FURTHER CHARACTERIZATION OF PANICUM MOSAIC VIRUS AND ITS ASSOCIATED SATELLITE VIRUS

by

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INTRODUCTION

Panicum mosaic virus (PMV) was first described by Sill and Pickett (28) 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, 21, 28, 29, 30). 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 (21) demonstrated that there were two centrifugal components associated with PMV. In preliminary reports Buzen and Niblett (5) and Buzen et al. (4) indicated that one component (42 S) was isometric, 17 nm in diameter and not infectious. It contained two RNA species (14 and 34 S), a single protein species (15,500 d), and was serologically unrelated to the other component (109 S). The 109 S component was isometric, 28-30 nm in diameter and infectious. It contained a single RNA species (28 S) and a single protein species (28,000 d). From these results Buzen and Niblett (3) concluded that the 42 S component was a satellite virus, designated satellite panicum mosaic virus (SPMV), which was dependent upon the 109 S component for its replication. The 109 S component is designated PMV, as it is capable of replicating in the absence of SPMV (3). The purpose of this research was to examine several isolates of PMV and SPMV to determine additional characteristics of these two viruses.

MATERIALS AND METHODS

The type strain of PMV and its associated SPMV (SPMV-type) were obtained from the virus collection at Kansas State University. Dr. G. Holcomb (Louisiana State University) kindly provided the isolates of PMV-SAD which

we designated as PMV-L1, PMV-L2, PMV-L3, and PMV-L4. Dr. R. Toler (Texas A & M University) kindly provided the isolate of PMV-SAD designated PMV-Tx and three antisera made to a mixture of satellite and helper viruses. Dr. 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 mottle virus (CfMV). Dr. J. K. Uyemoto (Kansas State University) 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 Dr. J. M. Clark (University of Illinois).

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 at a 1:3 (w/v) dilution using cold 0.02 M potassium phosphate buffer, pH 7.0 (KPO_4). For small scale inoculations, tissue was ground using a mortar and pestle and inoculated onto carborundum-dusted leaves. For large scale inoculations, tissue was homogenized in a Waring blendor 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 to 7 kg/cm^2 using a DeVilbiss No. 152 sprayer.

Purification.—Virus was purified as described by Niblett and Paulsen (21) with the following modifications. Millet was inoculated at the 4 to 5 leaf stage and both 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 at a 1:3 (w/v) dilution in cold 0.1 M KPO_4 plus 1% β-mercaptoethanol. Homogenized tissue was expressed through four layers of cheesecloth. The liquid was centrifuged for 10 min at 10,000 *g* and the supernatant was made 8% (w/v) polyethelene glycol 6,000 and 0.2 M NaCl. After stirring two hours at 4 C, the precipitate was collected

by centrifuging 10 min at 8,000 g. Pellets were resuspended overnight in 0.1 M KPO_4 at 1 ml per 2 g 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 then centrifuged for two hr at 363,000 g. Pellets were resuspended in 0.02 M KPO_4.

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. Components were collected using an ISCO fractionator. PMV and SPMV fractions were pooled separately, dialyzed overnight to remove sucrose, and centrifuged at 363,000 g for one hr (PMV) or two hr (SPMV). Pellets were resuspended in 0.02 M KPO_4. Individual viruses used for antiserum production, amino acid analysis, and RNA extraction were purified by two cycles of density gradient centrifugation.

Separation and activation of SPMV.—To properly designate SPMV as a satellite virus it must be demonstrated that it is dependent on PMV for its replication. This is termed activation. PMV also must be capable of replication in the absence of SPMV.

Starting with 1.0 mg/ml of purified PMV a series of 10-fold dilutions were made. Millet was inoculated and approximately 30 g tissue was harvested for each treatment. Virus was purified and the components assayed by density gradient centrifugation. Those preparations containing only 109S particles were used as inoculum and propagated through several cycles to ensure the absence of 42 S particles.

Activation of SPMV-type by PMV-type was tested by inoculating millet with SPMV-type, PMV-type, and a mixture of the two. Purified SPMV-type was used at 0.1 mg/ml. PMV-type was obtained from infected tissue derived from

the experiment above. For the mixture, tissue containing PMV-type was homogenized using purified SPMV instead of buffer. Experiments with MSV were performed similarly. Virus production was assayed as above.

Physical characteristics.—The size of the PMV and SPMV particles was determined by measuring electron micrographs of purified SPMV-type and PMV-type which had been negatively stained with ammonium molybdate.

The molecular weights of the capsid proteins were estimated using disrupted purified virus. Preparations were suspended in 62.5 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 a 11% polyacrylamide slab gel containing 0.2% SDS as described by Lugtenberg et al. (15). The molecular weight was determined by comparison to molecular weight standards and known viral proteins 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, 0.10 M NaCl, 0.001 M EDTA, 400 µg/ml bentonite, 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, 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). To further ensure purity, purified viruses were electrophoresed on 2.8% polyacrylamide gels as described by Semancik (26). Viral bands, located by comparison to a companion gel that had been fixed 5 min in 17.4 N acetic acid, were excised and mixed with an equal volume of distilled water. They were then placed in a syringe and extruded through a 23 gauge needle. An equal volume of adjuvant was mixed with the gel-water mixture and injected

intramuscularly into rabbits. The first injection utilized Freund's complete adjuvant and subsequent injections utilized Freund's incomplete adjuvant. Rabbits were injected with 0.5 mg (SPMV) or 1.0 mg (PMV) virus in a total volume not exceeding three ml, three times at two wk intervals, followed by a booster injection four wk after the third injection. Rabbits were bled two wk after the booster injection.

Immunodiffusion tests were performed in 0.75% ion agar containing 0.05 M Tris-HCl, 0.85% NaCl and 0.02% NaN_3. Reactants were arranged as recommended 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 Beckman model 121 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. (22). The preparation was then filtered through an Amicon PM 10 ultrafilter to remove the enzymes and analyzed by high performance liquid chromotography (HPLC) on a Micro Pak MCH 10 column using 3% methanol, 97% 10 mM perchloric acid, 5 mM heptane sulfonate. The flow rate was 2 ml/min. Nucleosides were detected at 260 nm. The peak area was determined using an intergrator 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 C^{14} leucine, 20 µl S 30 and 5 µg viral RNA in a final volume of 100 µl. The reaction was carried out at room temperature for 2 hr and was 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 counting cocktail.

For electrophoresis of the translation products the reaction was stopped by precipitation with one ml cold acetone. After 12 hr at -70 C, the precipitate was collected by centrifugation for 10 min at 1500 *g* and dried under N_2. The precipitate was resuspended in 100 µl of 62.5 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 salicilate 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 109 S PMV particles and no 42 S SPMV particles were recovered. These preparations were monitored through several cycles of PMV

purification and only PMV was detected. These preparations were considered to be 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 (Table 1). 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 having been reported in the United States, are capable of activating SPMV-type replication. Also it is interesting to note that no satellite virus was found associated with MSV and that two different SPMV serotypes are capable of replication in the presence of a single PMV serotype (Table 2). No selective activation similar to that demonstrated for TNV and STNV (13, 31) 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 SPMV-type 15-16 nm (Fig. 1). This is in reasonable agreement with our preliminary reports (4, 20).

The molecular weights of the viral capsid proteins were determined using SDS-polyacrylamide slab gel electrophoresis. When compared to molecular weight standards and capsid proteins of known viruses, values of about 16,000 d and 30,000 d were determined for the capsid proteins of SPMV and PMV, respectively (Fig. 2). The same molecular weights were obtained when other serotypes of SPMV and PMV were examined.

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

extraction procedure, only a single RNA sedimenting at 28 S (Fig. 3) was extracted from PMV. The number and sedimentation rate of RNAs extracted from SPMV depended on the extraction procedure used (Fig. 3). One RNA species (14 S) was always present, but the occurrence of the other RNA species (34 S) depended on the absence of a reducing agent. Incorporation of either β-mercaptoethanol or dithiothreitol into the extraction buffer removed the 34 S species quantitatively with a concomitant increase in the amount of 14 S RNA. We conclude that the 34 S species was composed of 14 S RNA and some remaining capsid protein. Apparently, the presence of a reducing reagent or phenol extraction causes complete removal of the capsid protein. The homogenicity of the 34 S species is noteworthy.

Serology.—Six PMV serotypes were differentiated using three antisera provided by Dr. R. Toler and antisera prepared above to monitor spur formation in agar double diffusion reactions. Examples are shown in Fig. 4 and complete results are shown in Table 2. SPMV was associated with all PMV isolates except MSV. Two serotypes of SPMV were differentiated. Antisera titers of 1/1024 (PMV) and 1/128 (SPMV) were obtained in agar double diffusion reactions using 0.25 mg/ml homologous antigen.

Using antisera prepared above and two antisera provided by Dr. H. Paul, a progressive serological relationship was observed. In terms of the intensity of the serological reaction, the order of progression was: PMV-type, PMV-L4, MSV, PhMV, and CfMV.

The serotypes of PMV 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 2; fig. 5). The serotypes of SPMV had the same apparent electrophoretic mobility.

When the two electrophoretic forms of PMV were tested by agar double diffusion reactions against antiserum that contained antibodies to both

electrophoretic forms, two distinct precipitin lines were formed (Fig. 4). The reaction nearest the center antiserum well was shown to be that of the slow-electrophoretic form and the reaction nearest the outer antigen well was shown to be 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 two different serotypes.

Agar double diffusion was used to test for serological relationships among PMV, SPMV, TNV and STNV. Tissue containing a mixture of TNV and STNV was tested against TNV, STNV, PMV and SPMV antisera. Also, tissue containing a mixture of PMV and SPMV as well as purified PMV and SPMV were tested against the same four antisera. No precipitin lines were formed between tissue containing TNV and STNV and antisera to PMV and SPMV. Conversely, no precipitin lines were formed between crude or purified preparations of PMV and SPMV and antisera to TNV and STNV. Purified PMV failed to react with SPMV antisera and purified SPMV failed to react with PMV antisera. Serological reaction did occur for each homologous antigen and antiserum tested.

SPMV, differentiated into two serotypes, was associated with all six PMV serotypes except MSV. The PMV serotypes could be separated into two groups by their relative electrophoretic mobility in polyacrylamide gel electrophoresis. Members of the PhMV group (12) are serologically related to PMV and a progressive serological relationship was observed among them. Perhaps the PhMV group would be better called the PMV group using PMV as the type member. Then the ability to activate SPMV replication also could be used to determine relationships within this group. No serological relationship was observed among PMV, SPMV, TNV, and STNV.

Amino acid analysis.—The amino acid compositions of three PMV and two satellite serotypes are shown in Table 3. No major discrepancies were

observed over the various hydrolysis times. The amino acid compositions of the three PMV serotypes are similar, but obvious differences are apparent (e.g. ASP, THR, ALA, VAL, MET, ILE and PHE). The compositions 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 amino acid compositions of three PMV serotypes, (type, L4, and MSV), are similar to one another but differ greatly from those of two satellite serotypes, (type and L1). The amino acid compositions of the two satellite serotypes also resemble one another. Apparently, the differences in amino acid composition occurring within the SPMV serotypes and the PMV serotypes are sufficient to impart the serological and electrophoretic differences within each group. The greater amino acid differences occurring between the two groups may partially explain their lack of serological relatedness. Undoubtly the secondary and tertiary structure of the capsid protein also is important in the serological differences observed. The amino acid compositions of PMV and SPMV differ greatly from those determined for TNV and STNV (23, 32). Thus the absence of any serological relationships among these four viruses is not unexpected.

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 4. TMV RNA was used as an internal control of the HPLC procedure. The base compositions for the three PMV serotypes showed a similar pattern, with approximately the same amount of each base although the amount of uridine was slightly low. 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/cytosine) content and a very low A (adenosine) content. The base composition for TMV agreed favorably with that reported by Zaitlin and Israel (33).

The base compositions for the RNAs of PMV-type, PMV-L4, and MSV are very similar to one another, with the amount of each base about equal, but differ from those of SPMV-type and SPMV-L1 which have a high G-C and a low A content. Mossop and Francki (19) showed that STNV RNA survived *in vivo* without replication up to 10 days in the absence of TNV. When TNV was subsequently inoculated, STNV RNA was then activated and replicated normally. They speculated that this stability could be attributed to a more extensive secondary structure resulting from base pairing. If true, SPMV should have an even greater *in vivo* stability due to its higher G-C content (60% compared to 42% for STNV).

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 Figure 2. To estimate their molecular weight the translation products were compared to authentic coat proteins and to molecular weight standards using polyacrylamide slab gel electrophoresis.

Incorporation of C^{14} 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 was only 10- and 15-fold, respectively.

Gel electrophoresis showed the several translation products expected for TMV and BMV RNAs (2, 27). Several labeled products also were observed for PMV RNA with two obvious peptides. One co-electrophoresed with unlabeled PMV coat protein (MW = 30,000) and the other was approximately 50,000 molecular weight. Also, a diffuse band of low molecular weight products (<20,000 d) was observed. Both STNV and SPMV RNAs directed synthesis of single, labeled translation products. The SPMV RNA translation product which co-migrated with authentic coat protein was of slightly lower molecular

weight than the STNV RNA translation product.

Several labeled proteins were synthesized when PMV RNA was used as the messenger RNA. One product which occurred in significant amounts co-electrophoresed with authentic PMV coat protein. The major product was a protein (or proteins) of <20,000 MW, which 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 monocystronic messenger, but resembles TMV RNA in its ability to direct the synthesis of several proteins. In contrast, a single labeled protein corresponding in electrophoretic mobility to authentic SPMV coat protein was translated for by SPMV RNA. Similarly, STNV RNA yielded a single labeled protein. The molecular weight of the STNV product was greater than that of SPMV RNA which is in agreement with the relative sizes of the coat proteins. Apparently, SPMV RNA resembles STNV RNA in that both are monocystronic messengers in the wheat germ translation system. Based on the molecular weight of the coat protein (16,000 d) and the estimated number of nucleotides ($\sim$1200) in the RNA, SPMV RNA could conceivably code for more than its coat protein, as could STNV RNA (24).

The relationship of PMV and SPMV represents a new satellite virus system. Biologically, PMV and SPMV mimic the relationship between TNV and STNV. However, no serological relationships exist among these viruses. Perhaps additional research may elucidate other relationships between PMV and SPMV that parallel those between TNV and STNV. For example, selective activation may be shown to exist between PMV and SPMV serotypes although none was demonstrated here. The longevity *in vivo* of PMV and SPMV RNA needs to be explored and perhaps it may rival that found for TNV and STNV (19). Clearly, PMV

and SPMV are a new and interesting satellite virus system which along with other satellite virus systems, may be used as a tool in research and may offer a unique system for studying specificity of plant viral replicases.

TABLE 1. ACTIVATION OF SATELLITE PANICUM MOSAIC VIRUS (SPMV) BY PANICUM MOSAIC VIRUS (PMV)

VIRUSES INOCULATED	VIRUSES RECOVERED
SPMV-type	None
PMV-type	PMV-type
MSV[a]	MSV
SPMV-type + PMV-type	SPMV-type + PMV-type
SPMV-type + MSV	SPMV-type + MSV

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

TABLE 2. CHARACTERISTICS OF SEVERAL ISOLATES OF PANICUM MOSAIC VIRUS (PMV) AND THEIR SATELLITE VIRUSES

ISOLATE	PMV SEROTYPES	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 serotype of PMV.

TABLE 3. AMINO ACID COMPOSITIONS OF SEVERAL 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 serotype of PMV.

[c]Expressed as mole percent of individual amino acids.

TABLE 4. BASE COMPOSITIONS OF THE RNA OF SEVERAL SEROTYPES OF PANICUM MOSAIC VIRUS (PMV) AND SATELLITE PANICUM MOSAIC VIRUS (SPMV)

	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 serotype of PMV; TMV = Tobacco mosaic virus.

[b]Results expressed as percent of each nucleoside, and the precision is ± 0.4%.

Figure 1. Electron micrographs showing purified A) PMV and B) SPMV particles. Preps were stained with ammonium molybdate. Magnification is 195,000 X.

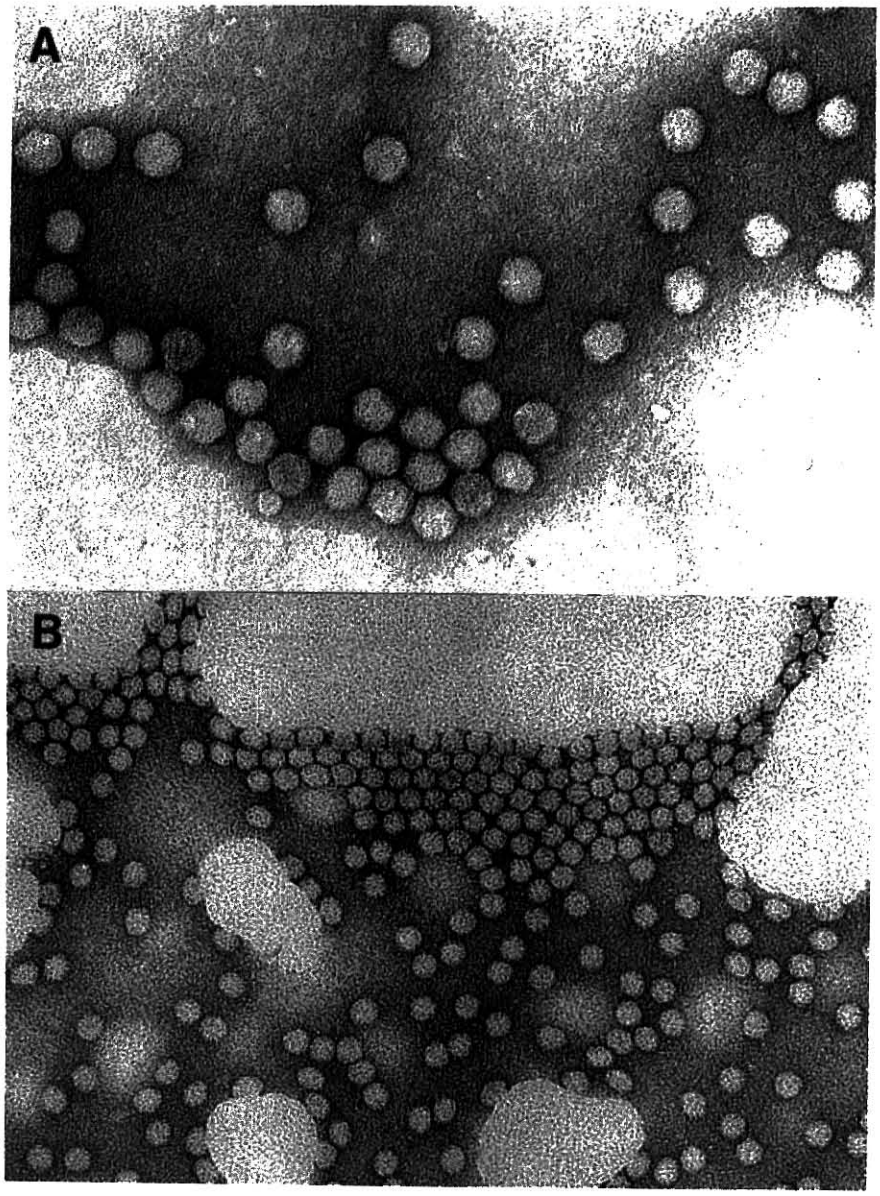
A
B

Figure 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 (reading top to bottom) of authentic coat protein from F) BMV and TMV, G) PMV and SPMV, and H) molecular weight standards (phosphorylase B, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, and lysozyme). Electrophoresis was performed for three hr in 11% acrylamide gels containing 0.2% sodium dodecyl sulfate.

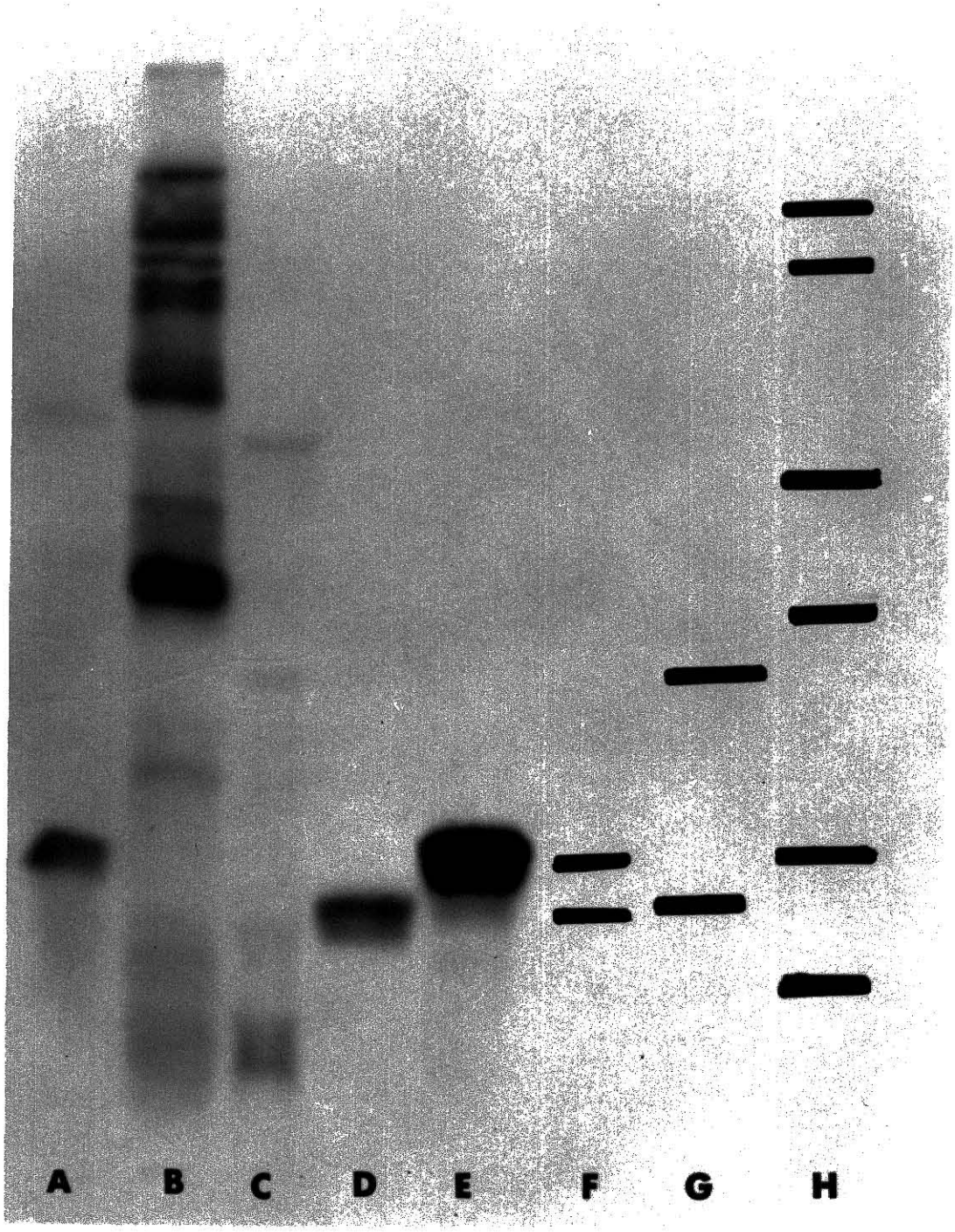
A
B
C
D
E
F
G
H

Figure 3. Sedimentation patterns of RNA extracted from A) SPMV by incubation overnight at room temperature in 0.02 M Tris, 0.10 M NaCl, 0.001 M EDTA, 400 µg/ml bentonite, 1% sodium dodecyl sulfate (SDS), pH 9.0, B) SPMV by boiling 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, C) PMV by either of the above methods. Samples (A_{260} = 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 arrow.

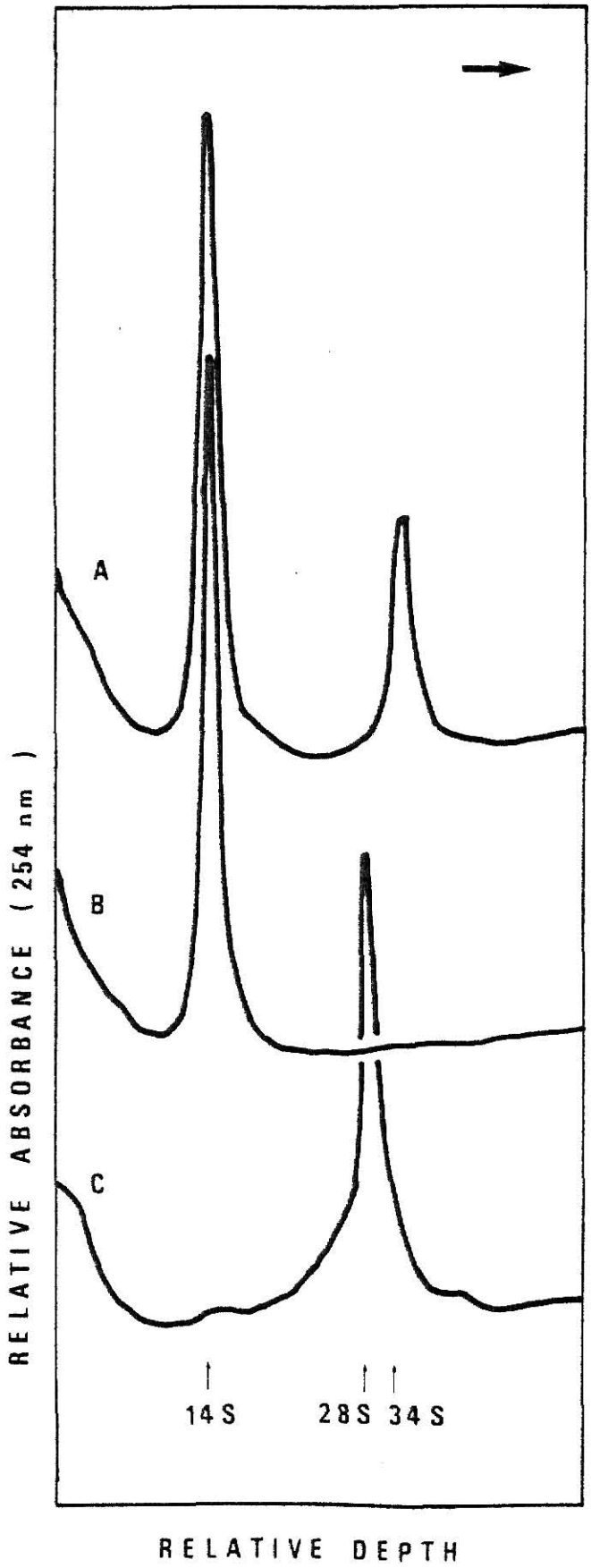
A
B
C
14S
28S
34S
RELATIVE ABSORBANCE (254 nm)
RELATIVE DEPTH

Figure 4. Precipitation patterns of agar double diffusion reactions of panicum mosaic virus (PMV). In Figure 1a, PMV-type (Ty) and PMV-L2 (L2) were reacted against antiserum made to a St. Augustine decline isolate of PMV (PMV-SAD; As 1). In Figure 1b, a fast electrophoretic migrating serotype, PMV-L4 (L4), a slow electrophoretic migrating serotype, PMV-type (Ty), a mixture of both serotypes (M), and PMV-Tx (Tx) were tested against a PMV-SAD antiserum (As 2) that contained antibodies to both the fast and slow electrophoretic serotypes.

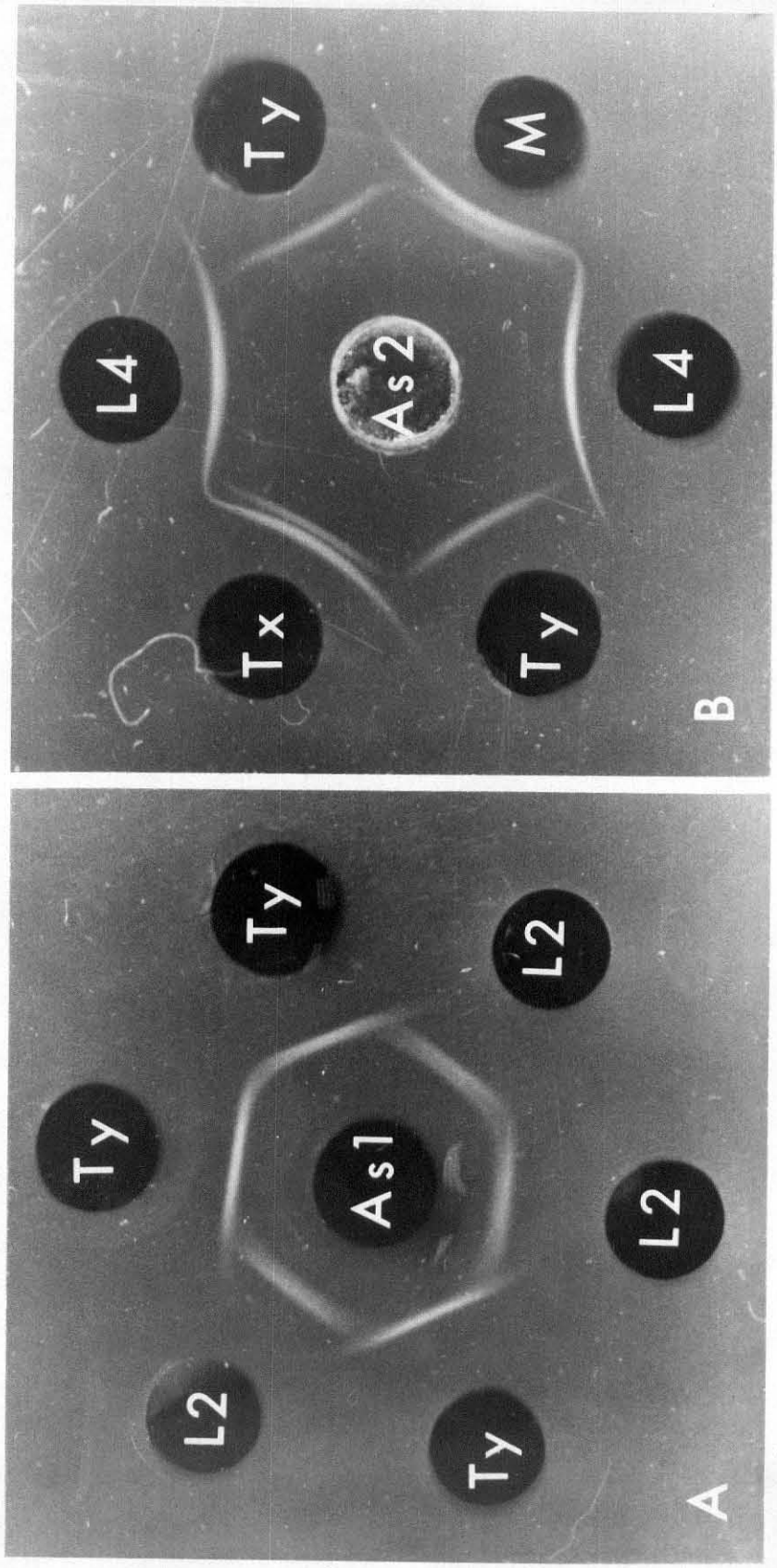
A
Ty
Ty
L2
As1
L2
Ty
L2
B
L4
Ty
Tx
As2
M
Ty
L4

Figure 5. Relative electrophoretic mobility of A) panicum mosaic virus St. Augustine decline strain L-4, B) panicum mosaic virus-type and C) molinia streak virus on 2.8% polyacrylamide gels electrophoresed for 2 hr at 200 v.


A
B
C

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FURTHER CHARACTERIZATION OF PANICUM MOSAIC VIRUS AND ITS ASSOCIATED SATELLITE VIRUS

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AN ABSTRACT OF A MASTER'S THESIS

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ABSTRACT

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 with two serotypes of SPMV and six of PMV. The serotypes were differentiated on the basis of their reactions with homologous and heterologous antisera. The PMV serotypes were separated into two groups based on their relative electrophoretic mobility. A progressive serological relationship exists between PMV and those members of the phleum mottle virus (PhMV) group tested. The order of the progression in terms of the intensity of the serological reaction was: the type strain of PMV, the St. Augustine decline strain of PMV, molinia streak virus (MSV), PhMV, and cocksfoot mottle virus. SPMV was associated with all PMV serotypes tested except 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 molecular weight, and amino acid composition. The RNA translation products for PMV and SPMV were compared. PMV RNA directed the synthesis of several C^{14} labeled proteins including one which co-electrophoresed with authentic PMV coat protein. SPMV RNA directed the synthesis of a single C^{14} labeled protein which co-electrophoresed with its authentic coat protein. While PMV and SPMV mimic the relationship between tobacco necrosis virus and its satellite virus, no serological relationships were found among these four viruses.