

## Double-stranded Ribonucleic Acid from Plants Infected with Viruses Having Elongated Particles and Undivided Genomes

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### ABSTRACT

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Double-stranded RNA (dsRNA) of several members of the poty-, carla-, potex-, and tobamoviruses was purified from 7–30 g of infected tissue by chromatography on CF-11 cellulose. Gel electrophoresis of dsRNA aliquots equivalent to one-tenth of the extracted dsRNA (approximately 0.5  $\mu$ g) normally resulted in detectable dsRNA bands. Lowest yield of dsRNA was from plants infected with some potyviruses. The size and number of the expected dsRNAs corresponding to the encapsidated single-stranded RNA genomes (the replicative forms) were diagnostic for each virus group analyzed. Other dsRNAs of smaller size (greater mobility) than the replicative form were consistently obtained with most viruses, and were well resolved by electrophoresis on 6% polyacrylamide gels. The presence or absence of additional dsRNAs, together with their size, were useful

characteristics for differentiating particular viruses, including strains. Use of appropriate marker dsRNAs is important if results are to be compared and interpreted. Infected plants incubated at 27 C and harvested 10 days postinoculation gave the highest dsRNA yields. Regardless of the host used for virus propagation, dsRNAs were obtained, and no effect of host was observed on dsRNA patterns for each virus tested. Unexpected dsRNAs were found in 6 of 24 uninoculated and apparently healthy plant species and cultivars. These dsRNAs had molecular weights similar to dsRNAs of plant viruses with single-stranded RNA genomes, and indicate infection by cryptic or latent viruslike agents. Their erratic distribution indicates the need for a careful choice of plant species to be used as disease-free controls.

The presence of high-molecular-weight, double-stranded ribonucleic acid (dsRNA) in extracts from plants infected with RNA viruses is well established (8,9). The isolation and description of dsRNAs from plants and fungi has been used to detect infections caused by characterized and uncharacterized viruses and viruslike agents (9,16,22). Simplification of extraction procedures, and the requirement for relatively low amounts of tissue, have led to the proposed use of dsRNA analysis as a diagnostic technique (2,8,9,22). Stained bands detected after gel electrophoresis of dsRNAs contain replicative form (RF) dsRNAs that correspond in size to twice that of the encapsidated single-stranded genomic RNAs (ssRNA), and frequently additional dsRNAs. The origin of the latter is obscure, but their presence in extracts from infected plants enhances the diagnostic value of this technique (8,9).

This study was initiated with the intention of taking a more systematic approach than in previous studies to the isolation, description, and evaluation of the dsRNAs that accumulate in plants infected with 20 viruses representing five well-characterized virus groups. The choice was restricted to elongated viruses with undivided (monopartite), positive sense, ssRNA genomes. This selection has been a first choice when new taxonomic approaches have been evaluated (5). This study is seen as a further step toward accomplishing a larger task, which is to describe and compare the dsRNAs of members of plant virus groups for which this approach should be valuable.

Elongated plant viruses with monopartite ssRNA genomes are placed in five main groups. Viruses in the closterovirus group have particles of variable length, 700–2,000 nm, which encapsidate ssRNA with molecular weights (MW) that range 2.3–6.5  $\times 10^6$  (1). An expected dsRNA with an MW twice that of the ssRNA genome and several additional dsRNAs of lower MW with distinct electrophoretic mobilities were detected for four closteroviruses

(8). Beet yellows virus and carnation necrotic fleck virus, which have particles of similar length, had very similar dsRNAs.

Most viruses of the potyvirus group have particles with lengths of 720–770 nm, which encapsidate ssRNA with MW = 2.7–3.5  $\times 10^6$  (15). Viruses of the carlavirus group have particles with lengths of 610–700 nm, which encapsidate ssRNA with MW = 2.3–3.0  $\times 10^6$  (19). Viruses of the potexvirus group have particles with lengths of 470–580 nm, which encapsidate ssRNA with MW = 2.0  $\times 10^6$  (18). No detailed description of dsRNAs of these groups has been published. The only report is that of the dsRNA of potato virus M (a carlavirus), which had an estimated MW of 5.0  $\times 10^6$  (24).

Viruses of the tobamovirus group have particles with lengths of approximately 300 nm, which encapsidate ssRNA with MW = 2.0  $\times 10^6$  (12). An expected dsRNA twice the size of the ssRNA genome, and three additional dsRNAs (MW = 2.1, 1.9, and 0.4  $\times 10^6$ ) have been detected in extracts from plants infected with the type strain of tobacco mosaic virus (TMV) (7,23,26). Additional dsRNAs, present in lesser amounts, have also been reported for this strain (7).

The objective of this investigation was to describe and compare the dsRNAs isolated from plants infected with one member of the closterovirus group, and with several members of the poty-, carla-, potex-, and tobamovirus groups, and to evaluate their diagnostic potential. The effect of host, temperature, and age of infection on the recovery and quality of dsRNA was also examined.

### MATERIALS AND METHODS

**Viruses.** Several members of the poty-, carla-, potex-, and tobamovirus groups were examined. Viruses and hosts in which they were propagated were: potyviruses—potato virus Y (PVY) (North Carolina isolate [NC-57]), tobacco etch virus (TEV) (California isolate), pepper mottle virus (PeMV) (Arizona isolate) in *Nicotiana tabacum* L. 'Xanthi-nc,' turnip mosaic virus (TuMV) in *N. benthamiana* Domin., soybean mosaic virus (SMV) (Arkansas isolate) in *Glycine max* (L.) Merr. 'Harosoy,' and dasheen mosaic virus (DMV) (California isolate) in *Philodendron selloum* C. Koch; carlaviruses—red clover vein mosaic virus (RCVMV) (American Type Culture Collection [ATCC], PV-110),

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pea streak virus (PSV) (Arizona isolate) in *Pisum sativum* L. 'Progress No. 9,' dandelion latent virus (DLV), potato virus S (PVS) (ATCC, PV-103) and kalanchöe latent virus (KLV) (isolate-1) in *Chenopodium quinoa* Willd., and eggplant mild mottle virus (EMMV) in *N. tabacum* 'Xanthi-nc'; potexviruses—potato virus X (PVX) (ATCC, PV-197) in *N. glutinosa* L., cactus virus X (CVX) (California isolate) in *C. quinoa*, clover yellow mosaic virus (CYMV) (ATCC, PV-200) in *C. amaranticolor* Coste & Reyn., and white clover mosaic virus (WCIMV) (ATCC, PV-190) in *P. sativum* 'Progress No. 9'; tobamoviruses—tobacco mosaic virus-type (TMV-type) (ATCC, PV-135), four strains of tomato mosaic virus (ToMV) (grey wall, fruit necrosis, purple, and white), TMV-U5 (California isolate) in *N. tabacum* 'Turkish,' cowpea strain of TMV (CPTMV) in *Vigna unguiculata* (L.) Walp. subsp. *unguiculata* 'Blackeye,' and odontoglossum ringspot virus (ORSV) (ATCC, PV-274) in *N. benthamiana*. Other viruses used were: a tymovirus—turnip yellow mosaic virus (TYMV) in *Brassica campestris* L. var. *chinensis* 'Chinese' and *B. campestris* 'Tendergreen'; a closterovirus—citrus tristeza virus (CTV), in *Citrus aurantifolia* (Christm.) Swingle (Mexican lime); a cucumovirus—cucumber mosaic virus (CMV), in *N. tabacum* 'Turkish'; and tobacco necrosis virus (TNV) in *N. tabacum* 'Xanthi-nc.' All viruses were identified and periodically checked to assure that the isolates were not contaminated with other isolates by biological assays to indicator hosts, examination of particle morphology after negative staining, and gel diffusion serological tests with specific antisera.

**DsRNA extraction and electrophoresis.** DsRNAs in STE (0.1 M NaCl, 0.05 M tris-HCl, 1.0 mM EDTA, pH 7.0), were purified from phenol-treated, buffered extracts from 7 to 30 g of leaf tissue by two cycles of fractionation on columns of Whatman CF-11 cellulose (2.5 g dry weight) in the presence of 16% ethanol (16,21). DsRNAs eluted from cellulose in ethanol-free STE were concentrated by precipitation with two volumes of cold 95% ethanol and 0.1 volume of 2.0 M sodium acetate, pH 5.5, and resuspended in 300  $\mu$ l of electrophoresis buffer. Aliquots of 30  $\mu$ l (approximately 0.5  $\mu$ g) were loaded onto individual channels of 6% polyacrylamide gels (1.5 mm  $\times$  7 cm  $\times$  8 cm, 40:1 acrylamide, bisacrylamide) in a vertical slab gel apparatus, or onto 1.2% agarose gels (5 mm  $\times$  5 cm  $\times$  7.5 cm) in a horizontal slab gel apparatus in 40 mM tris, 20 mM sodium acetate, 1 mM EDTA, pH 7.8. Electrophoresis was at constant voltage, 100 V for 3–4 hr (polyacrylamide gels) or 75 V for 1 hr (agarose gels). Gels were stained in ethidium bromide, 20 ng/ml. Some dsRNA samples were treated with DNase and RNase prior to electrophoresis by following procedures described by Jordan et al (16). Reference dsRNAs with MWs in parentheses (6–8,17) were

from plants infected with CTV (MW =  $13.3 \times 10^6$ ), TMV (MWs = 4.3, 2.1, 0.95, and  $0.42 \times 10^6$ ), TNV (MWs = 2.6, 1.05, and  $0.94 \times 10^6$ ), and CMV (MWs = 2.1, 2.0, 1.4, and  $0.6 \times 10^6$ ). New MWs were estimated by the graphical method of Bozarth and Harley (4), and were used to calculate MWs of unknowns.

**Effect of host and age of infection.** Selected members of three virus groups were inoculated to different plant species and cultivars. Pepper mottle virus was inoculated to *N. benthamiana*, *N. glutinosa*, *N. clevelandii* A. Gray, and *Capsicum annuum* L. 'Chili'; PVX was inoculated to *N. tabacum* 'Xanthi-nc,' 'Turkish,' *N. benthamiana*, and *N. glutinosa*; TMV-type was inoculated to *N. benthamiana*, *N. silvestris* Speg. & Comes, *N. clevelandii*, *Lycopersicon esculentum* Mill. 'Rutgers,' 'UC 82,' 'Peto 95,' and *C. annuum* 'Yolo Wonder'; and CPTMV was inoculated to *Phaseolus vulgaris* L. 'Top Crop,' 'Red Kidney,' 'Black Turtle,' and *P. sativum* 'Progress No. 9.' Leaf tissue from these infected plants was harvested at 10, 20, and 30 days after inoculation.

**Effect of temperature.** Plants inoculated with a member of each group (PeMV, DLV, PVX, and TMV-type) were kept in growth chambers at 20, 24, and 27 C with a 16-hr daylength (400  $\mu$ Ein/m<sup>2</sup>/sec). Tissues were harvested 10 days after inoculation.

## RESULTS

**Identity of viruses used.** The virus isolates exhibited the expected results from host range, serology, and electron microscopy tests. No contaminants were detected.

**Relative mobility of reference dsRNAs.** The MWs of reference dsRNAs and their relative mobilities are shown in Table 1. These MWs were calculated by using dsRNAs of CTV (8) and TMV (7) as standards.

**DsRNA detection.** One-tenth of the final 300- $\mu$ l sample purified from 7 g of infected tissue was usually all that was needed to detect dsRNAs in photographs of acrylamide or agarose gels stained with ethidium bromide after electrophoresis. Up to 30 g of tissue from plants infected with PVY, TEV, DMV, TuMV, or EMMV were necessary to obtain dsRNA yields comparable to that from 7 g of tissue infected with other viruses. Unless otherwise indicated, all gel channels illustrated contained the dsRNA isolated from 0.7 g of tissue.

The relative migration of the slowest dsRNA (usually the most heavily stained and assumed to be the RF of these viruses) was in the following order, from slowest to fastest: clostero-, poty-, carla-, potex-, and tobamovirus (Fig. 1). This is the same order as the MW of the encapsidated ssRNA of these viruses. The slowest-migrating dsRNA of each virus was clearly detected by both polyacrylamide and agarose gel electrophoresis. Polyacrylamide gels (Fig. 1a) were better than agarose gels (Fig. 1b) for the detection of additional dsRNAs, which migrated faster than their respective RFs.

Nucleic acids that remained at the surface of polyacrylamide gels after electrophoresis, and that fluoresced when stained with ethidium bromide, were common when samples were not pretreated with RNase. Treatment of samples with RNase in 0.15 M NaCl normally resulted in the loss of these nucleic acids, without any effect on the dsRNAs. Not all minor dsRNAs described in the following sections are clearly visible on the figure illustrations, but were obvious in the original photographs of stained gels.

**Potyvirus.** A major dsRNA (MW =  $6.5 \times 10^6$ ) was obtained with PVY, TEV, SMV, and TuMV (Fig. 2). This was by far the most heavily stained dsRNA detected and is assumed to be the RF of these viruses. It was the only dsRNA detected for TEV and SMV. Lightly stained faster migrating dsRNA bands were observed consistently in extracts from PVY (MWs = 5.0, and  $1.2 \times 10^6$ ) and TuMV (MWs 3.5–5.1 [several bands] and  $1.2 \times 10^6$ ) infected plants. The slowest migrating RF of dsRNA of PeMV and DMV had a faster mobility (MW =  $5.6 \times 10^6$ ) than the equivalent dsRNA of other potyviruses. An additional dsRNA (MW =  $5.2 \times 10^6$ ) with a slightly faster mobility than that of the slowest migrating dsRNA was also found for these two viruses. Two other dsRNAs (MWs =  $3.5 \times 10^6$  and  $2.5 \times 10^6$ ) were obtained with PeMV. A heavily stained dsRNA (MW =  $0.75 \times 10^6$ ) was obtained with DMV. Three other lightly stained dsRNAs were also detected.

TABLE 1. Relative migration during electrophoresis of dsRNAs of citrus tristeza virus (CTV), tobacco mosaic virus (TMV), cucumber mosaic virus (CMV), and tobacco necrosis virus (TNV) in 6.0% polyacrylamide gels, with current and previous estimates of their molecular weights

Virus dsRNA segment	Distance (cm) migrated	Molecular weight ( $\times 10^6$ )	
		Current	Previous
CTV-1	0.40	(13.30) <sup>a</sup>	13.30 <sup>b</sup>
TMV-1	0.80	(4.30)	4.30 <sup>c</sup>
TNV-1	1.30	2.30	2.60 <sup>d</sup>
TMV-2	1.39	(2.10)	2.10 <sup>e</sup>
CMV-1	1.40	2.00	2.02 <sup>e</sup>
CMV-2	1.45	1.90	1.78 <sup>e</sup>
CMV-3	1.90	1.30	1.36 <sup>e</sup>
TMV-3	2.40	(0.95)	0.95 <sup>e</sup>
TNV-2	2.80	0.78	1.05 <sup>d</sup>
TNV-3	3.30	0.65	0.94 <sup>d</sup>
CMV-4	3.75	0.55	0.66 <sup>e</sup>
TMV-4	5.00	(0.42)	0.42 <sup>e</sup>

<sup>a</sup> Molecular weights in parentheses are those used as standards to determine the current values.

<sup>b</sup> Dodds and Bar-Joseph (8).

<sup>c</sup> Dawson and Dodds (7).

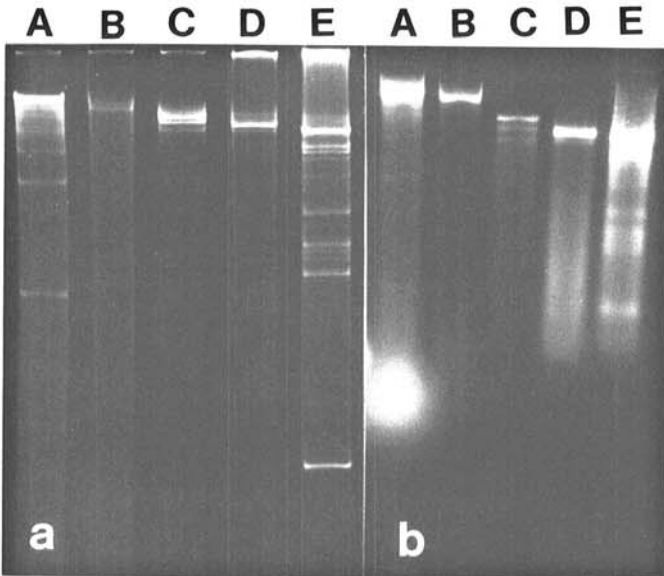
<sup>d</sup> Condit and Fraenkel-Conrat (6).

<sup>e</sup> Kaper and Diaz-Ruiz (17).

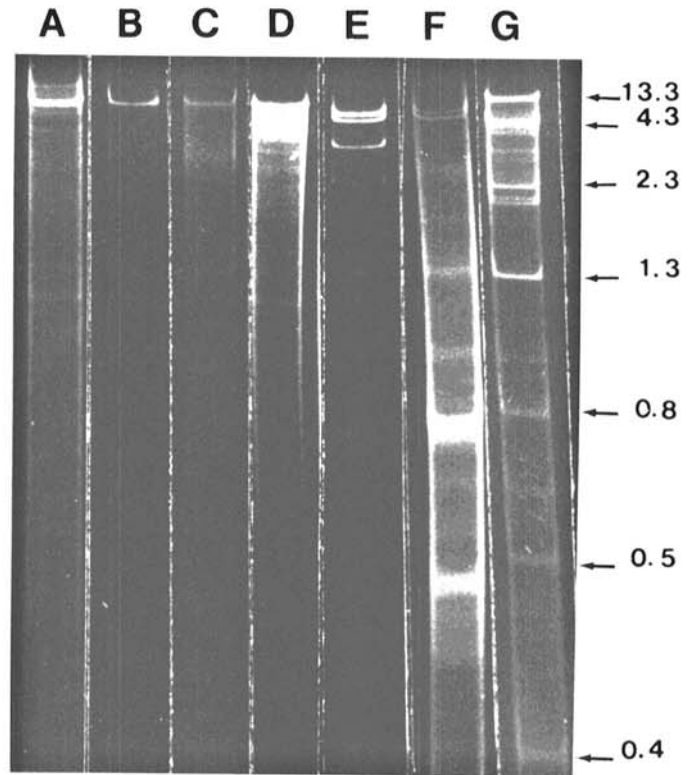
With the exception of TEV and SMV, all other potyviruses could be differentiated by their dsRNA banding patterns.

**Carlaviruses.** The slowest migrating dsRNA of all six carlaviruses that were examined had an MW of approximately  $5.5 \times 10^6$  (Fig. 3). A faint band above the RF was consistently obtained with EMMV, PVS, and DLV. Two additional high-MW dsRNAs were obtained with PSV, RCVMV, PVS, and DLV, and one with KLV. They were characteristic for each virus. The approximate MWs of these dsRNAs were  $5.2 \times 10^6$  and  $4.8 \times 10^6$  for PSV,  $4.8 \times 10^6$  and  $4.6 \times 10^6$  for RCVMV and PVS, and  $5.0 \times 10^6$  and  $4.8 \times 10^6$  for DLV. One additional dsRNA (MW =  $4.8 \times 10^6$ ) was obtained with KLV. Additional dsRNAs were not present in extracts from EMMV-infected plants.

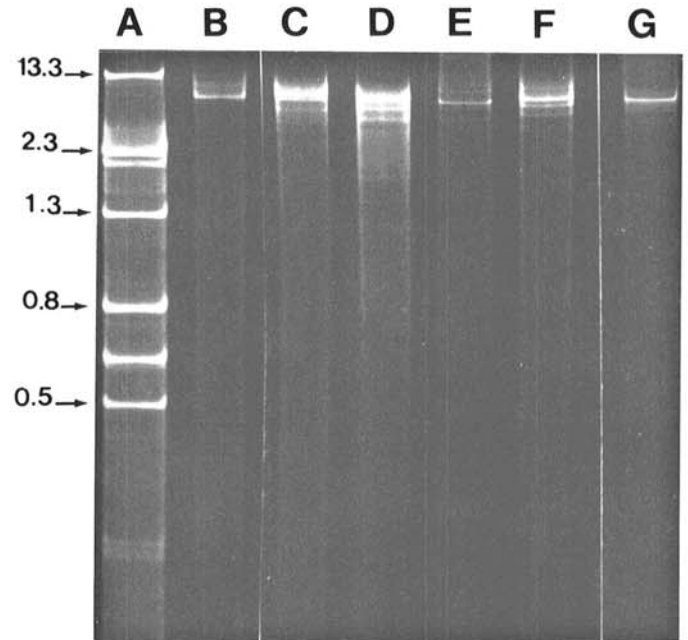
**Potexviruses.** The potexviruses had similar dsRNA patterns, with the exception of WCIMV. An intensely staining dsRNA (MW =  $5.0 \times 10^6$ ) and an additional dsRNA (MW =  $4.6 \times 10^6$ ) were detected in extracts of plants infected with PVX and CVX. The dsRNA of these two viruses could be differentiated by the presence of additional dsRNAs (MW =  $4.0 \times 10^6$  for PVX and MW =  $0.7 \times 10^6$  for CVX). The slowest migrating heavily stained dsRNA of CYMV (MW =  $5.2 \times 10^6$ ) migrated more slowly than the equivalent dsRNA of CVX and PVX. Additional dsRNAs (MW =  $4.8 \times 10^6$  and  $4.0 \times 10^6$ ) were also present in extracts from plants infected with CYMV. The dsRNAs detected for WCIMV were atypical when compared to the three other potexviruses. The most heavily stained dsRNA had a faster relative mobility (MW =  $4.0 \times 10^6$ ) than the equivalent dsRNA of the other three potexviruses. Two other minor dsRNAs (MW =  $5.2 \times 10^6$  and  $5.0 \times 10^6$ ) of slower mobility than the most heavily stained dsRNA, and two others with faster mobility (MW =  $3.8 \times 10^6$  and  $3.2 \times 10^6$ ) were also detected. Other very lightly stained dsRNAs (PVX, MW = 2.1, 1.3, 1.0, 0.57, 0.55; CVX, MW =  $1.4 \times 10^6$ ; CYMV, MW = 1.9- and  $0.75 \times 10^6$ ; and WCIMV, MW = 1.5, 0.57, and  $0.6 \times 10^6$ ) also were obtained consistently with all four viruses (these are not well illustrated in Fig. 4). The presence of a dsRNA with a slightly higher mobility than that of the RF seems to be a characteristic feature of the examined members of this group.



**Fig. 1.** Polyacrylamide and agarose gel electrophoresis of dsRNAs from plants infected with rod-shaped viruses with undivided genome. Lanes: A, citrus tristeza virus (closterovirus); B, tobacco etch virus (potyvirus); C, dandelion latent virus (carlavirus); D, potato virus X (potexvirus); and E, tobacco mosaic virus U5 (tobamovirus). **a**, A 6% polyacrylamide gel (1.5 mm  $\times$  7 cm  $\times$  8 cm); samples were analyzed by electrophoresis for 3 hr at 100 V in 0.04 tris, 0.02 sodium acetate, and 0.001 M EDTA, pH 7.8. **b**, A 1.2% agarose gel (5 mm  $\times$  5 cm  $\times$  7.5 cm); samples were analyzed by electrophoresis for 1 hr at 75 V in the same buffer system. Gels were stained in ethidium bromide (20 ng/ml). DsRNA (approximately  $0.5 \mu\text{g}$ ) loaded on gels was one-tenth of that obtained from 7 g of infected tissue for all viruses except TEV, which was obtained from 30 g.



**Fig. 2.** Polyacrylamide gel electrophoresis (6%) of dsRNAs from plants infected with six potyviruses. Lanes: A, potato virus Y; B, tobacco etch virus; C, soybean mosaic virus; D, turnip mosaic virus; E, pepper mottle virus; F, dasheen mosaic virus; and G, molecular weight standard ( $\times 10^6$ ) (mixture of dsRNAs of citrus tristeza virus, tobacco mosaic virus, and cucumber mosaic virus). Samples were analyzed by electrophoresis in a 1.5 mm  $\times$  7 cm  $\times$  8 cm slab gel for 3 hr at 100 V in 0.04 tris, 0.02 sodium acetate, and 0.001 M EDTA, pH 7.8. DsRNA (approximately  $0.5 \mu\text{g}$ ) loaded on gels was one-tenth of that obtained from 30 g of tissue for all viruses except pepper mottle virus, which was obtained from 7 g.



**Fig. 3.** Polyacrylamide gel electrophoresis (6%) of dsRNAs from plants infected with six carlaviruses. Lanes: A, molecular weight standard ( $\times 10^6$ ) (mixture of dsRNAs of citrus tristeza virus, tobacco necrosis virus, and cucumber mosaic virus); B, eggplant mild mottle virus; C, pea streak virus; D, red clover vein mottle virus; E, potato virus S; F, dandelion latent virus; and G, kalachöe latent virus. Gel channels contain one-tenth of the dsRNA obtained from 7 g of tissue. Samples were analyzed by electrophoresis in a slab gel for 3 hr at 100 V.

**Tobamovirus.** A single heavily stained dsRNA ( $MW = 4.3 \times 10^6$ ) was detected in plants infected with TMV-type, TMV-U5, CPTMV, ToMV, and ORSV. This was the most obvious dsRNA detected in gels and is assumed to be the RF of these viruses. A second ( $MW = 2.1 \times 10^6$  for TMV-type, TMV-U5, CPTMV, and ToMV,  $1.1 \times 10^6$  for ORSV) and third ( $MW = 0.42 \times 10^6$  for TMV-type, CPTMV, and ToMV,  $0.50 \times 10^6$  for TMV-U5 and  $0.58 \times 10^6$  for ORSV) dsRNA was readily detected. Other dsRNAs ( $MW = 1.4-4.0 \times 10^6$ ) were also detected and they varied in number and intensity from virus to virus. These differences, together with those noted above, made it possible to distinguish each virus by careful comparison of the dsRNAs.

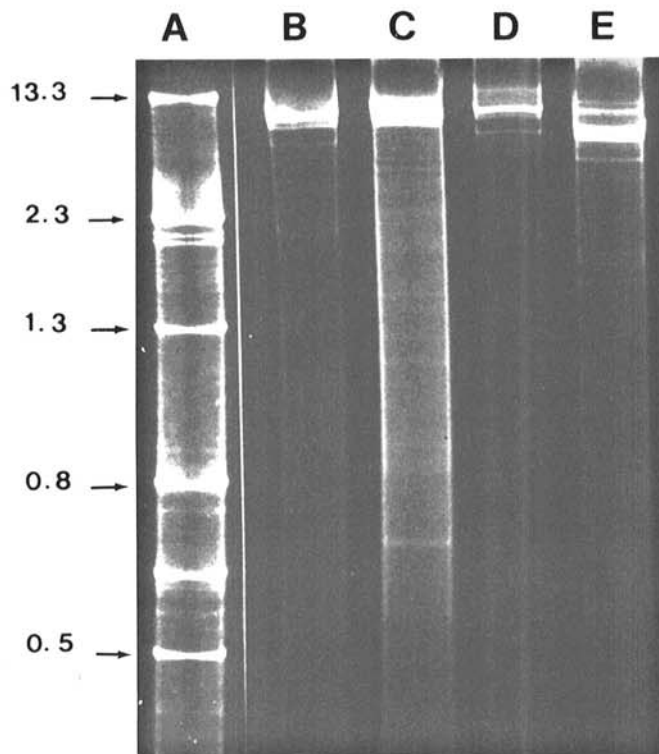
The dsRNAs of TYMV (Fig. 5A) were compared with those of the tobamovirus group (Fig. 5). An RF ( $MW = 4.3 \times 10^6$ ) similar in size to the RF of the tobamovirus was obtained. The next fastest migrating dsRNA ( $MW = 0.42 \times 10^6$ ) co-electrophoresed with the fastest migrating dsRNA of TMV-type, CPTMV, and ToMV. The number and mobility of several other dsRNAs were distinctive for TYMV.

The dsRNAs of four ToMV strains (Fig. 6) were similar. Minor differences were detected, and these were sufficient to distinguish the strains. Variable numbers and mobilities of dsRNAs ( $MW = 4.0-0.5 \times 10^6$ ) were observed.

Each of these viruses and strains (from all five groups) were extracted and analyzed at least 10 times. Differences described here were obtained consistently from all the extractions. Three separate purifications from Turkish tobacco of the dsRNAs of two isolates of ToMV are illustrated in Fig. 7.

**Host effect.** The use of different hosts did not affect the dsRNA banding patterns. Consistent dsRNA patterns were obtained from extracts of four PeMV-infected hosts (Fig. 8). A minor effect on yield of dsRNA was the only host effect observed in this experiment.

High-MW dsRNAs were detected in extracts from six uninoculated plant species and cultivars used in this study (Fig. 9).



**Fig. 4.** Polyacrylamide gel electrophoresis (6%) of dsRNAs from plants infected with four potexviruses. Lanes: A, molecular weight standard ( $\times 10^6$ ) (dsRNAs of citrus tristeza virus, tobacco necrosis virus, and cucumber mosaic virus); B, potato virus X; C, cactus virus X; D, clover yellow mosaic virus; and E, white clover mosaic virus. Gel channels contain one-tenth of the dsRNA obtained from 7 g of tissue. Samples were analyzed by electrophoresis in slab gels for 4 hr at 100 V.

The particular dsRNAs detected were characteristic of the cultivar or the species of plant tested. These six plants were not used to detect virus dsRNAs. High-MW dsRNAs were not detected by these methods in the 18 other plants used in this study.

**Effect of age of infection and temperature.** Yields of dsRNA were highest when tissue was harvested 10 days after inoculation. Older infections yielded lower amounts of dsRNA except for TMV infections which gave similar yields at 10, 20, and 30 days postinoculation. Plants incubated at 27 C gave the highest yields. Lower yields were obtained from plants incubated at 24 C, and even lower from those incubated at 20 C. Age of infection, and incubation temperature did not affect the quality of dsRNA patterns.

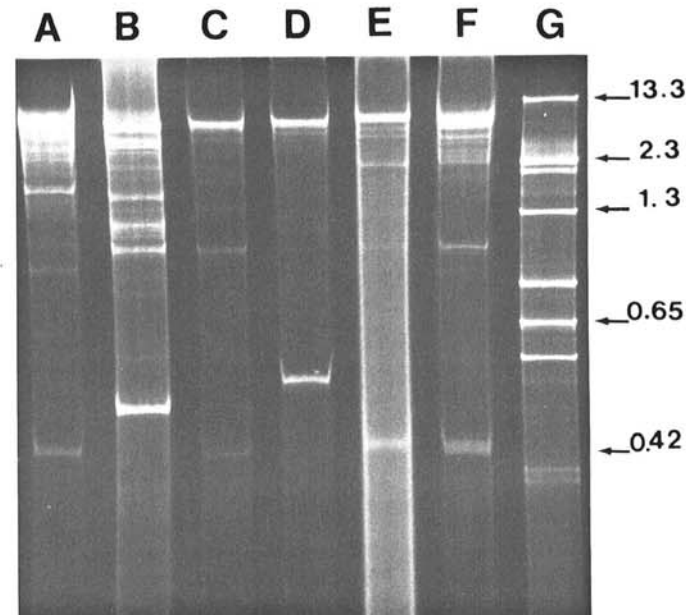
## DISCUSSION

All stated MWs of dsRNAs used as standards were estimated in our laboratory. Variations encountered between previous and current MW estimates are probably due to different experimental conditions. The MWs reported here are relative; this must be kept in mind when interpreting them. Relative mobility rather than the exact MW may be more useful for diagnostic purposes. Nevertheless, it is imperative that either MW or mobility standards be included in electrophoretic analysis.

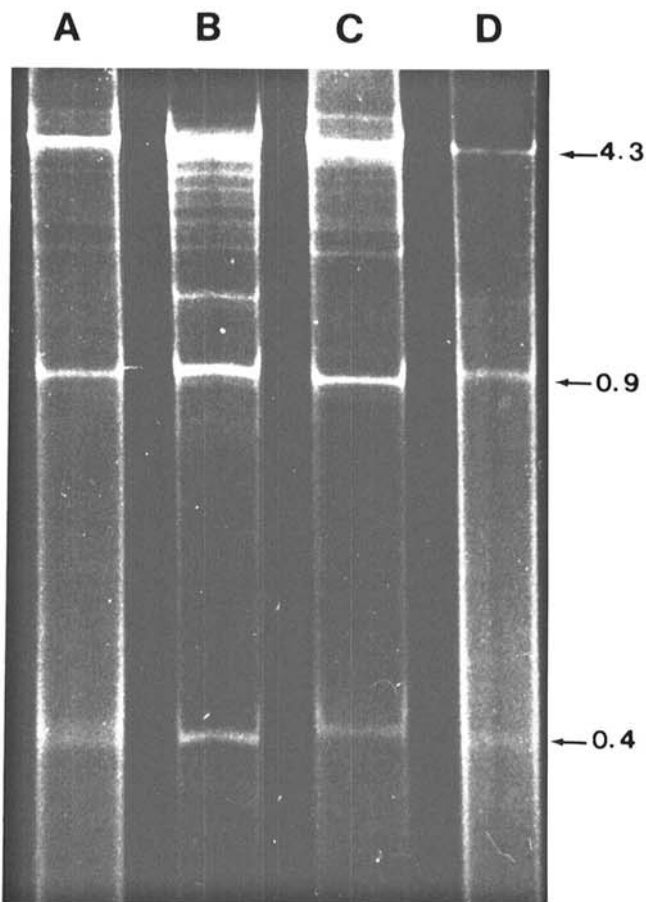
The results of this investigation indicate that dsRNAs can be detected readily in plants infected with 20 different rod-shaped viruses. With a few notable exceptions, no comparable dsRNA was detected in uninfected plants.

Different viral groups were clearly distinguishable by the similar size of the RFs of their members, which differed for each group. Interpretation of these RFs will be of primary importance when using this technique for diagnosis.

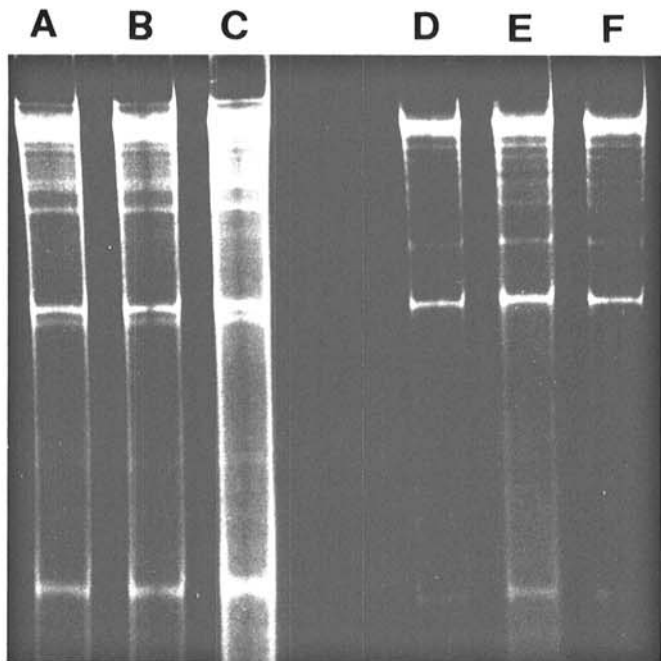
Additional dsRNAs of faster mobility than the RF have been found for several plant viruses (7,8,13,26). The additional dsRNAs described here, together with those reported for the closteroviruses (8) seem to divide the virus groups into two categories. The first (closterovirus, tobamovirus, and tymovirus) had readily detectable multiple high- and low-MW dsRNAs. The second (potyvirus,



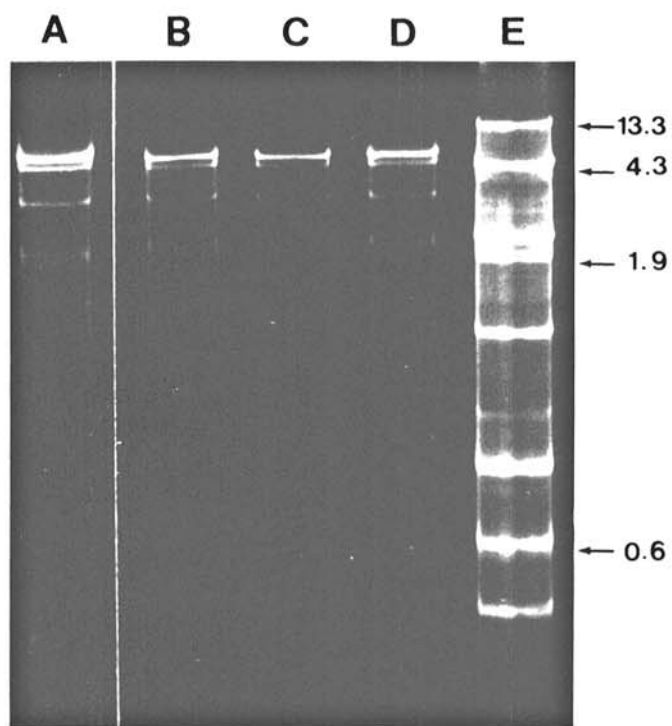
**Fig. 5.** Polyacrylamide gel electrophoresis (6%) of dsRNAs from plants infected with a tymovirus (lane A) and five tobamoviruses (lanes B-F). Lanes: A, turnip yellow mosaic virus; B, tobacco mosaic virus U5; C, tomato mosaic virus purple; D, odontoglossum ringspot virus; E, the cowpea strain of tobacco mosaic virus; F, tobacco mosaic virus type strain; and G, molecular weight standard ( $\times 10^6$ ) (dsRNAs of citrus tristeza virus, tobacco necrosis virus, and cucumber mosaic virus). Gel channels contain one-tenth of the dsRNA obtained from 7 g of tissue. Samples were analyzed by electrophoresis in slab gels for 3 hr at 100 V.



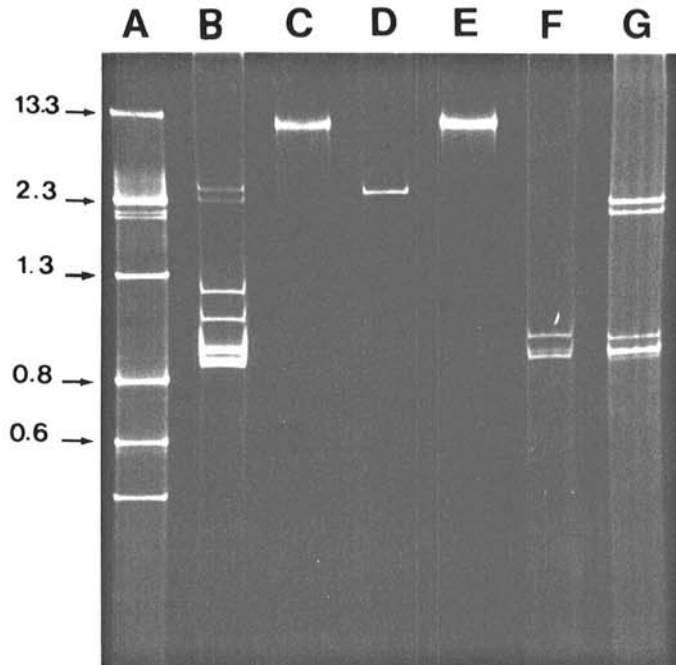
**Fig. 6.** Polyacrylamide gel electrophoresis (6%) of dsRNAs from *Nicotiana tabacum* 'Turkish' infected with four isolates of tomato mosaic virus (ToMV). Lanes: A, ToMV-grey wall; B, ToMV-purple; C, ToMV-fruit necrosis; and D, ToMV-white. Molecular weights  $\times 10^6$ . Gel channels contain one-tenth of the dsRNA obtained from 7 g of tissue. Samples were analyzed by electrophoresis in slab gels for 3 hr at 100 V.



**Fig. 7.** Polyacrylamide gel electrophoresis (6%) of dsRNAs obtained from six samples, independently purified, of two isolates of tomato mosaic virus (ToMV). Lanes: A-C, DsRNAs of three samples of ToMV-fruit necrosis. Lanes: D-F, DsRNAs of three samples of ToMV-purple. Gel channels contain one-tenth of the dsRNA obtained from 7 g of tissue. Samples were analyzed by electrophoresis in slab gels for 3 hr at 100 V.



**Fig. 8.** Polyacrylamide gel electrophoresis (6%) of dsRNAs extracted from different plant species infected with pepper mottle virus. Lanes: A, *Nicotiana tabacum* 'Xanthi-nc'; B, *N. glutinosa*; C, *Capsicum annuum* 'Chili'; D, *N. clevelandii*; and E, molecular weight standard ( $\times 10^6$ ) (mixture of dsRNAs of citrus tristeza virus, tobacco mosaic virus, tobacco necrosis virus, and cucumber mosaic virus). Gel channels contain one-tenth of the dsRNA obtained from 7 g of tissue. Samples were analyzed by electrophoresis in slab gels for 3.5 hr at 100 V.



**Fig. 9.** Polyacrylamide gel electrophoresis (6%) of dsRNAs extracted from six uninoculated plant species. Lanes: A, molecular weight standard ( $\times 10^6$ ) (mixture of dsRNAs of citrus tristeza virus, tobacco necrosis virus, and cucumber mosaic virus); B, *Beta vulgaris* 'Y905'; C, *Phaseolus vulgaris* 'Black Turtle'; D, *Lycopersicon esculentum* 'UC-82'; E, *Capsicum annuum* 'Yolo Wonder'; F, *Brassica campestris* 'Tendergreen,' seed lot A; and G, *B. campestris* 'Tendergreen,' seed lot B. Gel channels contain one-tenth of the dsRNA obtained from 7 g of tissue. Samples were analyzed by electrophoresis in slab gels for 3.2 hr at 100 V.

carlavirus, and potexvirus) had mainly small numbers of high-MW dsRNAs. The presence or absence of additional dsRNAs, together with their variable size, were useful characteristics for differentiating particular viruses. Interpretation of these minor dsRNAs, and their use for diagnostic purposes, requires that known viruses of the group be analyzed together with the unknown.

Relatively high yields of virus particles for a potyvirus are obtained when PeMV is purified (O. Abdalla, *personal communication*). Tissue affected with PeMV also yielded relatively large amounts of dsRNA compared to the other potyviruses that were tested. The generally low dsRNA yield for potyviruses may be related to the low titer of these viruses in their hosts. The detection of prominent dsRNAs in addition to the RF for some members of this group is an interesting observation. Two different replication strategies for TEV and PeMV has been suggested (10). This might be the reason for the striking difference in the dsRNA banding patterns of these two viruses. Of the potyviruses tested, only DMV had dsRNAs similar to those of PeMV. It also had several unique low-MW dsRNAs. These low-MW dsRNAs were not detected in the uninoculated plants of *P. sellowii* used to propagate DMV.

Expected RFs and several additional dsRNAs of faster mobility but high MW were readily detected with six carlaviruses. The dsRNA of EMMV did not have dsRNAs with faster mobility than the most prominent dsRNA. DsRNA of EMMV, like the potyviruses, was present in low amounts in the infected tissue. The MW of its dsRNAs distinguish EMMV from the potyviruses. Despite the similarities of the RFs among DMV, PeMV, and the carlaviruses, other properties of these two viruses indicate that they are potyviruses. We do not think that this presents a serious problem since the use of other diagnostic techniques is recommended to complement results from dsRNA analysis.

The dsRNAs of WCIMV differed from those of the other members of the potexvirus group, mainly because of the apparent low MW of what is presumed to be the RF. The degree to which secondary structure affects the migration of dsRNA in the polyacrylamide gels is not clearly understood, and this result may indicate a need for a study to examine this. Nevertheless, the dsRNA banding patterns of these four potexviruses in polyacrylamide gels were diagnostic. The lightly stained dsRNAs of the members of this group seem to be unique for each virus; but, as is the case for the other additional dsRNAs, their role in replication is uncertain. They covered a similar range of MWs (but were not as readily detected) as those associated with tobamoviruses and closteroviruses (8).

The dsRNA of the type member (TMV) of the tobamovirus group has been well characterized (7,23,26). The specific MW of the RF, and the presence of several readily detectable additional dsRNAs with a wide range of MWs down to  $0.4 \times 10^6$ , differentiated this group from other groups tested, except for the tymoviruses. The fastest migrating dsRNA ( $MW = 0.42 \times 10^6$ ) of TMV-type is presumed to contain a positive-sense molecule that is similar to the mRNA for the coat protein (7,23). If that is true for the other tobamoviruses, TMV-type, TMV-U5, and ORSV may each have a very different sized mRNA for their coat protein, while CPTMV, ToMV, and TMV-type may have one of similar size. Minor differences in the dsRNAs obtained with four ToMV strains amplify the diagnostic value of this technique for strain differentiation, as well as for virus differentiation. Other examples of this include differences in the dsRNAs of strains of barley yellow dwarf virus, beet western yellows virus, and CMV (11,13,22).

Similarities between the overall dsRNA profiles of TYMV and TMV-type were interesting. Despite their different particle morphology, the size of the TMV genome is similar to that of TYMV, and several subgenomic ssRNAs are involved in replication of both groups (20). Our results support the conclusion that these two viruses may have similar replication strategies.

The major and minor dsRNAs described for all viruses examined here were consistently obtained. Some were difficult to detect when yield of dsRNA was low or gels were inadequately loaded. Only occasional minor variations were related to the host, age of infection, or incubation temperature.

High-MW dsRNAs in uninoculated plants have been reported (14,25). Because of the similarity of their size and homogeneity to plant viral dsRNA, they can easily be confused with dsRNA of known viral origin. The isolation of such molecules from some of the hosts that we tested could lead to erroneous conclusions. This problem can be avoided by using appropriate healthy controls when extracting dsRNA. The use of a cultivar or seed lot identical to that of the infected plant may be necessary when field samples are analyzed. Prior selection and use of cultivars that lack dsRNA for virus propagation will be useful. It is possible that most of these unexpected dsRNAs are the consequence of infection with cryptic or latent viruslike agents (3).

The results obtained here strengthen the value of dsRNA patterns for diagnosis of RNA plant virus infections (9,21,22). We did not attempt to investigate their role in virus replication. Nevertheless, the contrasts and similarities described and discussed here should encourage further studies on the replicative strategies of these viruses.

#### LITERATURE CITED

1. Bar-Joseph, M., Murant, A. F. 1982. Closterovirus Group. No. 260 in: Descriptions of Plant Viruses. Commonw. Mycol. Inst., Assoc. Appl. Biologists, Kew, Surrey, England. 3 pp.
2. Bar-Joseph, M., Rosner, A., Moscovitz, M., and Hull, R. 1983. A simple procedure for the extraction of double-stranded RNA from virus infected plants. *J. Virol. Meth.* 6:1-8.
3. Boccardo, G., Lisa, V., and Milne, R. G. 1983. Cryptic viruses in plants. Pages 425-430 in: Double-Stranded RNA Viruses. R. W. Compans and D. H. L. Bishop, eds. Elsevier, New York.
4. Bozarth, R. F., and Harley, E. H. 1976. The electrophoretic mobility of double-stranded RNA in polyacrylamide gels as a function of molecular weight. *Biochem. Biophys. Acta* 432:329-335.
5. Brandes, J., and Wetter, C. 1959. Classification of elongated plant viruses on the basis of particle morphology. *Virology* 8:99-115.
6. Condit, C., and Fraenkel-Conrat, H. 1979. Isolation of replicative forms of 3' terminal subgenomic RNAs of tobacco necrosis virus. *Virology* 97:122-130.
7. Dawson, W. O., and Dodds, J. A. 1982. Characterization of subgenomic double-stranded RNAs from virus-infected plants. *Biochem. Biophys. Res. Commun.* 107:1230-1235.
8. Dodds, J. A., and Bar-Joseph, M. 1983. Double-stranded RNA from plants infected with closteroviruses. *Phytopathology* 73:419-423.
9. Dodds, J. A., Morris, T. J., and Jordan, R. L. 1984. Plant viral double-stranded RNA. *Annu. Rev. Phytopathol.* 22:151-168.
10. Dougherty, W. G., Allison, R. F., Parks, T. D., Johnston, R. A., Field, M. J., and Armstrong, S. B. 1986. Nucleotide sequence at the 3' terminus of pepper mottle virus genomic RNA: Evidence for an alternative mode of potyvirus capsid protein gene organization. *Virology* 145:(In press).
11. Falk, B. W., and Duffus, J. E. 1984. Identification of small single- and double-stranded RNAs associated with severe symptoms in beet western yellows virus-infected *Capsella bursa-pastoris*. *Phytopathology* 74:1224-1229.
12. Gibbs, A. J. 1977. Tobamovirus Group. No. 184 in: Descriptions of Plant Viruses. Commonw. Mycol. Inst., Assoc. Appl. Biologists, Kew, Surrey, England. 6 pp.
13. Gildow, F. E., Ballinger, M. E., and Rochow, W. F. 1983. Identification of double-stranded RNAs associated with barley yellow dwarf virus infections of oats. *Phytopathology* 73:1570-1572.
14. Grill, L. K., and Garger, S. J. 1981. Identification and characterization of double-stranded RNA associated with cytoplasmic male sterility in *Vicia fava*. *Proc. Nat. Acad. Sci., USA* 78:7043-7046.
15. Hollings, M., and Brunt, A. A. 1981. Potyvirus Group. No. 245 in: Descriptions of Plant Viruses. Commonw. Mycol. Inst., Assoc. Appl. Biologists, Kew, Surrey, England. 7 pp.
16. Jordan, R. L., Dodds, J. A., and Ohr, H. 1983. Evidence for viruslike agents in avocado. *Phytopathology* 73:1130-1135.
17. Kaper, J. M., and Diaz-Ruiz, J. R. 1977. Molecular weights of double-stranded RNAs of cucumber mosaic virus strain S and its associated RNA 5. *Virology* 80:214-217.
18. Koenig, R. 1978. Potexvirus Group. No. 200 in: Descriptions of Plant Viruses. Commonw. Mycol. Inst., Assoc. Appl. Biologists, Kew, Surrey, England. 5 pp.
19. Koenig, R. 1982. Carlavirus Group No. 259 in: Descriptions of Plant Viruses. Commonw. Mycol. Inst., Assoc. Appl. Biologists, Kew, Surrey, England. 4 pp.

20. Matthews, R. E. F. 1981. *Plant Virology*. 2nd ed. Academic Press, New York. 897 pp.
21. Morris, T. J., and Dodds, J. A. 1979. Isolation and analysis of double-stranded RNA from virus-infected plant and fungal tissue. *Phytopathology* 69:854-858.
22. Morris, T. J., Dodds, J. A., Hillman, B., Jordan, R. L., Lommel, S. A., and Tamaki, S. J. 1983. Viral specific dsRNA: Diagnostic value for plant virus disease identification. *Plant Mol. Biol. Rep.* 1:27-30.
23. Palukaitis, P., Garcia-Arenal, F., Sulzinski, M., and Zaitlin, M. 1983. Replication of tobacco mosaic virus. VII. Further characterization of single- and double-stranded virus-related RNAs from TMV-infected plants. *Virology* 131:533-545.
24. Tavantzis, S. T. 1984. Physicochemical properties of potato virus M. *Virology* 133:427-430.
25. Wakarchuk, D. A., and Hamilton, R. I. 1984. Two double-stranded RNAs of unknown origin in vegetative tissues of bean. (Abstr.) *Phytopathology* 74:860.
26. Zelcer, A., Weaber, K., Balazs, E., and Zaitlin, M. 1981. The detection and characterization of viral-related double-stranded RNAs in tobacco mosaic virus-infected plants. *Virology* 113:417-427.