THE RELATIONSHIP BETWEEN BEAN YELLOW MOSAIC VIRUS AND PEA MOSAIC VIRUS

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Summary

The host range, symptomatology, serology, and electron microscopy of one isolate of bean yellow mosaic virus (BYMV) and of three isolates of pea mosaic virus (PMV1, PMV2, and PMV3) were compared. All had similar host ranges, but could be differentiated by symptomatology. BYMV, PMV1, and PMV2 were closely related serologically.

The normal particle length of PMV1 was consistently in the range 787–800 mμ, whereas with BYMV it varied according to the host plant. From legumes it was within the range 742–756 mμ and from *Chenopodium amaranticolor* 794–800 mμ. PMV2 and PMV3 had particles of similar length to PMV1.

Morphological differences were observed between some particles of BYMV and PMV1 from leguminous hosts, but not from *C. amaranticolor*. The pitfalls of using particle length measurements as a criterion of strain relationship are discussed. It is concluded that all the isolates should be regarded as strains of BYMV.

I. INTRODUCTION

Bean yellow mosaic virus (BYMV) and pea mosaic virus (PMV) have similar physical properties, are readily sap transmissible, and are stylet borne by the same vectors (Smith 1957; Kennedy, Day, and Eastop 1962). A close serological relationship between the two viruses has been demonstrated by Goodchild (1956*b*) and Bercks (1960). However, Goodchild (1956*a*) considered that they should be regarded as distinct viruses because some isolates from *Pisum sativum* do not infect *Phaseolus vulgaris* and these he differentiated as PMV. However, Fry (1953) and Shroeder and Providenti (1966) reported infection of *Ph. vulgaris* by isolates that cause strong mosaic symptoms on *P. sativum*.

Brandes and Wetter (1959) reported that both BYMV and PMV have a particle length of about 750 mμ, which is considerably shorter than the 790 mμ reported by Bancroft and Kaesburg (1959). Bos (1964) suggested that the relationship of these viruses should be re-investigated and our work was done for this purpose.

II. MATERIALS AND METHODS

(*a*) *Virus Isolates*

The virus isolates collected from field plants are described in the following tabulation:

Isolate	Source Plant	Locality	Symptoms on Source Plant
BYMV	*Phaseolus vulgaris* L. (cv. Brown Beauty)	Eastern Victoria	Strong systemic yellow mosaic

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Isolate	Source Plant	Locality	Symptoms on Source Plant
PMV1	*Pisum sativum* L. (cv. 99L)	Southern Victoria	Strong dark green to light green mosaic
PMV2	*Pisum sativum* L. (cv. Victory Freezer)	South-eastern New South Wales	Strong dark green to light green mosaic
PMV3	*Vicia faba* L. (cv. Leviathan Longpod)	Southern Victoria	Strong dark green to light green mosaic. Marked growth reduction

The BYMV isolate was maintained in *Ph. vulgaris* cv. Brown Beauty (syn. The Prince) and the PMV isolates in *V. faba* cv. Leviathan Longpod. For host range studies and for producing virus for purification, plants were inoculated with the forefinger wetted with inoculum obtained by grinding infected tissue in phosphate buffer (0·05M, pH 7·6) in the approximate proportions 1:10 (w/v). The plants were dusted with carborundum before inoculation and the inoculum rinsed off with tap water immediately after. All plants were grown in an insect-free glasshouse at temperatures fluctuating between 65 and 85°F.

(*b*) *Virus Purification*

BYMV isolates were increased in *Ph. vulgaris* cv. Brown Beauty and PMV isolates in *V. faba* cv. Leviathan Longpod, except when particle lengths were to be measured from a common host. In this case *P. sativum* cv. Greenfeast or *Chenopodium amaranticolor* Costye & Reyne was used. Two to three weeks after inoculation 200–400 g of leaf and stem tissue were harvested and cooled to 4°C, and maintained at this temperature throughout the purification process. The tissue was homogenized in borate buffer (0·5M, pH 8·2; H_3BO_4–NaOH) (Thomlinson 1964) containing 0·1% thioglycollic acid, kept overnight at 4°C, and then squeezed through cheesecloth. The extract was emulsified with chloroform (1 : 4) and the emulsion broken by centrifugation (10,400 *g*, 20 min). The virus was sedimented from the supernatant fluid by centrifuging (78,000 *g*, 90 min), resuspended in borate buffer (0·05M, pH 8·2), and the preparation clarified by centrifugation (12,000 *g*, 10 min). These partially purified preparations were often used as test antigens, but for antigens used for injection or for electron microscopy they were given a further cycle of ultracentrifugation (105,000 *g*, 45 min). The second high-speed pellet was resuspended and clarified as described above, except that phosphate buffer was used when the pellet was to be emulsified in adjuvant, because preparations in borate buffer emulsified poorly in Freund's incomplete adjuvant. Phosphate could not be used routinely, as it caused end to end aggregation of the particles.

(*c*) *Serology*

Antisera to isolates BYMV, PMV1, and PMV3 were prepared by injecting rabbits at intervals of 10 days. The initial injection consisted of a 1 : 1 emulsion of partially purified virus in Freunds' incomplete adjuvant; 2 ml were injected intramuscularly into each hind leg and 4 ml subcutaneously into the flank. Two further injections each of 2 ml were given intravenously. The rabbits were bled by cardiac puncture 30–40 days after the first injection and the antisera mixed with equal parts of glycerol and stored. In initial tests the antisera were absorbed with sap from healthy plants; however, in most tests unabsorbed antisera were used because in microprecipitin tests there was no evidence of reactions between host components and their antibodies.

The microprecipitin technique was used in all tests. Droplets of twofold serial dilutions of antisera and normal serum were positioned evenly across a plastic Petri dish and virus preparations or preparations from healthy bean were then added. The volume of the antigen droplets was about half that of the antisera droplets. The Petri dishes were then placed on an orbital shaker and agitated at 60 r.p.m. for 10–20 min. Precipitin reactions were observed through a dissecting microscope using side illumination and a dark background. Final records were made within 30 min of commencing the tests, because non-specific precipitates gradually developed after this time, making the reactions progressively more difficult to read.

(*d*) *Electron Microscopy*

All mounts were prepared by the negative staining technique. The stain used was 2 or 4% phosphotungstic acid neutralized with 1N NaOH; 4% phosphotungstic acid was more satisfactory. Preparations for mounts were made either directly from leaves, by drawing epidermal strips or small pieces of cut leaf tissue through a droplet of stain on a glass slide, or from freshly purified preparations, which were mixed at a range of dilutions with equal volumes of stain. The droplets were then placed on parlodion-coated, carbon-reinforced copper grids, the excess fluid was removed with filter paper, and the mount air-dried before examination at a magnification of 40,000 in a Siemens 1A electron microscope at 80 kV.

The particle lengths were determined by using tobacco mosaic virus (TMV) as a reference and also by using the magnification scale of the microscope after calibration with a standard aperture. The TMV reference consisted of particles mounted directly from tobacco or tomato leaves, or mounted from preparations obtained by density gradient centrifugation of unclarified sap of very young (4–6 weeks old) tobacco plants. The virus was dialysed against tap water for 24 hr after removal from the sucrose density gradient. This technique yielded excellent preparations of non-aggregated virus (Fig. 1). Most particles were of the same length (300 mμ).

All photographs for each series of measurements were taken on the one day, to minimize variations in the magnification of the microscope. The effect of any variations was compensated for by alternating the viruses being examined every fifth or sixth photograph. When TMV was used as an external standard, it was recorded on every sixth plate. TMV was included in each photograph when used as an internal standard.

Particles were measured directly from the glass plate negatives when viewed against a strong light. Dividers sharpened to very fine points were used to transfer the lengths to a steel rule. The accuracy of the measurements was within $\pm 6 \cdot 5$ mμ. Three or four steps of the dividers were necessary when measuring some flexuous particles. Any particle requiring more than four steps was ignored as atypical because it represented less than 2% of the particles of BYMV photographed and did not occur at all in the PMV isolates.

Preliminary measurements indicated that most of the particles from sap or from purified preparations fell within a narrow size range. The remainder consisted of very short particles of uneven length, or of particles twice the normal length. The small particles were not measured whereas the longer particles were accepted as double particles and the measurement was halved and recorded as two separate particles.

III. Results

(*a*) *Host Ranges*

Various plants were inoculated to see whether the isolates differed in pathogenicity and whether those from *P. sativum* would infect cultivars of *Ph. vulgaris*. The results in Table 1 show that the isolates of PMV differed from each other and from BYMV and that the PMV isolates infected the cultivars of *Ph. vulgaris* listed, except that PMV1 failed to infect the variety Brown Beauty.

The PMV3 isolate obtained from *V. faba* differed from all others in causing severe stunting and leaf roll in *P. sativum*. All isolates caused identical symptoms in *C. amaranticolor*.

(*b*) *Serology*

The results in Table 2 show that the four isolates are closely related serologically as the titres of the antisera were similar whether titrated against homologous or heterologous antigens. However, the antiserum prepared against PMV1 had a slightly

TABLE 1

REACTIONS INDUCED BY BYMV, PMV1, PMV2, AND PMV3 IN A RANGE OF PLANTS

Host Species	BYMV	PMV1	PMV2	PMV3
Ph. vulgaris				
cv. Brown Beauty	Bright systemic yellow mosaic	No reaction	Slight systemic chlorotic rings and mild vein necrosis	Occasional chlorotic rings on inoculated leaves. Systemic vein clearing, mild mottle
cv. Red Kidney	Chlorotic rings on inoculated leaves. Strong yellow mosaic	Chlorotic rings on inoculated leaves	Chlorotic rings on inoculated leaves	Chlorotic rings on inoculated leaves
cv. Stringless Blue Lake	Necrotic local lesions on inoculated leaves. Stunting. Systemic vein clearing and vein necrosis	Red rings on inoculated leaves. Epinasty of trifoliate leaves with systemic vein clearing	Necrotic spotting on inoculated leaves. Systemic vein clearing with chlorotic rings	Necrotic rings on inoculated leaves. Systemic vein necrosis and vein clearing with leaf distortion
P. sativum				
cv. Greenfeast	Faint systemic mottle	Strong mosaic	Strong mosaic	Strong mosaic. Severe leaf rolling. Stunting
cv. Victory Freezer	Faint systemic mottle	Mosaic	Strong mosaic	Strong mosaic. Stunting and severe leaf rolling
cv. Canners 75	Mild mottle	Mosaic	Strong mosaic	Strong mosaic. Stunting and severe leaf rolling
V. faba				
cv. Coles Dwarf Prolific	Mild mottle	Strong mottle	Strong mottle	Strong mosaic. Stunting
C. amaranticolor	Chlorotic local lesions and systemic chlorosis, leaf malformation and top necrosis	As for BYMV	As for BYMV	As for BYMV

smaller titre with BYMV than with the PMV isolates, which may indicate a minor difference between BYMV and the other isolates.

TABLE 2

TITRES OF ANTISERA PREPARED AGAINST BYMV, PMV1, AND PMV3 WHEN TITRED AGAINST BYMV, PMV1, PMV2, AND PMV3

+ indicates a positive reaction. Controls were mixtures of normal sera and virus; reactions of antisera with preparations from healthy host plants were always negative

Antigen	Antiserum	Reciprocal of Antiserum Dilution			
		64	128	256	512
BYMV	BYMV	+	+	–	–
PMV1	BYMV	+	+	–	–
PMV2	BYMV	+	+	–	–
PMV3	BYMV	+	+	–	–
BYMV	PMV1	+	+	–	–
PMV1	PMV1	+	+	+	–
PMV2	PMV1	+	+	+	–
PMV3	PMV1	+	+	+	–
BYMV	PMV3	+	–	–	–
PMV1	PMV3	+	–	–	–
PMV2	PMV3	+	–	–	–
PMV3	PMV3	+	–	–	–

TABLE 3

ANALYSIS OF PARTICLE LENGTH MEASUREMENTS OF BYMV AND PMV1 IN SAP AND PURIFIED PREPARATIONS FROM VARIOUS HOSTS

Test for significance of measurements as indicated in text. P, partially purified preparation; S, sap. Hosts from which particles were measured: series 1–3, *V. faba* (PMV1) and *Ph. vulgaris* (BYMV); series 4–6, *C. amaranticolor*; series 7–8, *P. sativum*

Series*	No. of Particles		Mean Length (mμ)		Mean Difference	2 S.E.	Standard Deviation	
	PMV1	BYMV	PMV1	BYMV	PMV1 – BYMV		PMV1	BYMV
1(P)	89	97	790·5	742·5	−48·0	8·0	20·8	32·8
2(P)	86	104	790·0	750·3	−30·6	9·0	25·5	36·0
3(S)	48	66	787·5	747·8	−39·7	6·8	10·8	24·0
4(P)	81	84	799·0	799·5	+ 0·50	6·5	23·3	18·8
5(P)	81	82	794·1	794·4	+ 0·30	6·1	13·3	24·0
6(S)	52	56	799·8	798·5	− 1·3	5·1	10·0	15·8
7(P)	115	118	798·2	780·9	−17·3	5·1	15·1	23·2
8(S)	71	116	795·4	756·9	−38·3	8·4	15·3	41·3

* Magnification determined with TMV reference (series 1–4), and from microscope scale (series 5–8).

(c) *Particle Lengths*

Measurements were made of BYMV and PMV1 particles from sap and from purified preparations — see Table 3 for a statistical analysis of these. As the distributions of particle lengths are not normal, the usual tests for significance cannot

be applied. However, because so many particles were measured, a satisfactory test for significance consists of comparing twice the standard error of the mean with the difference of the means.

The particle length of PMV1 was constant, irrespective of the host, and whether the measurements were made on sap or a purified preparation (see Table 3). By contrast the length of BYMV varied and was significantly shorter than PMV1 from the leguminous hosts, but not from *C. amaranticolor*; also its mean length in a purified preparation from *P. sativum* was greater than in sap from this host. The greater variability of the length of BYMV, except for a purified preparation from *C. amaranticolor* (Table 3, series 4), is also apparent from the larger standard deviations of the values obtained for the measurements on this isolate.

The length obtained from PMV1 by reference to a TMV standard (Table 3, series 1–4), or by using the microscope magnification scale (Table 3, series 5–8) agreed closely. However, to verify these values, photographs were taken of 81 PMV1 particles in *V. faba* sap extracts with a similar number of TMV particles included as an internal standard. The length measurements obtained for each virus were averaged and a value of 798 mμ was obtained for the length of PMV1. To compare the length of PMV2 and PMV3 with PMV1, 50 particles of PMV2 and 55 of PMV3 were measured from sap of *V. faba* using TMV as an internal standard. The means of the measurements obtained were 800 mμ for PMV2 and 795 mμ for PMV3, indicating that in *V. faba* the particle lengths of these isolates and those of PMV1 were similar.

(*d*) *Morphology of Particles in BYMV and PMV Isolates*

The host plant seemed not to affect the morphology of the PMV isolates. Most of the particles of this virus were of one length and clearly differentiated in the stain (Fig. 2). Many particles were almost straight, particularly when the mount was a dip preparation (Fig. 3). The only exception to this generalization was that occasional particles of PMV1 in purified preparations were of uneven width and their appearance suggested that the protein coat was disintegrating (Fig. 4). This was also occasionally observed in BYMV mounts prepared from either leaf extracts or purified preparations.

The particles of BYMV from *C. amaranticolor* were identical with those of the PMV isolates. However, when BYMV originated from *Ph. vulgaris* or *P. sativum*, although some particles were of the PMV type, most differed sufficiently to be differentiated in photographs. The greater variability in the length of BYMV particles was obvious when purified preparations were used and several particles could be seen side by side (Fig. 5). The particles of BYMV were rarely as clearly differentiated in the background stain as those of the PMV isolates, and one end of many particles of BYMV seemed to merge into the strain and was malformed or broken (Fig. 5). This type of particle was rarely seen in PMV1 mounts.

All mounts of all isolates contained from 1 to 5% of particles twice the normal length (Fig. 6). This indicated that such particles either occur in infected plants or are joined by aggregation immediately the cells are ruptured. They are not artifacts formed during purification.

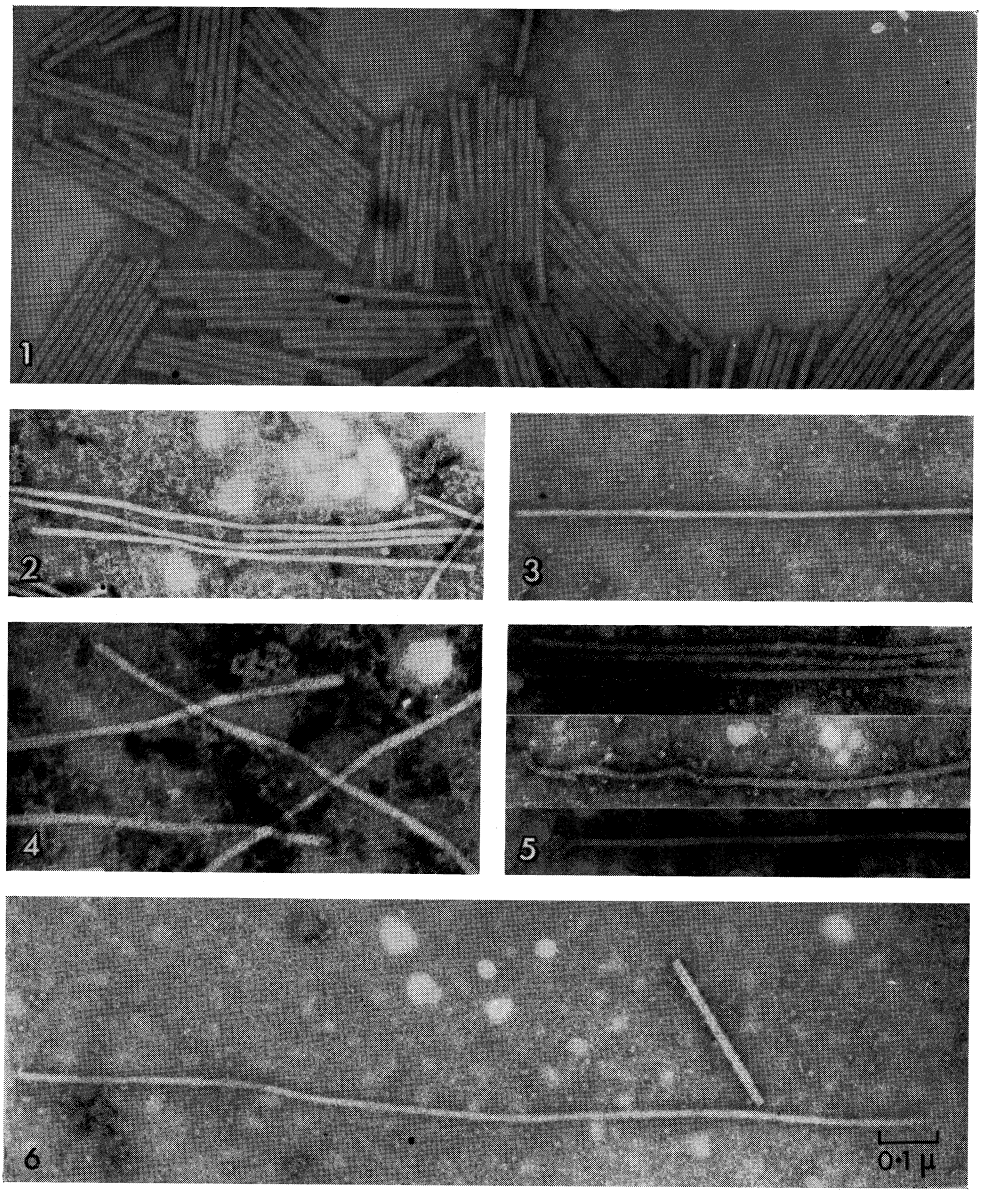

Fig. 1.—TMV used as standard for measurements.
Fig. 2.—PMV1 from purified preparation.
Fig. 3.—PMV1 from sap preparation.
Fig. 4.—PMV1 disintegrating particles from purified preparation.
Fig. 5.—BYMV — note uneven length, poor staining, and abnormality of ends.
Fig. 6.—Double particle of PMV1 with TMV reference.

IV. Discussion

The Terminology Committee of the Canadian Phytopathological Society, when considering viruses, defined a strain as "a virus that can be recognized whenever isolated, by its properties and behaviour, and that is shown to be related to a characterized virus, serologically or by interactions in its hosts" (Welsh 1961). This definition has been widely accepted and, if applied to the virus isolates we have compared, indicates that they must be regarded as strains of BYMV, the name that has precedence (Pierce 1934). They are very closely related serologically and the variations in their host reactions are typical of those expected between a range of isolates of any virus. The present work and that of Fry (1953) and Schroeder and Providenti (1966) indicate that some isolates of BYMV that cause a mosaic disease of *P. sativum* will also infect *Ph. vulgaris*. The differentiation between BYMV and PMV made by Goodchild (1956*a*) placed too much emphasis on host reactions which are notoriously variable and less reliable than serological reactions for determining strain relationships. There is no record of two viruses being closely related serologically and not sharing many other properties; often it is only the different reactions they produce in hosts that allows them to be differentiated.

Brandes and Wetter (1959) and Brandes and Bercks (1965) stated that the normal length, i.e. the average of the most common particle lengths of any virus, is an intrinsic characteristic of the virus not influenced by the host plant. Therefore, particle length could be a useful criterion for determining strain relationships. However, the normal length of BYMV was about 5% less than that of PMV1 when its particles came from leguminous hosts, whereas for *C. amaranticolor* the particles of both viruses were of similar length. There have been two reports of distantly related strains of the one virus having different particle lengths (Harrison and Woods 1966; Corbett 1967) and one report of closely related strains of the one virus having different particle lengths (von Wechmar and Hahn 1967), but there seems to be no previous report of the particle length of a virus being influenced by the host it infects. The particles of BYMV in legumes may be shorter than from *C. amaranticolor* because of some effect of the constituents from ruptured leguminous cells, but this seems unlikely in view of the constant length of PMV1 particles. The strain of BYMV used in our work may be atypical; to test this we compared the length of a strain of BYMV from Queensland with PMV1. The Queensland isolate was serologically related to, but not identical to, our BYMV. Its particles were uniform in length and appearance when obtained from *Ph. vulgaris*, but were about 50 mμ shorter than PMV1. When obtained from *C. amaranticolor* its particles were very variable in length and peaks occurred in the frequency distributions at about 600–650 and 700–750 mμ; the most common length was 700–750 mμ. It appears, therefore, that strains of the one virus can vary in particle length and that there are pitfalls in using this characteristic as a criterion of strain relationships.

We were primarily concerned with comparative rather than absolute measurements of particle lengths. However, in view of the consistency of our measurements, we consider that the normal particle length of the strains of PMV is in the range 785–800 mμ, which is substantiated by the agreement between our measurements of

Victorian and Queensland strains of BYMV in *Ph. vulgaris* and those previously reported for BYMV (Brandes and Quantz 1955). An inconsistent measurement was that of 781 mμ obtained for BYMV from a purified preparation from *P. sativum*, which was significantly shorter than the 798 mμ obtained for PMV1, but considerably larger than the 757 mμ obtained for the measurement of BYMV in sap from *P. sativum*. As the same plants were used for both series of measurements which were made during the same week, it seems probable that a greater proportion of the shorter particles were lost during the purification process. This result emphasizes the necessity of making measurements from mounts of virus particles in sap whenever their concentration is sufficient for this method to be practical.

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VI. References

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