Further Characterization of Panicum Mosaic Virus and Its Associated Satellite Virus

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ABSTRACT

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Panicum mosaic virus (PMV) replicates in the absence of satellite panicum mosaic virus (SPMV), whereas SPMV requires PMV for its replication. PMV and SPMV are unrelated serologically. Presently, two serotypes of SPMV and six of PMV have been differentiated on the basis of their reactions with homologous and heterologous antisera. A serological relationship exists between PMV and members of the phleum mottle virus (PhMV) group that were tested. The PMV serotypes were classified into two groups based on their relative electrophoretic mobility. SPMV was found associated with all PMV serotypes tested. It was not associated with MSV. Both PMV-type and MSV were capable of directing the replication of SPMV. PMV differs from SPMV in particle size, RNA content and base composition, capsid protein molecular weight, and amino acid composition. The in vitro translation products for PMV and SPMV were compared. PMV RNA directed the synthesis of several ^{14}C-labeled proteins, including one that coelectrophoresed with authentic PMV coat protein. SPMV RNA directed the synthesis of a single ^{14}C-labeled protein which coelectrophoresed with its authentic coat protein. Although the relationship between PMV and SPMV mimics the relationship between tobacco necrosis virus and its satellite virus, no serological relationships were found among these four viruses.

Panicum mosaic virus (PMV) was first described by Sill and Pickett (30) on switchgrass in Kansas in 1953. They and others were able to mechanically transmit the virus and found it had a narrow host range in the Gramineae (14,17,20,29–31). The type strain of PMV has been found only in Kansas, where it causes no serious economic losses; however, the St. Augustine decline strain (PMV-SAD) causes severe losses in St. Augustine grass lawns in Texas and Louisiana (11,17). Several serological variants of PMV were detected by Holcomb (10).

Niblett and Paulsen (20) demonstrated that there were two centrifugation components associated with PMV. Buzen and Niblett (3) and Buzen et al (5) indicated that one component (42S) is isometric, 17 nm in diameter, and not infectious. It contains two

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RNA species (14 and 34S) and a single protein species (15,500 daltons), and is serologically unrelated to the other component (109S). The 109S component is isometric, 28–30 nm in diameter, and infectious. It contains a single RNA species (28S) and a single protein species (28,000 daltons). From these results, Buzen and Niblett (4) concluded that the 42S component was a satellite virus, (they designated it satellite panicum mosaic virus [SPMV]), which was dependent upon the 109S component for its replication. The 109S component is designated PMV because it is capable of replicating in the absence of SPMV (4).

The purpose of this research was to examine several isolates of PMV and SPMV to determine additional characteristics.

MATERIALS AND METHODS

The type strain of PMV and its associated SPMV (SPMV-type) were obtained from the virus collection at Kansas State University. G. Holcomb (Louisiana State University, Baton Rouge) kindly provided four isolates of PMV-SAD, which we designated as PMV-L1, PMV-L2, PMV-L3, and PMV-L4. R. Toler (Texas A&M University, College Station) kindly provided the isolate of PMV-SAD designated PMV-Tx and three antisera made to a mixture of satellite and helper viruses. H. Paul (Germany) kindly provided molinia streak virus (MSV), which we now consider to be a serotype of PMV, and antisera to phleum mottle virus (PhMV) and cocksfoot mild mosaic virus (CMMV). J. K. Uyemoto (Kansas State University, Manhattan) kindly provided inoculum of a mixture of tobacco necrosis virus (TNV) and satellite tobacco necrosis virus (STNV), and antisera made to each of these viruses. STNV RNA was kindly provided by J. M. Clark (University of Illinois, Urbana).

All isolates of PMV and SPMV were maintained on pearl millet (*Setaria italica* (L.) Beauv.) German strain R. Inoculum was prepared by grinding 14- to 21-day infected leaves 1:3 (w/v) in cold 0.02 M potassium phosphate buffer, pH 7.0 (KPO_4). For small-scale inoculations, tissue was ground with a mortar and pestle and inoculated onto Carborundum-dusted leaves. For large-scale inoculations, tissue was homogenized in a Waring blender and filtered through four layers of cheesecloth. Carborundum was added to the filtrate at 1 g per 20 ml and sprayed onto millet at 6–7 kg/cm^2 from a DeVilbiss No. 152 sprayer.

Purification. Virus was purified as described by Niblett and Paulsen (20) with the following modifications. Millet was inoculated at the four- to five-leaf stage, and inoculated and systemically infected leaves were harvested after 14 to 21 days. Leaves were cut into 2- to 3-cm segments and either used fresh or frozen for later purification. Leaves were ground 1:3 (w/v) in cold 0.1 M KPO_4 plus 1% β-mercaptoethanol. Homogenized tissue was expressed through four layers of cheesecloth. The liquid extract was centrifuged for 10 min at 10,000 *g* and the supernatant was made 8% (w/v) polyethylene glycol 6,000 and 0.2 M NaCl. After the mixture had been stirred for 2 hr at 4 C, the precipitate was collected by centrifugation for 10 min at 8,000 *g*. Pellets were resuspended overnight in 0.1 M KPO_4 (1 ml per 2 g of tissue). The resulting suspension was mixed with an equal volume of a cold 1:1 mixture of chloroform and butanol and kept at 4 C for 30 min. The emulsion was broken by centrifugation for 10 min at 2,000 *g*. The aqueous phase was drawn off, centrifuged 10 min at 10,000 *g*, and the supernatant was centrifuged for 2 hr at 363,000 *g*. Pellets were resuspended in 0.02 M KPO_4. Virus yield was ~0.1 mg of virus per gram fresh weight of tissue.

Virus was further purified by sedimentation in a 0–30% sucrose density gradient for 20 min in a Sorvall TV850 vertical rotor at 237,000 *g*. Fractions were collected using a model D ISCO fractionator. Fractions containing PMV and SPMV were pooled separately, dialyzed overnight to remove sucrose, and centrifuged at 363,000 *g* for 1 hr (PMV) or 2 hr (SPMV). Pellets were resuspended in 0.02 M KPO_4. Virus used for antiserum production, amino acid analysis, and RNA extraction was purified by two cycles of density gradient centrifugation.

Separation and activation of SPMV. To be a satellite virus, SPMV must be dependent on PMV for its replication or activation. PMV also must be capable of replication in the absence of SPMV.

Millet was inoculated with a series of 10-fold dilutions starting with purified PMV at 1.0 mg/ml. Approximately 30 g of tissue was harvested from each treatment 14 days after inoculation. Virus was purified and the presence of components was determined by density gradient centrifugation. Preparations containing only 109S particles were used as inoculum and subcultures free of 42S particles were selected.

Activation of SPMV-type by PMV-type was tested by inoculating millet with each separately and with a mixture of the two. Purified SPMV-type was used at 0.1 mg/ml. PMV-type was obtained from infected tissue derived from subcultures free of 42S particles. For the mixture, tissue containing PMV-type was homogenized with buffer containing purified SPMV. Experiments with MSV were performed similarly. Virus production was determined as above.

Physical characteristics. The size of the PMV and SPMV particles was determined by using electron microscopy, purified SPMV-type, and PMV-type, which had been negatively stained with unbuffered 0.5% ammonium molybdate and air-dried.

Molecular weights of the capsid proteins were estimated by disrupting purified virus in 63 mM tris-HCl, pH 6.8, 2% sodium dodecyl sulfate (SDS), 10% glycerol, 0.001% bromphenol blue, and 5% β- mercaptoethanol and boiled for 5 min. Electrophoresis was performed on an 11% polyacrylamide slab gel containing 0.2% SDS as described by Lugtenberg et al (15). Molecular weight standards and known viral proteins were prepared by the same procedure.

The sedimentation coefficients for the RNA of PMV and SPMV were determined using linear-log sucrose gradients as described by Brakke and Van Pelt (1). Purified viruses were disrupted by incubation overnight at room temperature in Brakke's dissociation buffer (0.02 M tris, 0.1 M NaCl, 0.001 M EDTA, 400 μg of bentonite per milliliter, and 1% SDS, pH 9.0) or by boiling for 2 min in 0.08 M tris, 0.04 M Na acetate, 0.002 M EDTA, 2.4% SDS, and 1% β-mercaptoethanol, pH 7.0.

Serology. Antisera prepared to PMV-type, PMV-L4, MSV, SPMV-type, and SPMV-L4 were produced in rabbits as described by McMillen and Consigli (18), except virus was not treated with SDS. To further ensure purity, viruses were electrophoresed on 2.8% polyacrylamide gels as described by Semancik (27). Viral bands were excised after comparison to a companion gel fixed 5 min in 17.4 N acetic acid. They were mixed with distilled water and extruded through a 0.64-mm-diameter (23-gauge) syringe needle. The gel-water mixture was mixed with an equal volume of Freund's complete adjuvant for the first injection and subsequent injections contained Freund's incomplete adjuvant. Rabbits were injected three times at 2-wk intervals with 0.5 mg (SPMV) or 1.0 mg (PMV) of virus in 3 ml of suspension. A booster injection identical to previous injections was given 4 wk after the third injection. The rabbits were bled 2 wk later.

Immunodiffusion tests were performed in 0.75% Ionagar containing 0.05 M tris-HCl, 0.85% NaCl, and 0.02% NaN_3. Reactants were arranged as described by Grogan et al (9) to study both homologous and heterologous reactions.

Amino acid analysis. Purified virus (1.0 mg per sample) was exhaustively dialyzed in distilled water, taken to dryness, and then hydrolyzed with 6.0 N HCl for 24, 48, or 72 hr in sealed nitrogen-filled tubes. The hydrolysate was taken to dryness and resuspended in 0.2 M Na-citrate buffer, pH 2.2, and analyzed on a model 121 Beckman amino acid analyzer equipped with an integrator. Duplicate analyses were performed for each hydrolysis time.

Base composition analysis. RNA was extracted from purified virus by the method of Bruening et al (2) or Clark and Klein (7). The RNA was enzymatically hydrolyzed to nucleosides as described by Randerath et al (23). The preparation was then filtered through an Amicon PM 10 ultrafilter to remove the enzymes and analyzed by high-performance liquid chromatography (HPLC) in a mixture of 3% methanol, 97% 10 mM perchloric acid, and 5 mM heptane sulfonate on a Micro Pak MCH 10 column. The flow rate was 2 ml/min. Nucleosides were detected at 260 nm. The peak area was determined by using an integrator with known concentrations of

standards. Results represent the average of several analyses, generally from two different RNA preparations.

Translation of viral RNA. RNA was extracted as above. In vitro translation was performed according to Marcu and Dudock (16) with the following modifications. Wheat germ was a gift from the Wall-Rogalsky Milling Co., McPherson, KS 67460. The buffer used in the initial extraction of the wheat germ was 20 mM tris-acetate (Ac), pH 7.6, 1 mM MgAc, 2 mM $CaCl_2$, 50 mM KCl, and 0.5 mM dithiothreitol (DTT). The buffer used in gel filtration chromatography of the 30,000 *g* supernatant (S 30) was 25 mM tris-Ac, pH 8.0, 4.5 mM MgAc, 90 mM KAc, and 0.5 mM DTT.

The reaction mixture contained 25 mM tris-Ac, pH 8.0, 2.5 mM MgAc, 90 mM KAc, 0.5 mM DTT, 1 mM ATP, 0.2 mM GTP, 0.04 mg/ml creatine phosphokinase, 5 mM creatine phosphate, 0.03 mM of all 19 unlabeled amino acids, 0.1 μCi ^{14}C leucine, 20 μl S 30 and 5 μg viral RNA, all in a final volume of 100 μl. The reaction was carried out at room temperature for 2 hr and stopped by the addition of 1 ml cold 10% trichloroacetic acid. The reaction mixture was cooled on ice for 15 min, then heated to 75 C for 15 min. After cooling to 0 C, the precipitates were collected on glass fiber filters, dried, and counted in a toluene-based counting cocktail.

For electrophoresis of the translation products, the reaction was stopped by precipitation with 1 ml acetone. After 12 hr at −70 C, the precipitate was collected by centrifugation for 10 min at 1,500 *g* and dried under N_2. The precipitate was resuspended in 100 μl of 63 mM tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 0.001% bromphenol blue, and 5% β-mercaptoethanol and boiled for 5 min. The sample was electrophoresed on an 11% polyacrylamide slab gel as above and stained according to Fairbanks et al (8). Chamberlain's (6) method of fluorography utilizing Na-salicylate was used. The X-ray film was developed after 48 hr exposure at −70 C.

RESULTS AND DISCUSSION

Separation and activation of SPMV. That PMV was able to replicate in the absence of SPMV was demonstrated using a serial dilution procedure. At several dilutions, only the 109S PMV particles and no 42S SPMV particles were recovered. These preparations were monitored by density gradient centrifugation through several subcultures and only PMV was detected. These preparations were considered free of SPMV and were used to demonstrate the requirement of PMV for the replication of SPMV. Millet was inoculated with SPMV-type, PMV-type, and a mixture of both. When virus production was assayed, no virus was recovered from plants inoculated with SPMV-type, PMV-type was recovered from PMV-type inoculated plants, and both viruses were recovered from plants inoculated with the mixture. In similar experiments, MSV also was capable of activating the replication of SPMV-type when mixed inocula were used.

Both PMV-type and MSV, a geographically distinct virus not reported in the United States, are capable of activating SPMV-type replication. It is interesting to note that SPMV was found associated with all PMV serotypes tested. It was not found associated with MSV. Also of interest is the fact that two different SPMV serotypes are capable of replication in the presence of a single PMV serotype (serotype 5, Table 1). No selective activation similar to that demonstrated for TNV and STNV (13,33) was observed. However, selective activation of SPMV by PMV cannot be ruled out until many more isolates of each have been tested.

Physical characteristics. About 250 and 180 negatively stained particles of PMV-type and SPMV-type, respectively, were measured. The average diameter for the PMV-type particles was 29–30 nm and for the SPMV-type it was 15–16 nm (Fig. 1). This is in reasonable agreement with our preliminary reports (5,21).

The molecular weights of the viral capsid proteins were determined using SDS-polyacrylamide gel electrophoresis. By comparison to molecular weight standards and capsid proteins of known viruses, values of about 16,000 and 30,000 daltons were determined for the capsid proteins of SPMV and PMV, respectively (Fig. 2). Similar molecular weights were obtained when other serotypes of SPMV and PMV were examined. Paul et al (22) reported a molecular weight of 25,000 daltons for PMV, but few runs were made, and all were with mixed virus samples, never PMV alone.

The sedimentation coefficients for the RNAs of the PMV-type and the SPMV-type were determined by density gradient

TABLE 1. Serotyping and electrophoretic mobility of several isolates of panicum mosaic virus (PMV) and their satellite viruses

Isolate	PMV serotypes[b]	Relative electrophoretic mobility of the PMV serotypes	Presence of satellite	Satellite serotypes
PMV-type	1	Slow	+	Type
PMV-L1	2	Slow	+	L1
PMV-L2	3	Slow	+	L1
PMV-Tx	4	Slow & Fast	+	L1
PMV-L3	5	Fast	+	Type
PMV-L4	5	Fast	+	L1
MSV[a]	6	Fast	−	None

[a] MSV = Molinia streak virus, a serotype of PMV.

[b] Numbers are used to designate different serotypes. The same number appearing for two different isolates indicates that they are not serologically different.

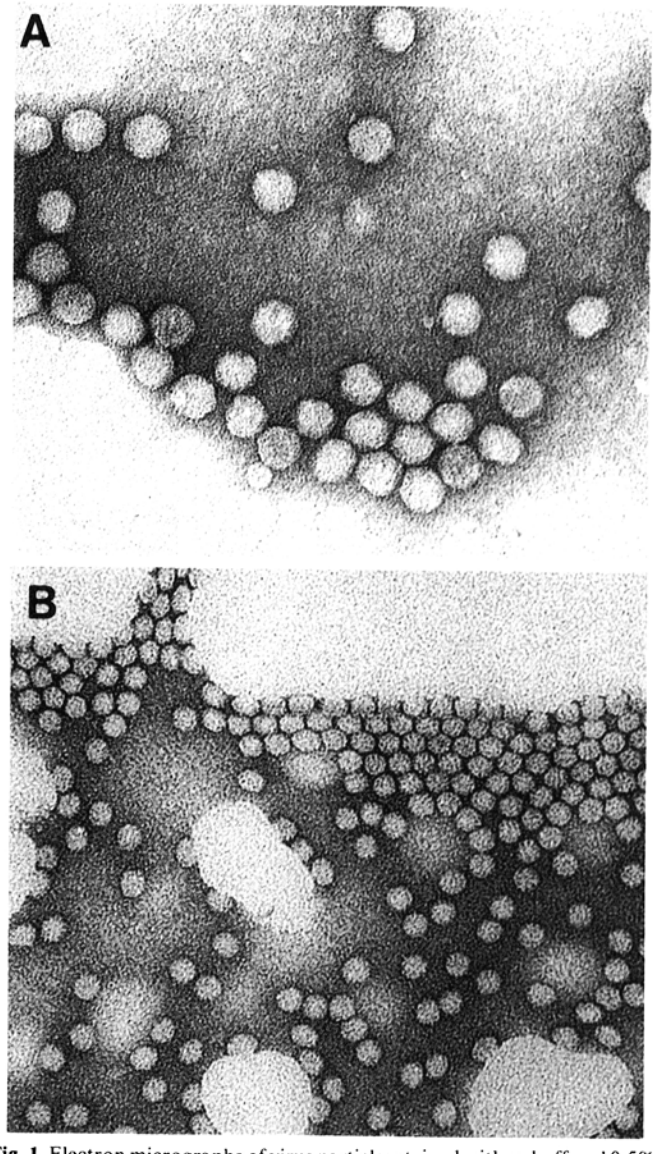

Fig. 1. Electron micrographs of virus particles stained with unbuffered 0.5% ammonium molybdate. **A,** Panicum mosaic virus; **B,** satellite panicum mosaic virus (×195,000).

centrifugation. Regardless of the extraction procedure, only a single RNA sedimenting at 28S (Fig. 3) was obtained from PMV. The number and sedimentation rate of RNAs extracted from SPMV depended on the extraction procedure used (Fig. 3). One RNA species (14S) was always present, but the second RNA species (34S) depended on the absence of a reducing agent. Incorporation of either β-mercaptoethanol or dithiothreitol into Brakke's dissociation buffer caused the 34S species to diminish quantitatively, with a concomitant increase in the amount of 14S RNA. Further, only the 14S RNA was observed when SPMV was phenol extracted. We conclude that the 34S species was composed of 14S RNA and some remaining capsid protein. Apparently, the presence of a reducing reagent or phenol extraction causes complete removal of the capsid protein. The homogeneity of the 34S species is noteworthy.

Serology. In agar double diffusion tests, six PMV serotypes were differentiated using three antisera provided by R. Toler and the antisera prepared as described above. SPMV, associated with all PMV isolates except MSV, was differentiated into two serotypes based on spur formation in agar double diffusion serology. Complete results are reported in Table 1. Antisera titers of 1/1,024 (PMV) and 1/128 (SPMV) were obtained in agar double diffusion reactions using 0.25 mg of homologous antigen per milliliter.

Using antisera prepared above and antisera to PhMV and CMMV provided by H. Paul, our results with PMV-type, PMV-L4, and MSV agree with those of Paul et al (22) who subdivided these viruses into two main clusters typified by PMV and CMMV. They reported MSV to be distantly related to PhMV and CMMV, whereas we obtained strong serological reactions indicative of a closer relationship. It would be interesting to determine if PhMV

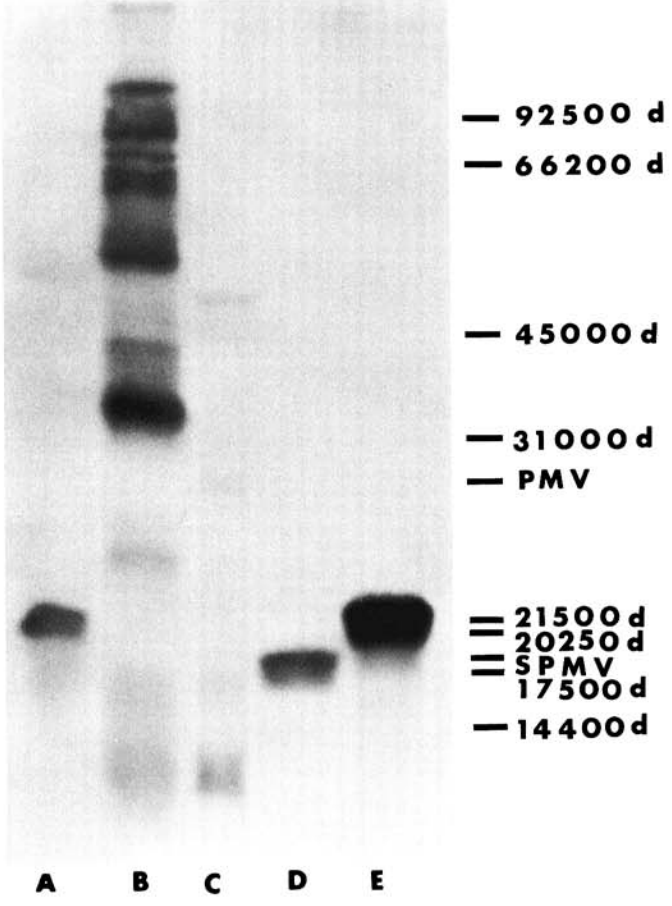

Fig. 2. Fluorogram showing the translation products from the RNA of **A,** brome mosaic virus (BMV); **B,** tobacco mosaic virus (TMV); **C,** panicum mosaic virus (PMV); **D,** satellite panicum mosaic virus (SPMV), and **E,** satellite tobacco necrosis virus compared to markers showing the position of molecular weight standards and authentic coat protein.

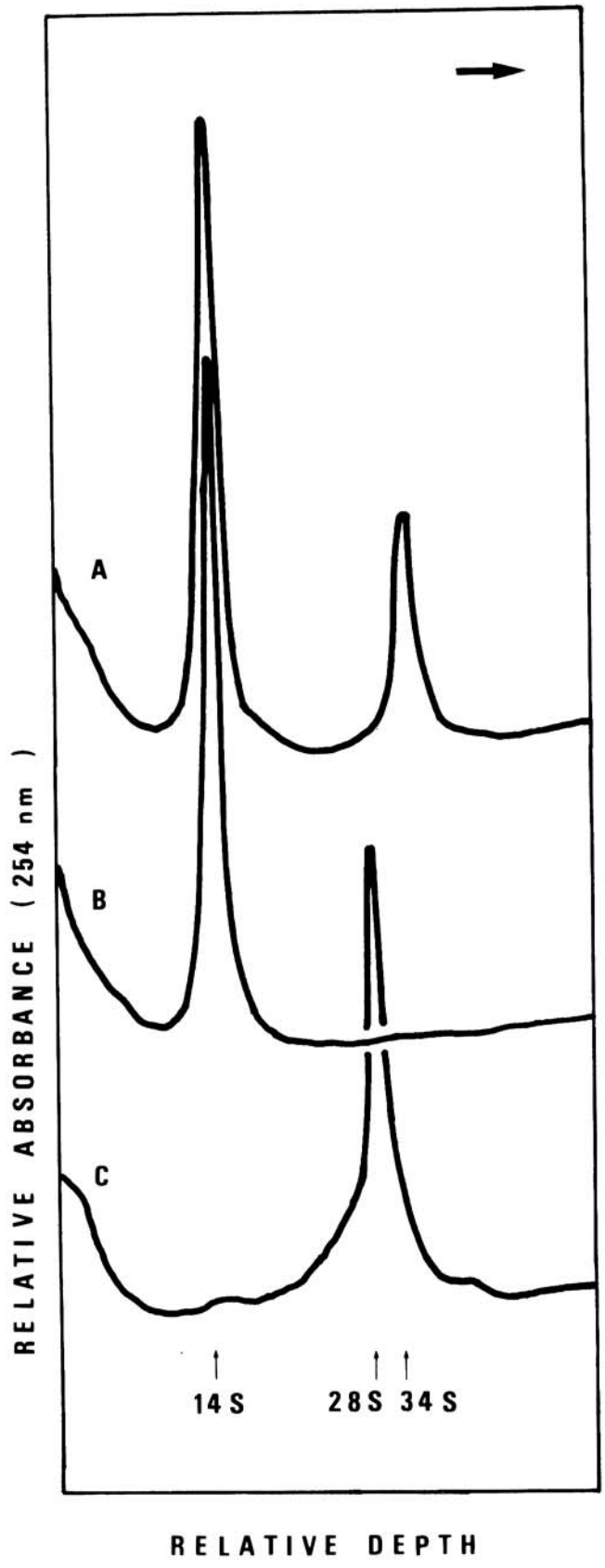

Fig. 3. Sedimentation profiles of RNA extracted from **A,** satellite panicum mosaic virus (SPMV) by incubation overnight at room temperature in 0.02 M tris, 0.10 M NaCl, 0.001 M EDTA, 400 μg/ml bentonite, and 1% sodium dodecyl sulfate (SDS), pH 9.0; **B,** SPMV by boiling for 2 min in 0.08 M tris, 0.04 M Na acetate, 0.002 M EDTA, 2.4% SDS, 1% β-mercaptoethanol, pH 7.0, and **C,** panicum mosaic virus by either of the above methods. Samples ($A_{260\ nm}$ = 0.5 to 1.0) were centrifuged through sucrose linear-log density gradients for 6.0 hr at 283,000 *g* and 15 C. Direction of sedimentation is indicated by the arrow.

and CMMV could activate SPMV replication.

Agar double diffusion tests were used to determine serological relationships among PMV, SPMV, TNV, and STNV. Positive reactions occurred only with homologous reactants; no heterologous reactions were observed.

Electrophoresis. PMV serotypes could be further differentiated on the basis of their relative electrophoretic mobility in 2.8% polyacrylamide gels, with one group migrating faster than the other (Table 1; Fig. 4). The two serotypes of SPMV had the same apparent electrophoretic mobility.

When the two electrophoretic forms of PMV were tested by agar double diffusion reactions with antiserum that contained antibodies to both electrophoretic forms, two distinct precipitin lines were formed. The reaction nearest the center antiserum well was that of the slow-electrophoretic form, and the reaction nearest the outer antigen well was that of the fast-electrophoretic form. Based on electrophoretic mobility and the precipitation pattern in agar double diffusion, PMV-Tx was shown to be a mixture of two electrophoretic forms, which reasonably could be considered a mixture of two different serotypes.

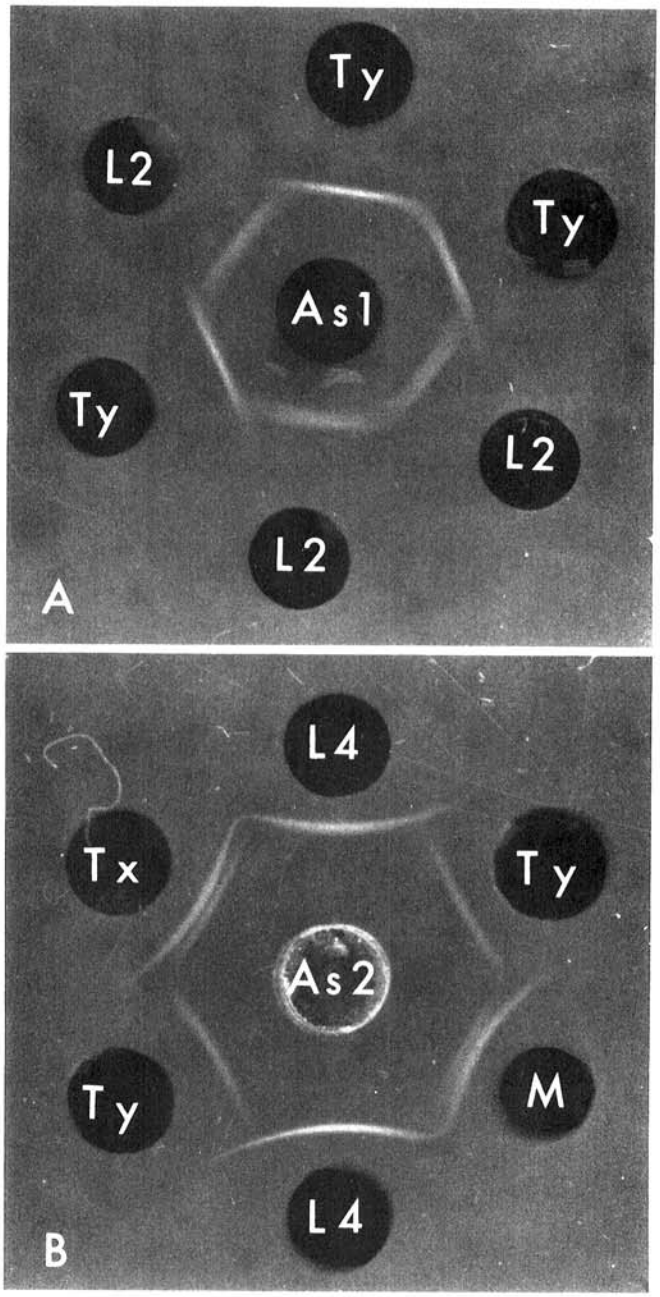

Fig. 4. Relative electrophoretic mobilities of **A,** panicum mosaic virus (PMV)-St. Augustine decline strain L4; **B,** PMV-type, and **C,** molinia streak virus on 2.8% polyacrylamide gels electrophoresed for 2 hr at 200 V. The arrow indicates the direction of migration.

Amino acid analysis. The amino acid compositions of three PMV and two satellite serotypes are shown in Table 2. No major differences were observed over the various hydrolysis times; hence the 24-hr values are reported. The amino acid compositions of the three PMV serotypes are similar, but obvious differences are apparent (eg, Asp, Thr, Ala, Val, Met, Ile, and Phe). The composition of the SPMV serotypes also resemble one another, but differ greatly from those of PMV (especially Lys, His, Asp, Thr, Pro, Ala, Val, Ile, and Phe).

The differences in amino acid composition occurring within and between the SPMV serotypes and the PMV serotypes are sufficient to explain the serological and electrophoretic differences detected.

Base composition analysis. Base compositions determined for the RNA of MSV, PMV-L4, PMV-type, SPMV-type, SPMV-L1, and tobacco mosaic virus (TMV) are shown in Table 3. TMV RNA was used as an internal control of the HPLC procedure, and the base composition agreed favorably with that reported by Zaitlin and Israel (34). The base compositions for the three PMV serotypes showed similar patterns, with approximately the same amount of each base although the amount of uridine was slightly lower. The base compositions for the two satellite serotypes were very similar to one another, but differed from the PMV serotypes by having a high G-C (guanosine/cytidine) content and a very low A (adenosine) content.

Translation of viral RNA. The RNA translation products for PMV-type and SPMV-type are shown in comparison to one another and to the RNA translation products from brome mosaic virus (BMV), STNV, and TMV in Fig. 2. To estimate their molecular weights, the translation products were compared to authentic coat proteins and to molecular weight standards using

TABLE 2. Amino acid compositions of serotypes of panicum mosaic virus (PMV) and satellite panicum mosaic virus (SPMV)[a]

Amino acid	MSV[b]	PMV-L4	PMV-type	SPMV-type	SPMV-L1
Lys	3.80[c]	4.08	4.20	2.26	2.67
His	1.51	1.50	1.61	0.75	0.88
Arg	9.49	8.68	9.99	9.22	8.09
Asp	5.07	6.87	6.70	8.53	9.45
Thr	10.94	11.76	8.07	10.55	11.12
Ser	7.99	8.94	9.65	9.41	8.74
Glu	8.76	8.72	7.79	8.08	8.19
Pro	8.36	8.04	8.57	5.94	6.34
Gly	9.11	9.90	10.08	8.43	8.99
Ala	8.42	5.56	7.14	10.85	10.98
Val	7.26	6.01	5.42	7.42	7.65
Met	2.06	1.31	1.63	1.83	0.77
Ile	3.76	5.07	5.62	2.34	2.37
Leu	8.60	8.30	8.65	7.18	7.30
Tyr	2.93	2.10	2.92	2.47	2.16
Phe	1.93	3.15	1.93	4.76	4.32
Total	99.99	99.99	99.97	100.02	100.02

[a] Values are for 24 hr hydrolysis.
[b] MSV = Molinia streak virus, a serotype of PMV.
[c] Expressed as mole percent of individual amino acids.

TABLE 3. Base composition of the RNS of several serotypes of panicum mosaic virus (PMV) and satellite panicum mosaic virus (SPMV)

Nucleoside	MSV[a]	PMV-L4	PMV-type	SPMV-type	SPMV-L1	TMV[a]
Uridine	21.3[b]	21.4	21.7	20.7	21.0	28.1
Cytidine	26.8	27.3	26.5	32.1	30.2	18.1
Guanosine	25.6	25.1	26.4	28.4	29.7	25.6
Adenosine	26.4	26.3	25.6	18.9	19.2	28.3

[a] MSV = molinia streak virus, a serotype of PMV; TMV = tobacco mosaic virus.
[b] Results expressed as percent of each nucleoside. Precision is ± 0.4%.

polyacrylamide slab gel electrophoresis.

Incorporation of ^{14}C-leucine into the translation products of BMV, STNV, and TMV RNA was 23-, 50-, and 95-fold over that of controls, respectively. With PMV and SPMV RNA the incorporation as only 10- and 15-fold, respectively.

Gel electrophoresis showed the several translation products expected for TMV and BMV RNAs (2,28). Several labeled products also were observed for PMV RNA. One coelectrophoresed with unlabeled PMV coat protein (30,000 daltons), and another had a molecular weight of ~50,000 daltons. The major product, a diffuse band of low-molecular-weight products (<20,000 daltons), migrated at or near the buffer front. These proteins may represent incomplete peptides which result from incomplete translation or the translation of degraded RNA, and may in part explain the low levels of incorporation when PMV RNA was used as a message. The role of the translation products was not investigated. Obviously, PMV RNA is not a monocistronic messenger but resembles TMV RNA in its ability to direct the synthesis of several proteins. Both STNV and SPMV RNAs directed synthesis of a single, labeled translation product. The SPMV RNA translation product, which comigrated with authenic coat protein, was of slightly lower molecular weight (MW = 16,000 daltons) than the STNV RNA translation product (MW = 23,000 daltons). Apparently, SPMV RNA resembles STNV RNA in that both are monocistronic messages in the wheat germ system. Based on the molecular weight of the coat protein (16,000 daltons) and the estimated number of nucleotides in the RNA (~1,200), SPMV RNA could conceivably code for more than its coat protein, as could STNV RNA (25).

PMV and SPMV represent a new satellite virus system. Biologically, they mimic the relationship between TNV and STNV. However, no serological relationships exist among these viruses. Further research may elucidate other relationships between PMV and SPMV that parallel those between TNV and STNV. For example, selective activation may exist between PMV and SPMV serotypes although none was demonstrated here. Clearly, PMV and SPMV are a new and interesting satellite virus system which, with other satellite virus systems, may be used as a research tool.

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