Pepper Mild Mottle Virus, a Tobamovirus Infecting Pepper Cultivars in Sicily

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ABSTRACT

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A tobamovirus named pepper mild mottle virus (PMMV) was isolated from tobacco mosaic virus (TMV)-resistant peppers in Sicily, Italy. It could be distinguished by symptomatology and host range from other tobamoviruses. Infected pepper plants yielded up to 1.3 g of purified virus per kilogram of tissue. The virus formed a single band in rate-zonal density gradient centrifugation in sucrose (10–50%). The ultraviolet absorption spectrum had maxima and minima at 260 and 248 nm with ratios A_{max}/A_{min} and $A_{260\ nm}/A_{280\ nm}$ of 1.11 and 1.21, respectively. The specific extinction coefficient was $E^{0.1\%}_{1\ cm,\ 260\ nm} = 3.18$. From the length-distribution histogram a normal length of 312 nm was determined. Angled layer aggregates were observed in the cytoplasm of infected pepper leaf cells. Antisera to PMMV had homologous titers of 1:2,048 and 1:4,096. After absorption with eight heterologous wild strains of TMV, the PMMV antisera still reacted strongly with the homologous virus. No cross reactivity with heterologous strains was observed in the double antibody sandwich form of ELISA, but the indirect ELISA method showed cross reactions between PMMV and other tobamoviruses. The amino acid composition indicated that PMMV is distinct from all other well-established tobamovirus species.

In the last decade, the culture of peppers (*Capsicum annuum* L.) has increased considerably in Sicily, Southern Italy. In 1980, the pepper-growing area was 2,600 hectares, with more than half of it under plastic or glass. Pepper cultivars and hybrids resistant to tobacco mosaic (TMV) and tomato mosaic (ToMV) viruses have been widely adopted, because these are the most damaging viruses in this crop (7,8). In addition to the above-mentioned TMV strains, para-tobacco mosaic virus (PTMV or U2 strain) was also recently found to infect pepper in Italy (7, and C. Wetter, M. Conti, and M. Marte, *unpublished*).

During the winter 1979/1980, unusual viruslike symptoms were observed in a line of cultivar Lamuyo pepper, which is resistant to the common TMV and ToMV strains. The symptoms were most obvious on the fruits, and consisted of reduction in size, malformation and chlorotic mottling with occasional necrosis. Crops planted during October showed no leaf symptoms while crops planted later showed mottling, deformation, and slight twisting of the leaves.

From samples of such plants, kindly sent to us by G. Cartia, Institute of Plant Pathology, University of Catania, Italy, we isolated a new tobamovirus for which we propose the name pepper mild mottle virus (PMMV).

MATERIALS AND METHODS

Virus sources. In addition to PMMV, eight other wild strains of the tobamovirus group (21) were used for comparative host range and serological studies. These and their original sources are listed in Table 1.

Host range and symptomatology. Sap was extracted from pepper leaves and fruits in the presence of 0.01 M phosphate buffer, pH 7.0, and mechanically inoculated to carborundum or Celite-dusted leaves of several indicator plants. These had been grown from seed in a glasshouse, at 18–24 C and 75–90% relative humidity, in steam-sterilized soil. The plants were given supplemental light to provide a 12-hr photoperiod between October and March. PMMV was easily recovered and maintained in *Nicotiana clevelandii* Gray, from which it was inoculated to the following range of plants: Amaranthaceae—*Gomphrena globosa* L.; Chenopodiaceae—*Chenopodium amaranticolor* Coste & Reyn. and *C. quinoa* Willd.; Leguminosae—*Vigna unguiculata* (L.); Labiatae—*Ocimum basilicum* L.; Solanaceae—*Capsicum annuum* L., 'Haubners Szegediner,' 'Liebesapfel,' 'Frühzauber,' and 'Cadice,' *C. chinense* Jacq., *C. frutescens* L., *Datura stramonium* L., *Lycopersicon esculentum* Mill. 'Costoluto di Cambiano,' 'Haubners Vollendung,' 'Improved V. F.,' 'Marmande,' 'Marmolada,' 'Roma,' 'Sweet 100 F1 Hybrid,' and 'Rutgers,' *Nicotiana africana* Merx., *N. debneyi* Domin., *N. glauca* R. Grah., *N. glutinosa* L., *N. megalosiphon* Huerck & Muell., *N. rustica* L., *N. sylvestris* Speg & Gomes, *N. tabacum* L. 'Havana,' 'Samsun Bashi Bagli,' 'Samsun Cavala,' 'White Burley NN' (necrotic line), and SL (systemic line), and 'Xanthi-nc,' *Petunia hybrida* Vilm., and *Solanum melongena* L.; and Umbelliferae—*Eryngium planum* L. After inoculation, the plants were held in the glasshouse for at least 1 mo to observe the presence and appearance of virus symptoms. All plants were checked for PMMV both in inoculated and in uninoculated young leaves by either back inoculation to local lesion hosts (mainly *C. quinoa*, *D. stramonium*, *N. glutinosa*, and *N. megalosiphon* or electron microscopy.

TABLE 1. Tobamoviruses used for comparative host range and serological studies[a]

Virus	Original source	
	Investigator	Country
Cucumber mosaic virus 4 (CV4)	C. A. Knight	USA
Odontoglossum ringspot virus (ORSV)	C. Wetter	Germany
Ohio III strain (O III)	L. J. Alexander	USA
Para-tobacco mosaic virus (PTMV) (= U2 strain)	E. Köhler	Germany
Ribgrass mosaic virus (HRV)	F. O. Holmes	USA
Sunnhemp mosaic virus (SHMV) (cowpea strain)	F. C. Bawden	England
Tobacco mosaic virus (TMV) (U1 = common strain = vulgare)	H. G. Wittmann	Germany
Tomato mosaic virus (ToMV) (dahlemense strain)	G. Melchers	Germany

[a] These strains were obtained from the collection of one laboratory (C. Wetter).

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PPMV, TMV, and ToMV were also tested for their ability to infect *C. frutescens* 'Tabasco' different cultivars of *C. annuum*, and some lines of *C. chinense*. Most seeds of these pepper cultivars and lines, listed in Table 2, were kindly supplied by F. Saccardo, Plant Genetics Laboratory, E.N.E.A., Rome.

In vitro properties. The thermal inactivation and dilution end points of PMMV were determined using *N. clevelandii* as virus source and *N. glutinosa* as indicator plant.

Purification and physical properties. PMMV was purified from fresh leaves of *C. annuum* cultivars Haubners Szegediner, Liebesapfel and Frühzauber, by the method of Boedtker and Simmons (1) (B & S method) or by polyethylene glycol (PEG) precipitation (15) followed by differential centrifugation. Density gradient centrifugation was in 10–50% sucrose in 0.01 M phosphate buffer, pH 7.0, for 2 hr (2). The isoelectric point of purified virus (25) and the specific absorption coefficient and other optical data (24) were determined. In vitro crystallization of PMMV was done as described previously (19).

Electron microscopy. Electron microscopic examination was conducted with a Philips 201 C electron microscope. The magnification of the instrument was calibrated with TMV as a standard of 300 nm. Inoculated leaves were tested for the presence of virus by the leaf-dip or the epidermal strip method, using 2% aqueous uranyl acetate.

For particle measurements, carbon films on mica were floated on virus preparations which were obtained from density gradient centrifugation after dialysis against distilled water. For negative staining, 2% uranyl acetate was used. The normal length of the particles was calculated by determining the arithmetical mean from the main maximum of the length distribution of virions and by determining the mode of the length distribution (4). For thin sectioning, leaf pieces of systemically infected Frühzauber pepper were fixed in a mixture containing 2% formaldehyde and 2.5% glutaraldehyde and postfixed with 1% osmium tetroxide. Enbloc staining was done with 1% uranyl acetate. After dehydration in a graded ethanol series, the samples were embedded in Araldite. Ultrathin sections were stained with uranyl acetate and lead citrate.

Serology. Two rabbits were immunized with PMMV by giving each six intravenous injections containing 1 mg/ml PMMV in saline solution during 2 wk as primary stimulus. Booster injections were given 6–8 wk later with virus emulsified in Freund's incomplete adjuvant (36). Bleedings were taken at monthly intervals. Antisera to the viruses listed in Table 1 were from laboratory (C. Wetter) stocks. Antiserum titers were determined with purified virus by precipitin drop tests on slides (36). Double diffusion gel tests and intragel absorption tests (31) were carried out in a layer of gel on slides of 9 × 4 cm. The gel layer of 0.6% agar (3.5 ml) in 0.01 M phosphate buffer, containing 0.02% Cialit as preservative, was ~1.2 mm in thickness and covered an area of 7 × 4 cm. The slides were stored at high humidity. The double-antibody sandwich ELISA was carried out as described by Clark and Adams (5). Polystyrene microtiter plates (M 129 B, Dynatech, Germany, D-7306 Denkendorf) were used. Wells were coated at 37 C (2 hr) with 5 μg antivirus rabbit immunoglobulin per milliliter. After rinsing, the plates were incubated with antigens overnight in the refrigerator at 10 C. Conjugated antivirus globulins were added for 4 hr incubation at 37 C. The bound enzyme conjugate was determined by adding 200 μl of *p*-nitrophenylphosphate substrate at 1.5 mg/ml. After 1 hr of hydrolysis at room temperature, 50 μl of 3 M NaOH were added and the $A_{405\ nm}$ was read in a Dynatech Minireader. The indirect ELISA was carried out as described previously (34).

Amino acid analysis. Coat protein for the amino acid analysis was prepared by the acetic acid method of Fraenkel-Conrat (12); the protein was freeze-dried and resuspended in 0.1 M pH 8.0 tris-buffer. The amino acid composition of the protein was established by combining the results of several hydrolysis methods. Most residues were determined by hydrolysis in hydrochloric acid: samples of 2 nM of protein were hydrolyzed for 24 hr at 110 C in constant boiling hydrochloric acid (5.7 M) containing 0.02% 2-mercaptoethanol in tubes sealed under nitrogen. After evaporation the sample was analyzed on a Durrum D500 analyzer. Resistant peptide bonds were cleaved under the same hydrolysis conditions for 120 hr. Cysteine was determined as cysteic acid and methionine as methionine sulfone after oxidation in performic acid followed by hydrolysis, according to the method of Reinbolt et al (28). Tryptophan was determined after hydrolysis of the protein with 50 μl of 3 N methanesulfonic acid containing 0.2% 3-(2-aminoethyl)indole for 20 hr at 110 C (20). The solution was diluted with 100 μl of 0.2 M sodium citrate buffer pH 2.2 and analyzed on a Durrum D500 analyzer.

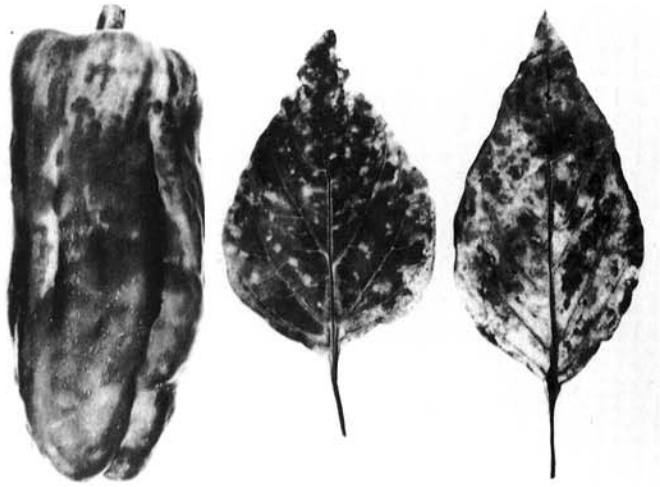

Fig. 1. Symptoms induced by pepper mild mottle virus on a field-infected pepper fruit of cultivar Lamuyo (*left*) and on apical leaves of an artificially inoculated plant of pepper cultivar Quadrato d'Asti 12 days after inoculation (*right*).

TABLE 2. Reactions of different cultivars and lines of *Capsicum* spp. to pepper mild mottle virus (PMMV), tobacco mosaic virus (TMV), and tomato mosaic virus (ToMV) infections

Pepper lines or cultivars	Viruses[a]		
	PMMV	TMV	ToMV
Capsicum annuum 'Corno di Toro'	M	M	M
C. annum 'Cuneo'	M	M	M
C. annum 'Lamuyo'	M	NLL	NLL
C. annum 'Quadrato d'Asti'	M	M	M
C. annum 'Yolo Wonder'	M	NLL	NLL
C. chinense PI 315000	M	M	NLL
C. chinense 'Surinam'	M	NLL	M
C. chinense PI 159236	M	NLL	NLL
C. chinense PI 152225	M	NLL	NLL
C. chinense 'Surinam-4'	M	NLL	M
C. chinense Rn 72-292	M	NLL	NLL
C. frutescens 'Tabasco'	M	NLL	NLL

[a] Abbreviations for symptoms: M = mosaic or mottle, systemic; NLL = necrotic local lesions, not systemic.

RESULTS

Host range and symptomatology. The samples sent from Sicily consisted of some branches of infected peppers bearing a few fruits near maturity. These were slightly deformed and mottled, with large, discolored areas more frequent near the top, and some depressed yellow whitish spots or stripes (Fig. 1). Different cultivars of pepper (*C. annuum*) infected in the glasshouse developed a transient mottle on the top leaves 10–12 days after inoculation (Fig. 1). Later, mottle symptoms and some necrosis appeared on some apical leaves. The infected plants were slightly stunted compared to healthy controls. The reactions of indicator plants inoculated with PMMV and, for comparison with TMV, ToMV, and PTMV, are listed in Table 3. *N. tabacum* cultivars Havana, Xanthi-nc, *N. megalosiphon*, and *S. melongena* reacted

with the formation of local lesions. The necrotic local lesions induced by PMMV were whitish and significantly smaller than those induced by the other tobamoviruses. Systemic infection occurred with all pepper cultivars tested (Table 2), with *N. clevelandii* (severe mottle), and *N. debneyi* (very mild mottle). The following plant species could not be infected with PMMV: *E. planum*, *G. globosa*, *N. africana*, *N. glauca*, *N. tabacum* 'Cavala,' *L. esculentum* (all cultivars tested), and *V. unguiculata*.

PMMV can clearly be distinguished from other wild strains of TMV by the following host reactions: PMMV does not systemically infect Samsun tobacco, but multiplies in the inoculated leaves only, without causing any symptoms. This host reaction is similar to that observed in Samsun tobacco inoculated with ORSV (26), although ORSV does not infect pepper (18). In contrast with ToMV, PMMV does not infect tomato. In this respect it resembles PTMV. The latter virus, however, becomes systemic in Samsun tobacco. In addition, PTMV infects *N. glauca* which is immune to PMMV.

In vitro properties. PMMV was still infective when sap was heated at 90 C but not at 95 C for 10 min, and diluted at 10^{-10}, but not at 10^{-11} with distilled water.

Purification and biophysical properties of PMMV. Fresh leaf tissue of the pepper cultivars Haubners Szegediner, Liebesapfel, and Frühzauber, which were used for propagation, yielded ~0.5 g of virus per kilogram of tissue when the B & S method of purification was used. The PEG method resulted in virus yields up to 1.3 g/kg tissue. Rate-zonal centrifugation of purified virus and of clarified plant sap produced a single band. Virus, purified by the B & S method and suspended in 0.01 M phosphate buffer, pH 7.5, had an ultraviolet absorption spectrum with maxima and minima at 260 and 248 nm. The ratios A_{max}/A_{min} and A_{260}/A_{280} were 1.11 and 1.21, respectively. The specific extinction coefficient was determined to be $E^{0.1\%}_{1\ cm\ 260\ nm} = 3.18$. These values were not corrected for light scattering.

Three determinations of the isoelectric point (I. P.) gave values of pH 3.70, 3.71, and 3.80 when virus purified according to the B & S method was used. Virus purified by PEG precipitation had a significantly lower I. P. of 3.38 although the same concentration of virus (3 mg/ml) dialyzed against distilled water was used. The values 3.7–3.8 are in the range of those for other tobamoviruses (13).

Two-dimensional crystals of PMMV similar to those obtained with other tobamoviruses (Table 1) (19) were produced, but they were stable for only several weeks in contrast to the crystals of other strains which were stable for more than 3 yr (C. Wetter, *unpublished*).

Electron microscopy. Rod-shaped particles typical of tobamoviruses were easily detected in the crude sap of infected plants. For normal length (NL) determinations, virus subjected to rate zonal density gradient centrifugation was used. The NL was determined from length measurements of 334 particles (Fig. 2). Of these, 267 (80%) belonged to the main maximum of the length distribution curve from which an NL of 312 nm (arithmetical mean) was calculated. The value for the mode was 311 nm.

PMMV induced angled-layer aggregates in leaf tissue of pepper Frühzauber (Fig. 3). The angles between the layers ranged from ~60 to 80 degrees, similar to the value found for the aucuba strain of TMV (35). Besides these inclusions, true two-dimensional crystals typical for type TMV were observed.

Serology. The two anti-PMMV sera had homologous titers of 1:2,048 and 1:4,096 when tested by the slide precipitin test. These titers and those with the cross-reacting antigens are summarized in Table 4. The SDI values between the more closely related strains TMV, ToMV, and PTMV are in accordance with those already known (32). The values obtained from tests with PMMV antiserum and the heterologous antigens gave much higher SDI values. Similar results were obtained in the reciprocal tests. Since there is a correlation between the SDI values and the sequence homology of the coat protein (32), these results indicated that PMMV belongs to the more distantly related tobamoviruses.

Gel diffusion tests in agar showed a strong spur formation when

TABLE 3. Comparison of symptoms induced by pepper mild mottle virus (PMMV), tobacco mosaic virus (TMV), tomato mosaic virus (ToMV), and para-tobacco mosaic virus (PTMV) in some indicator plants

Indicator plants	Viruses[a]			
	PMMV	TMV	ToMV	PTMV
Capsicum annum 'Lamuyo'	M	NLL	NLL	NLL/SL
Chenopodium amaranticolor	CLL	CLL	CLL/M	CLL
C. quinoa	CLL	CLL	CLL/M	CLL
Datura stramonium	NLL	NLL	NLL	NLL
Lycopersicon esculentum 'Marmande'	0	M	M	0
Nicotiana clevelandii	M	M	M	M
N. glutinosa	NLL	NLL	NLL	NLL
N. sylvestris	NLL	M	NLL	NLL
N. tabacum 'White Burley' SL	NLL	M	NLL	NLL
N. tabacum 'White Burley' NN	NLL	NLL	NLL	NLL
N. tabacum 'Samsun'	SL	M	M	M
Ocimum basilicum	SL	CLL	CLL	CLL
Petunia hybridia	M/SS	M	NLL	NLL

[a] Abbreviations for symptoms: CLL = chlorotic local lesions, nonsystemic; NLL = necrotic local lesions, nonsystemic; M = mosaic or mottle, systemic; SL = symptomless local infection; SS = symptomless systemic infection; 0 = no infection.

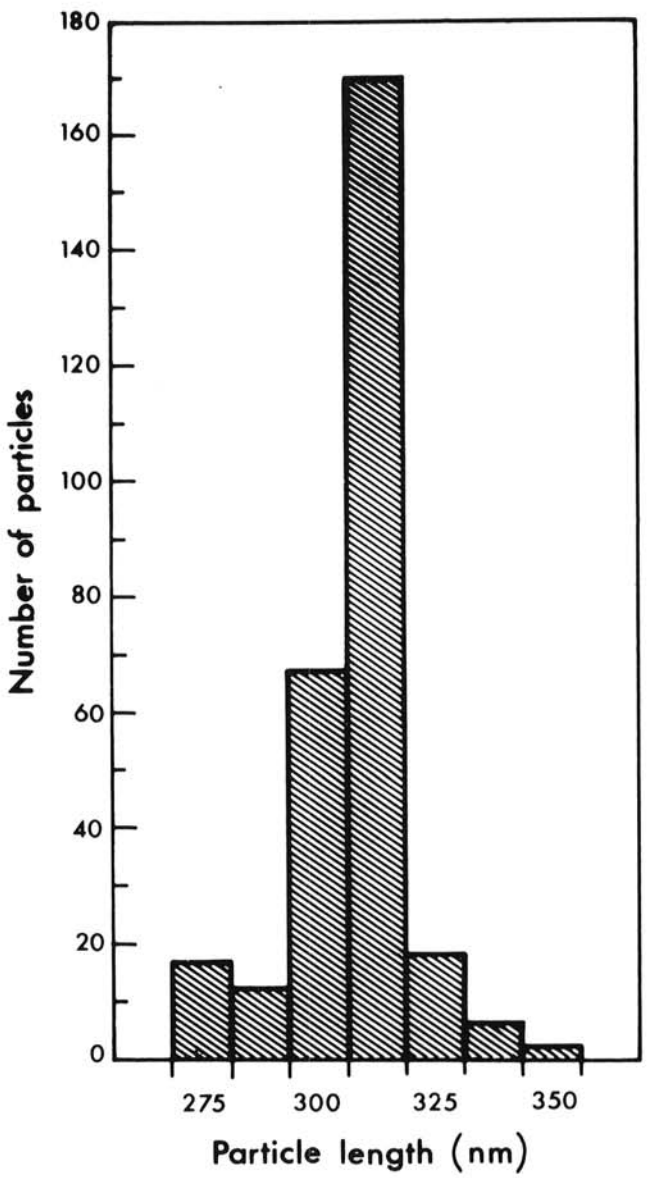

Fig. 2. Histogram of particle length distribution of pepper mild mottle virus from a preparation obtained by density gradient centrifugation.

PMMV was tested with heterologous antigens or in reciprocal tests (Fig. 4A and B). PMMV antiserum reacted with seven heterologous TMV strains (Fig. 4C). In intragel cross absorption tests, PMMV antiserum that had been absorbed with these heterologous antigens still produced a strong homologous reaction with PMMV (Fig. 4D).

According to the results of precipitin tests (Table 4), it was expected that no cross reactions between PMMV and the other tobamoviruses would occur in the double-antibody sandwich form of ELISA. This was indeed found, and the results (Fig. 5A) confirm the very narrow strain specificity of this test (34). By means of the indirect method of ELISA, however, it was possible to obtain reactions with other tobamoviruses and their antisera (Fig. 5B and C); and these results also are in agreement with earlier findings (34).

Amino acid composition. The amino acid composition of PMMV was found to be very different from that reported for other

TABLE 4. Serological relationships between pepper mild mottle virus (PMMV), para-tobacco mosaic virus (PTMV), tobacco mosaic virus (TMV), and tomato mosaic virus (ToMV)

	Antigen			
Antiserum to:	PMMV	TMV	PTMV	ToMV
PMMV	*2,048*[0][a]	32[6]	64[5]	128[4]
PMMV	*4,096*[0]	32[7]	128[5]	256[4]
TMV	128[5]	*4,096*[0]	512[3]	1,024[2]
PTMV	16[6]	256[2]	*1,024*[0]	128[3]
ToMV	16[7]	512[2]	256[3]	*2,048*[0]

[a] Reciprocal values of antisera dilutions followed by the serological differentiation index in brackets.

TABLE 5. Amino acid composition of coat proteins of pepper mild mottle virus (PMMV), tobacco mosaic virus (TMV), tomato mosaic virus (ToMV), and para-tobacco mosaic virus (PTMV)

	PMMV					
Amino acid	Hydrolysis time 24 hr	Hydrolysis time 120 hr	Calculated composition	TMV[a]	ToMV	PTMV
Asp	18.0	18.1	18	18	17	22
Thr	19.7	18.6	20	16	17	19
Ser	9.6	8.1	10	16	16	10
Glu	17.5	17.3	18	16	19	16
Pro	5.7	6.0	6	8	8	10
Gly	7.5	7.6	8	6	6	5
Ala	16.8	17.1	17	14	11	17
Cys (A)	0.85[b]	···	1	1	1	1
Val	13.3	13.7[c]	14	14	15	12
Met(s)	0.9[b]	···	1	0	1	2
Ile	3.9	4.6[c]	5	9	7	8
Leu	16.2	15.7[c]	16	12	13	11
Tyr	4.0	···	4	4	5	6
Phe	7.0	7.0	7	8	8	8
His	···	···	0	0	0	0
Lys	2.0	2.0	2	2	2	1
Trp	1.8[d]	···	2	3	3	2
Arg	9.0	9.0	9	11	9	8
Total			158	158	158	158

[a] The data for TMV, ToMV, and PTMV (U2) are from Van Regenmortel (33).
[b] Met and Cys were determined after oxidation with performic acid.
[c] Values used for calculating the composition.
[d] Trp was determined after hydrolysis with methane sulfonic acid.

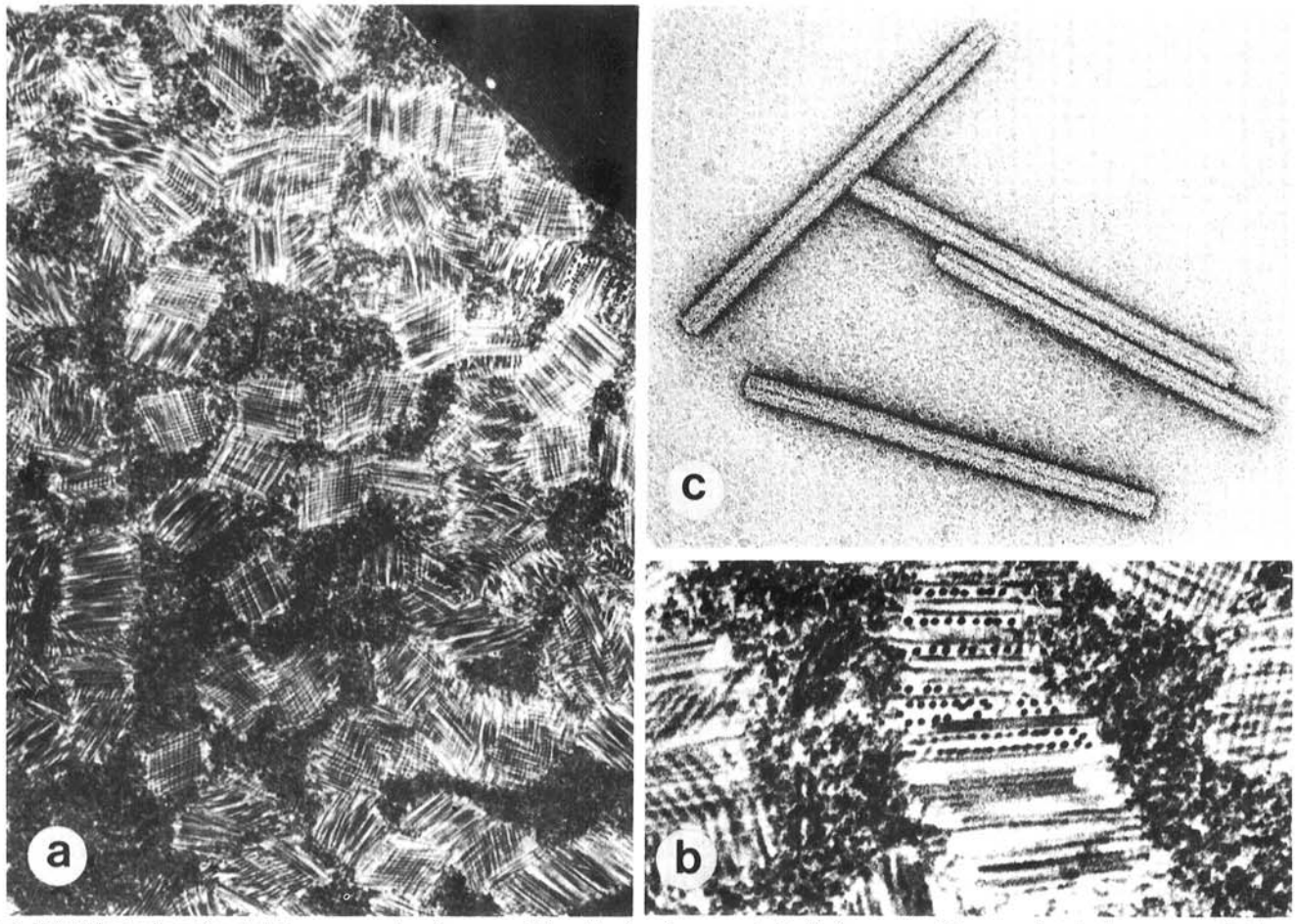

Fig. 3. Ultrathin sections of angled-layer aggregates of pepper mild mottle virus in a mesophyll cell of pepper. **a,** Many crosshatched aggregates of virions are embedded in the cytoplasm (×28,000). **b,** Angled-layer aggregates at higher magnification. The rows of black dots are cross-sectioned virions oriented at right angles between layers of virions sectioned longitudinally (×86,000). **c,** Micrograph of pepper mild mottle virus virions obtained by density gradient centrifugation (×185,000). The lower virion has a length of ~312 nm.

tobamoviruses (33). The composition of PMMV, TMV, ToMV, and PTMV is presented in Table 5. The number of exchanges relative to TMV are: PMMV/TMV = 16, PMMV/ToMV = 15, PMMV/PTMV = 15. Higher rates of exchange exist between PMMV and some more remote strains of TMV: PMMV/SHMV = 21, PMMV/HRV = 22, and PMMV/CV4 = 26. The number of exchanges correlates with the serological differences as expressed in SDI values (32).

DISCUSSION

Our results demonstrate that PMMV is a new member of the tobamovirus group (4,21,33). This is the fourth virus of the group which has been found to infect pepper under natural conditions. In addition to type TMV (17), ToMV was found to infect pepper crops in Illinois (23) and recently, PTMV (U2), which is widespread in tobacco crops in Germany (37), was detected in field-grown pepper both in Piedmont and Umbria in Italy (7, and C. Wetter, M. Conti, and M. Marte, *unpublished*). In many cases, isolates described as TMV have been obtained from diseased pepper, without a clear demonstration of which strain was involved.

Host reactions of PMMV are similar to those described by McKinney (22) for a virus which later was studied by Greenleaf et al (14). This pepper virus was called 'Samsun latent tobacco mosaic virus' (SLTMV) even though it was found to be poorly adapted to tobacco. In contrast, SLTMV could infect 141 commercial cultivars and breeding lines of pepper and induced systemic mottle in all of them (14). Recent results indicate that SLTMV and PMMV are very closely related; they were indistinguishable in immunodiffusion tests (C. Wetter, *unpublished*).

There is also some resemblance between PMMV and an unusual strain of TMV described from pepper in Argentina (11). Whereas the reactions of tobacco and tomato show some similarity to PMMV infection, the reactions of peppers are definitively different. All peppers (*C. annuum, C. frutescens*, and *C. chinense*) that we tested with PMMV were systemically infected by the virus and developed a mild mosaic or mottle as is the case with SLTMV. In contrast, the virus from Argentina has been reported to cause only local lesions in some peppers, including *C. frutescens*.

Recently, Rast (27) and Tóbiás et al (29) have studied the resistance of pepper species and cultivars, and described TMV strains differing in host reaction and in serological properties. Further studies are necessary before these strains can be definitely classed as members of the tobamovirus group.

The morphology of PMMV particles was very similar to that of type TMV (NL = 300 nm), but the NL of PMMV was found to be somewhat longer (312 nm). Slight differences in NL of tobamoviruses were reported for Sammons' opuntia virus with an NL of 317 nm (3) and for an orchid strain of TMV with an NL of 320 nm (9). Further studies are needed to establish whether the difference in NL is a significant feature of these viruses. The angled layer aggregates are of special interest, since such inclusions were first reported in the case of pepper plants infected with an unidentified strain of TMV in South America (16). They were never observed with type TMV, ToMV, or PTMV. It is possible,

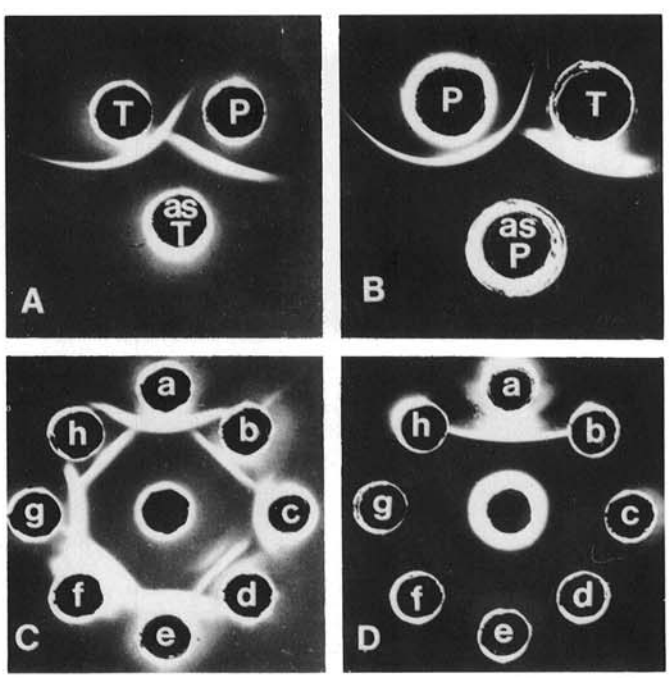

Fig. 4. Results of gel diffusion reactions of pepper mild mottle virus, tobacco mosaic, and other tobamoviruses with homologous and heterologous antisera. **A and B,** Antigens and antisera (as) are abbreviated as follows: T = TMV; P = PMMV. **C and D,** Results of gel diffusion **(C)** and intragel cross absorption tests **(D)** with PMMV antiserum. **C,** The central well was charged with PMMV antiserum and the wells contained: a = PMMV, b = ToMV, c = PTMV, d = HRV, e = O III, f = SHMV, g = CV4, and h = TMV. **D,** The central well was charged initially with antigens b to h and 2 hr later with PMMV antiserum. The surrounding wells were then charged as in C.

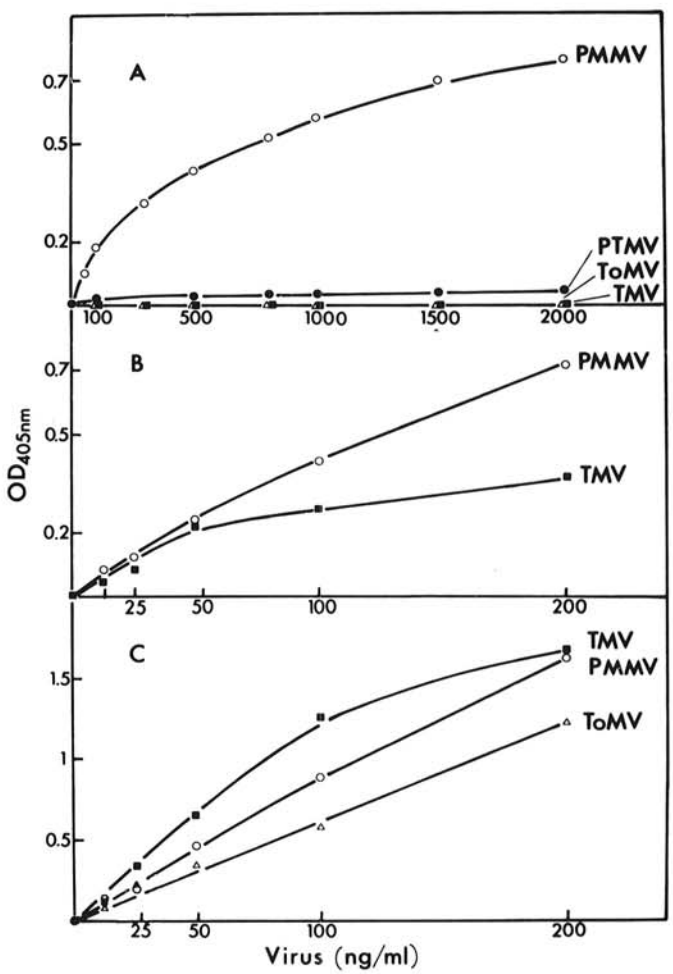

Fig. 5. Reactions of pepper mild mottle virus (PMMV) and other tobacco mosaic virus (TMV) strains in different ELISA procedures. **A,** Double-antibody sandwich ELISA with PMMV and three other tobamoviruses. Coating of wells was done with 5 μg/ml rabbit anti-PMMV globulins for 2 hr. The viruses being tested were incubated for 12 hr. The rabbit anti-PMMV conjugate, diluted 1:1,000, was incubated for 4 hr. Substrate hydrolysis time was 1 hr. **B,** Indirect ELISA with PMMV and TMV. Coating of wells was done with 1 μg/ml goat anti-TMV globulins for 2 hr at 37 C. The two viruses were incubated for 3 hr at 37 C and detected with PMMV rabbit antiserum diluted 1:4,000 (2 hr at 37 C) followed by conjugated goat anti-rabbit globulin (2 hr at 37 C). Substrate hydrolysis time was 1 hr. **C,** Indirect ELISA with PMMV, TMV, and ToMV. Wells were coated with the viruses for 3 hr at 37 C. Rabbit antiserum to PMMV (diluted 1:4,000) was incubated for 2 hr followed by conjugated goat anti-rabbit globulin (2 hr at 37 C). Substrate hydrolysis time was 1 hr.

therefore, that PMMV is related to the unidentified virus (16), which would indicate a worldwide distribution of this virus.

Tobamoviruses that infect pepper cause serious economic problems in field-grown crops as well as in plastic- or glass-protected cultures (6). They are transmitted mechanically and may be seedborne to a considerable degree (10,22,30). Proper identification of the etiological agent is thus of considerable importance, and is a prerequisite for the control of these diseases. Our results demonstrate that at least four different tobamoviruses that are easily differentiated by serology are able to cause disease in pepper.

LITERATURE CITED

1. Boedtker, H., and Simmons, N. S. 1958. The preparation and characterization of essentially uniform tobacco mosaic virus particles. J. Am. Chem. Soc. 80:2550-2556.
2. Brakke, M. K. 1967. Density-gradient centrifugation. Pages 93-118 in: Methods in Virology. Vol. 2. K. Maramorosch and H. Koprowski, eds. Academic Press, New York. 682 pp.
3. Brandes, J., and Chessin, M. 1965. An electron microscope study on the size of Sammons' opuntia virus. Virology 25:673-674.
4. Brandes, J., and Wetter, C. 1959. Classification of elongated plant viruses on the basis of particle morphology. Virology 8:99-115.
5. Clark, M. F., and Adams, A. N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34:475-483.
6. Conti, M., and Lovisolo, O. 1983. Virus problems in protected vegetable crops. Acta Hortic. 127:83-99.
7. Conti, M., and Marte, M. 1983. Virus, virosi e micoplasmosi del peperone. Ital. Agric. 120:132-152.
8. Conti, M., and Masenga, V. 1977. Identification and prevalence of pepper viruses in Northwest Italy. Phytopathol. Z. 90:212-222.
9. Corbett, M. K. 1967. Some distinguishing characteristics of the orchid strain of tobacco mosaic virus. Phytopathology 57:164-172.
10. Demski, J. W. 1981. Tobacco mosaic virus is seedborne in pimiento peppers. Plant Dis. 65:723-724.
11. Feldman, J. M., and Oremianer, S. 1972. An unusual strain of tobacco mosaic virus from pepper. Phytopathol. Z. 75:250-267.
12. Fraenkel-Conrat, H. 1957. Degradation of tobacco mosaic virus with acetic acid. Virology 4:1-4.
13. Gordon, R. B., and Price, W. C. 1953. Differentiation of tobacco mosaic virus strains by differences in pH of maximum optical density. Arch. Biochem. Biophys. 45:117-123.
14. Greenleaf, W. H., Cook, A. A., and Heyn, A. N. J. 1964. Resistance to tobacco mosaic virus in *Capsicum*, with reference to the Samsun latent strain. Phytopathology 54:1367-1371.
15. Hebert, T. T. 1963. Precipitation of plant viruses by polyethylene glycol. Phytopathology 53:362.
16. Herold, F., and Munz, K. 1967. An unusual kind of crystalline inclusion of tobacco mosaic virus. J. Gen. Virol. 1:375-378.
17. Holmes, F. O. 1934. Inheritance of ability to localize tobacco mosaic virus. Phytopathology 24:984-1002.
18. Kado, C. I., Van Regenmortel, M. H. V., and Knight, C. A. 1968. Studies on some strains of tobacco mosaic virus in orchids. Virology 34:17-24.
19. Kreibig, U., and Wetter, C. 1980. Light diffraction of in vitro crystals of six tobacco mosaic viruses. Z. Naturforsch. 35C:750-762.
20. Liu, T. Y. 1972. Determination of tryptophan. Meth. Enzymol. 25:44-55.
21. Matthews, R. E. F. 1982. Classification and nomenclature of viruses. Fourth Rep. Int. Comm. Taxon. Viruses. Intervirology 17:1-200.
22. McKinney, H. H. 1952. Two strains of tobacco mosaic virus, one of which is seed-borne in an etch-immune pungent pepper. Plant Dis. Rep. 36:184-187.
23. Miller, P. M., and Thornberry, H. H. 1958. A new viral disease of tomato and pepper. Phytopathology 48:665-669.
24. Noordam, D. 1973. Identification of plant viruses. Cent. Agric. Publ. Doc. (PUDOC) Wageningen, The Netherlands. 207 pp.
25. Oster, G. 1951. The isoelectric points of some strains of tobacco mosaic virus. J. Biol. Chem. 190:55-59.
26. Paul, H. L., Wetter, C., Wittmann, H. G., and Brandes, J. 1965. Untersuchungen am *Odontoglossum* ringspot virus, einem Verwandten des Tabakmosaik-Virus. Z. Vererbungsl. 97:186-203.
27. Rast, A. Th. B. 1982. Resistance of *Capsicum* species to tobacco, tomato and pepper strains of tobacco mosaic virus. Neth. J. Plant Pathol. 88:163-169.
28. Reinbolt, J., Tritsch, D., and Wittmann-Liebold, B. 1979. The primary structure of ribosomal protein S 7 from *E. coli* strains K and B. Biochimie 61:501-522.
29. Tóbiás, I., Rast, A. Th. B., and Maat, D. Z. 1982. Tobamoviruses of pepper, eggplant and tobacco: Comparative host reactions and serological relationships. Neth. J. Plant Pathol. 88:257-268.
30. Tošić, M., Šutić, D., and Pešić, Z. 1980. Transmission of tobacco mosaic virus through pepper (*Capsicum annuum* L.) seed. Phytopathol. Z. 97:10-13.
31. Van Regenmortel, M. H. V. 1967. Serological studies on naturally occurring strains and chemically induced mutants of tobacco mosaic virus. Virology 31:467-480.
32. Van Regenmortel, M. H. V. 1975. Antigenic relationships between strains of tobacco mosaic virus. Virology 64:415-420.
33. Van Regenmortel, M. H. V. 1981. Tobamoviruses. Pages 541-564 in: Handbook of Plant Virus Infections and Comparative Diagnosis. E. Kurstak, ed. Elsevier/North Holland Biomedical Press, Amsterdam. 943 pp.
34. Van Regenmortel, M. H. V., and Burckard, J. 1980. Detection of a wide spectrum of tobacco mosaic virus strains by indirect enzyme-linked immunosorbent assays (ELISA). Virology 106:327-334.
35. Warmke, H. E. 1968. Fine structure of inclusions formed by the aucuba strain of tobacco mosaic virus. Virology 34:149-159.
36. Wetter, C. 1965. Serology in virus-disease diagnosis. Annu. Rev. Phytopathol. 3:19-42.
37. Wetter, C. 1980. Occurrence of para-tobacco mosaic virus in field tobacco in South-West Germany. Z. Pflanzenkrankh. Pflanzenschutz 87:150-154.