl	ov	ov	nc	ov	ov
rh:rc	ns	ov	•	•	•
hp:fl	•	ov	ov	ov	ov

[a]Grapevine fanleaf virus was the only isolate that reacted with fanleaf serum in these tests. The hop-247:red currant and fanleaf:red currant pairs were not tested.
[b]Abbreviations of virus isolates: dw = dogwood; rh = rhubarb; hp = hop-247; fl = fanleaf; rc = red currant.
[c]Abbreviations for reactions: ov indicates that reaction of antigen on left spurs over that of antigen on right of a pair; un indicates that reaction of antigen on left is under the spur of that of antigen on right of a pair; ns = no spur but confluent reaction lines; ds = double spur; nc = reactions not confluent; • = test not done.

DISCUSSION

The ArMV subgroup of nepoviruses contains several strains of ArMV and GFV (25). Historically, GFV has been recognized as being different from ArMV and is listed as a separate virus by the International Committee on Taxonomy of Viruses (23). Based on the presence of epitopes, GFV was more like ArMV than was DMV. This determination justifies distinction of this isolate as a new virus, which we named DMV. Previous references to DMV as an ArMV strain were made before the critical analysis of epitopes presented here (1,2,15).

Other ArMV isolates that form spurs in Ouchterlony serological tests have been reported—for example, cucumber stunt mottle and raspberry yellow dwarf isolates (19), hop and strawberry isolates (7), strawberry, hop, ivy, and woodland isolates (14), and

TABLE 3. Minimal epitope content of viruses and corresponding paratope composition of antisera inferred from Ouchterlony serological reactions

Virus isolates[a]	Epitopes[b] a	b	c	d	e	f	g	h	i
dw	+	+	−	+	−	−	+	−	−
rh	+	+	+	+	+	+	−	+	−
rc	+	+	+	+	+	+	−	−	−
hp	+	+	+	−	+	−	−	−	−
fl	+	−	+	−	−	−	−	−	+
	Paratopes[b,c]								
Antisera	A	B	C	D	E	F	G	H	I
dw	*	?*	−	?*	−	−	*	−	−
rh	*	−	−	*	*	−	−	*	−
hp	*	*	−	−	*	−	−	−	−
ty	*	*	−	*	−	*	−	−	−
iv	*	−	*	−	*	−	−	−	−
fl	*	−	−	−	−	−	−	−	*

[a]Abbreviations for virus isolates and antisera: dw = dogwood; rh = rhubarb; hp = hop-247; fl = fanleaf; rc = red currant; iv = ivy; ty = type AB-10.
[b]+ or * indicates presence and − indicates absence of the epitope or paratope.
[c]?* indicates the presence of either the B or D paratope in dogwood antiserum.

TABLE 4. Similarities among virus isolates based on presence of epitopes

	rh[a]	rc	hp	dw	fl
rh[a]	(7)[b] 100[c]	80.0	57.1	28.6	25.0
rc	(6)	(6) 100	75.0	50.0	44.4
hp	(4)	(4)	(4) 100	50.0	57.1
dw	(3)	(3)	(2)	(4) 100.0	28.6
fl	(2)	(2)	(2)	(1)	(3) 100

[a]Abbreviations for virus isolates: rh = rhubarb; rc = red currant; hp = hop-247; dw = dogwood; fl = fanleaf.
[b]Numbers in parentheses are counts of epitopes (Table 3) common to isolates in each pairing.
[c]Percent similarities (100 − % dissimilarity), above the diagonal, are derived from epitope counts. For nine possible epitopes,

$$\% \text{ dissimilarity} = 100\,(a + b - 2c)/(18 - a - b) \text{ if } (a + b) > 9$$
$$= 100\,(a + b - 2c)/(a + b) \text{ if } (a + b) < 9$$

where a is the count of epitopes in the first member of the pair, b is the count of epitopes in the second member of the pair, and c is the count of epitopes common to the pair.

red currant and type isolates (6); therefore, other isolates possibly should be listed as viruses in this subgroup.

ArMV has a wide experimental host range (24). Most isolates have similar host ranges but symptom intensity varies considerably among isolates (10,11). Systemic infection of *Petunia hybrida* is considered characteristic of ArMV and can be used to differentiate ArMV from GFV (12). Cadman et al (12) used severe and mild symptoms on petunia to divide several isolates of ArMV into two groups. Symptom severity on *Cucumis sativus* and *Nicotiana tabacum* also varies with ArMV isolates. For instance, the Cambridge isolate (34), raspberry yellow dwarf isolate (16), German strawberry isolate (21), Forsythia, Ligustrum, and Laburnum isolates (31,32), cucumber stunt mottle isolate (19), lettuce chlorotic stunt isolate (38), narcissus isolate (20), and the lily isolate (22) caused rather strong symptoms in *C. sativus* and/or *N. tabacum* and generally caused symptoms in *P. hybrida*. Isolates of ArMV from rhubarb (36) and red currant (6) either did not infect *N. tabacum* or did so without symptoms. A hop isolate (7) failed to infect common indicator hosts except *Chenopodium amaranticolor* and *C. quinoa*. ArMV isolates from gladiolus form two groups: one with a wide host range like ArMV-type, and another with a narrow host range similar to hop isolates. Gladiolus isolates with narrow host ranges contained a fourth nucleoprotein component, whereas many gladiolus plants infected with the wide host range isolates also were infected with bean yellow mosaic virus (5). The host range of DMV is distinctly different from that of ArMV-type isolate but is similar to that of the red currant isolate; both DMV and ArMV-red currant cause local lesions on *C. sativus*, infect *P. hybrida* with difficulty, and infect *N. tabacum* with difficulty or not at all.

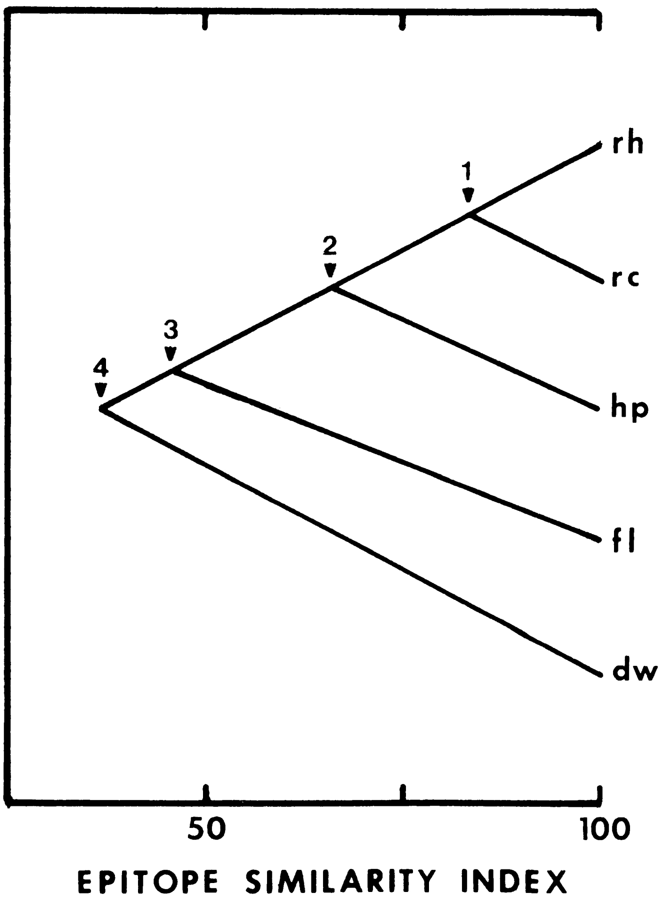


Fig. 12. Dendrogram of similarities among the five Arabis mosaic virus subgroup isolates, using percent similarities of isolate pairs derived from Ouchterlony reactions. Arrows denote points of isolate divergence. The corresponding similarity index values are read from the horizontal axis as follows: 1) 80, 2) 66, 3) 46, and 4) 37. rh = rhubarb isolate; rc = red currant isolate; hp = hop-247 isolate of Arabis mosaic virus; fl = grapevine fanleaf virus; and dw = dogwood mosaic virus.

Persistence of DMV infectivity in expressed sap was similar to that of the red currant isolate (6), other ArMV isolates (24), and other nepoviruses (17). Particle size, presence of T, M, and B components, molecular weight of the capsid protein, and serology place DMV in the ArMV subgroup of the nepoviruses (17,24,25,28). DMV was not transmitted by *Xiphinema diversicaudatum* Thorne from a Scottish population (D. J. F. Brown, *unpublished*). However, this result is inconclusive because ArMV-type strain was transmitted with different efficiencies by populations of *X. diversicaudatum* from different geographical areas (8).

The electrophoretic virus components found in DMV are similar to those found in ArMV by Clark (13). However, the two electrophoretic components of DMV were not detected in freshly purified preparations but occurred only after exposure to EDTA or after the preparations were aged for several weeks. The results of Quacquarelli et al (28) show that heat treatment either can change ArMV M and B components to T component by removing RNA from intact capsids or can degrade the capsid into RNA and structural units. These phenomena also might occur in other environments or during storage, which would explain the different component ratios in various purified preparations.

LITERATURE CITED

1. Barnett, O. W. 1981. Arabis mosaic virus from *Cornus florida* in South Carolina. Page 237 in: Fifth International Congress of Virology, Abstracts. Strasbourg, France.
2. Barnett, O. W., and Baxter, L. W., Jr. 1976. Arabis mosaic virus from a wild dogwood in South Carolina. (Abstr.) Proc. Am. Phytopathol. Soc. 3:249.
3. Barnett, O. W., and Gibson, P. B. 1975. Identification and prevalence of white clover viruses and the resistance of *Trifolium* species to these viruses. Crop Sci. 15:32-37.
4. Barnett, O. W., Randles, J. W., and Burrows, P. M. 1987. Relationships among Australian and North American isolates of the bean yellow mosaic potyvirus subgroup. Phytopathology 77:791-799.
5. Bellard, M. G., Canova, A., and Gelli, C. 1986. Comparative studies on gladiolus isolates of Arabis mosaic virus (ArMV). Phytopathol. Mediterr. 25:85-91.
6. Bercks, R., Krczal, H., and Querfurth, G. 1976. Untersuchungen uber einen aus Roter Johannisbeere (*Ribes rubrum*) isolierten Stamm des Arabis mosaic virus. Phytopathol. Z. 85:139-148.
7. Bock, K. R. 1966. Arabis mosaic and Prunus necrotic ringspot viruses in hop (*Humulus lupulus* L.). Ann. Appl. Biol. 57:131-140.
8. Brown, D. J. F. 1986. The transmission of two strains of Arabis mosaic virus from England by populations of *Xiphinema diversicaudatum* (Nematoda: Dorylaimoidea) from ten countries. Rev. Nematol. 9:83-87.
9. Bruening, G., Beachy, R. N., Scalla, R., and Zaitlin, M. 1976. In vitro and in vivo translation of the ribonucleic acids of a cowpea strain of tobacco mosaic virus. Virology 71:498-517.
10. Cadman, C. H. 1959. Some properties of an inhibitor of virus infection from leaves of raspberry. J. Gen. Microbiol. 20:113-128.
11. Cadman, C. H. 1960. Studies on the relationship between soil-borne viruses of the ringspot type occurring in Britain and continental Europe. Virology 11:653-664.
12. Cadman, C. H., Dias, H. F., and Harrison, B. D. 1960. Sap-transmissible viruses associated with diseases of grape vines in Europe and North America. Nature 187:577-579.
13. Clark, M. F. 1976. Electrophoretic heterogeneity of the sedimenting components of Arabis mosaic virus. J. Gen. Virol. 32:331-335.
14. Clark, M. F., Coles, C. L., and Hassam, D. 1976. Arabis mosaic virus. Pages 124-125 in: East Malling Research Station Report for 1975. East Malling Research Station, Maidstone, England.
15. Handley, M. K., and Barnett, O. W., Jr. 1981. Purification and partial characterization of a South Carolina isolate of Arabis mosaic virus. (Abstr.) Phytopathology 71:223.
16. Harrison, B. D. 1958. Raspberry yellow dwarf, a soil-borne virus. Ann. Appl. Biol. 46:221-229.
17. Harrison, B. D., and Murant, A. F. 1977. Nepovirus group. No. 185 in: Descriptions of plant viruses. Commonw. Mycol. Inst., Assoc. Appl. Biol., Kew, Surrey, England. 4 pp.
18. Harrison, B. D., and Nixon, H. L. 1960. Purification and electron microscopy of three soil-borne plant viruses. Virology 12:104-117.
19. Hollings, M. 1963. Cucumber stunt mottle, a disease caused by a strain of Arabis mosaic virus. J. Hortic. Sci. 38:138-149.

20. Iwaki, M., and Komuro, Y. 1974. Viruses isolated from Narcissus (*Narcissus* spp.) in Japan. V. Arabis mosaic virus. Ann. Phytopathol. Soc. Jpn. 40:344-353.
21. Lister, R. M., and Krczal, H. 1963. Uber das Auftreten des Arabis-Mosaicks bei der Erdbeere in Deutschland. Phytopathol. Z. 45:190-199.
22. Marani, F., and Faccioli, G. 1974. Caratteristiche di un ceppo del virus del mosaico dell' Arabis, isolato da Lilium tigrinum Ker-Grawl. Phytopathol. Mediterr. 13:101-107.
23. Matthews, R. E. F. 1982. Classification and nomenclature of viruses. Report of the International Committee on Taxonomy of Viruses. Intervirology 17:163-165.
24. Murant, A. F. 1970. Arabis mosaic virus. No. 16 in: Descriptions of Plant Viruses. Commonw. Mycol. Inst., Assoc. Appl. Biol., Kew, Surrey, England. 4 pp.
25. Murant, A. F. 1981. Nepoviruses. Pages 197-238 in: Handbook of Plant Virus Infections, Comparative Diagnosis. E. Kurstak, ed. Elsevier/North Holland Biomedical Press, Amsterdam.
26. Murant, A. F., Mayo, M. A., Harrison, B. D., and Goold, R. A. 1972. Properties of virus and RNA components of raspberry ringspot virus. J. Gen. Virol. 16:327-338.
27. Murant, A. F., Taylor, M., Duncan, G. H., and Raschke, J. H. 1981. Improved estimates of molecular weight of plant virus RNA by agarose gel electrophoresis and electron microscopy after denaturation with glyoxal. J. Gen. Virol. 53:321-332.
28. Quacquarelli, A., Gallitelli, D., Savino, V., and Martelli, G. P. 1976. Properties of grapevine fanleaf virus. J. Gen. Virol. 32:349-360.
29. Reddick, B. B., Barnett, O. W., and Baxter, L. W., Jr. 1979. Isolation of cherry leafroll, tobacco ringspot, and tomato ringspot viruses from dogwood trees in South Carolina. Plant Dis. Rep. 63:529-532.
30. Reddick, B., Barnett, O. W., and Baxter, Luther W., Jr. 1980. Viruses of dogwoods: Symptomology, isolation, and damage. Ornamentals South 2:4-7.
31. Schmelzer, K. 1962. Untersuchungen an Viren der Zier- und Wildgeholze. 2. Mitteilung: Virosen an Forsythia, Lonicera, Ligustrum, und Laburnum. Phytopathol. Z. 46:105-138.
32. Schmelzer, K. 1963. Untersuchungen an Viren der Zier- und Wildgeholze: Verserche zur Differenzierung und Identifizierung der Ringfleckenviren. Phytopathol. Z. 46:315-342.
33. Scott, S. W., and Barnett, O. W. 1984. Some properties of an isolate of broad bean wilt virus from dogwood (*Cornus florida*). Plant Dis. 68:983-985.
34. Smith, K. M., and Markham, R. 1944. Two new viruses affecting tobacco and other plants. Phytopathology 34:324-329.
35. Steere, R. L. 1956. Purification and properties of tobacco ringspot virus. Phytopathology 46:60-69.
36. Tomlinson, J. A., and Walkey, D. G. A. 1967. The isolation and identification of rhubarb viruses occurring in Britain. Ann. Appl. Biol. 59:415-427.
37. van Regenmortel, M. H. V. 1982. Page 93 in: Serology and Immunochemistry of Plant Viruses. Academic Press, New York.
38. Walkey, D. G. A. 1967. Chlorotic stunt of lettuce caused by Arabis mosaic virus. Plant Pathol. 16:20-22.
39. Waterworth, H. E., and Lawson, R. H. 1973. Purification, electron microscopy, and serology of the dogwood ringspot strain of cherry leafroll virus. Phytopathology 63:141-146.
40. Waterworth, H. E., and Povish, W. R. 1972. Tobacco ringspot virus from naturally infected dogwood, autumn crocus, and Forsythia. Plant Dis. Rep. 56:336-337.
41. Weber, K., and Osborn, M. 1969. The reliability of molecular weight determination by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. 244:4406-4412.