RELATIONSHIPS OF LEGUME VIRUSES IN AUSTRALIA

I. STRAINS OF BEAN YELLOW MOSAIC VIRUS AND PEA MOSAIC VIRUS

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Summary

Two strains of bean yellow mosaic virus and two strains of pea mosaic virus are shown to be present in Australia, the physical properties and host ranges are reported, and the relationship between these and previously described strains is discussed.

In determining the presence of the viruses use is made of a series of nine differential hosts with which it is possible to differentiate the reported sap-transmissible virus diseases of peas and beans.

The economic significance of these diseases appears to be slight in crop plants, but could be of importance in pasture legumes, and, in particular, subterranean clover (*Trifolium subterraneum* L.).

Similarities in physical properties and symptom expression suggest a close relationship between bean yellow mosaic virus and pea mosaic virus.

I. INTRODUCTION

Sap-transmissible virus diseases of leguminous plants have been reported in Australia on previous occasions. Norris (1943) records the presence of pea mosaic in Western Australia, where species of lupins (*Lupinus* spp.) and subterranean clover (*Trifolium subterraneum* L.) were infected in the field. Aitken and Grieve (1943) record a mosaic virus disease of subterranean clover in Victoria, and Watson (1949) confirmed the presence of this virus, but suggested it to be a strain of bean yellow mosaic virus (B.Y.M.V.), of which more than one strain appeared to be present. Butler (1949, unpublished data) has recorded B.Y.M.V. in New South Wales, and Stubbs (1949, unpublished data) in Victoria, both on the basis of symptom expression on bean (*Phaseolus vulgaris* L.).

More recently Hutton and Peak (1954) described a B.Y.M.V. of subterranean clover which they considered, on a host-range basis, to be similar to that described by Pierce (1934).

For some years crops of peas and beans in New South Wales have shown a certain percentage of plants with mosaic symptoms, but no systematic study has been undertaken to determine the virus, or viruses, present. The economic aspects of the disease appeared to be slight, which is in accordance with observations made by Norris (1943) and Norris and Hutton (1943) on the effect of pea mosaic virus (P.M.V.) on the yield of seed from mechanically-inoculated pea plants in the field. Here, aphid populations were considered to be the limiting factor in the spread of this virus and also the fact that the variety Gem, or William Massey, a popular commercial variety, was immune.

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TABLE 1

SAP-TRANSMISSIBLE VIRUS DISEASES OF PEAS AND BEANS, SHOWING REACTIONS ON THE HOSTS NECESSARY FOR THEIR DIFFERENTIATION AND CLASSIFICATION

N = Necrosis; + = mottle; − = no reaction; LL = local lesions; CL = chlorotic local lesions; W = wilt

Virus	Authority	*Vicia faba* L. var. Windsor Long Pod	*Pisum sativum* L. var. Greenfeast†	*Phaseolus vulgaris* L. var. (*a*)‡(*b*)‡(*c*)‡	*Trifolium pratense* L.	*Trifolium incarnatum* L.	*Glycine max* (L.) Merrill	*Datura stramonium* L.	*Nicotiana glutinosa* L.	*Nicotiana tabacum* L.
Bean mosaic*	Weiss 1945	+	−	+(*c*)	−	−		−	−	−
Bean southern mosaic*	Zaumeyer & Harter 1943	−	−	LL+(*a*)	−	−	+	−	−	−
Bean yellow stipple	Zaumeyer & Thomas 1950	−	+(*p*)	+(*b*)	−	−	LL+			
Bean red node	Thomas & Zaumeyer 1950	N	N	N	+	+	+	+	−	+
Bean yellow mosaic*	Pierce 1934	+	+	+(*a*)			+	−	−	−
(Pea mosaic)	Zaumeyer & Wade 1936	+	+	+	−		−	−	−	−
(Pisum 2)	Osborn 1937	+	+	+	+	+				
(Alsike clover mosaic)	Zaumeyer 1940	+	+	+(*a*)	−	+	−			
(Severe pea)	Johnson & Jones 1937	+	+(*p*)	+(*a*)	+					−
(Pea mosaic 4)	Zaumeyer 1940	+	+	+	+	+	−	−	−	−
Bean yellow mosaic*	McWhorter *et al.* 1947	+	−	N	+	+	−			
(Subterranean clover mosaic)	Aitken & Grieve 1943	+	+	+(*a*)	+	+	+			
(Type)	Grogan & Walker 1948	+	+	+(*b*)	−	+	+	−	−	−

* These names taken from the "Virus Index" in *Rev. Appl. Mycol.* Vol. 24, No. 13, 1945.

† (*p*) = Perfection variety used in addition to Greenfeast.

‡ (*a*) = Corbett's Refugee; (*b*) = Blue Lake; (*c*) = Canadian Wonder.

TABLE 1 (*Continued*)

Virus	Authority	*Vicia faba* L. var. Windsor Long Pod	*Pisum sativum* L. var. Greenfeast†	*Phaseolus vulgaris* L. var. (*a*)‡(*b*)‡(*c*)‡	*Trifolium pratense* L.	*Trifolium incarnatum* L.	*Glycine max* (L.) Merrill	*Datura stramonium* L.	*Nicotiana glutinosa* L.	*Nicotiana tabacum* L.
(Pod-distorting)	Grogan & Walker 1948	−	+	N[(b)]	−	+	+	−	−	−
(Severe yellow)	Thomas & Zaumeyer 1953	+	+	N[(b)]	−	+	+	−	−	CL
(Necrotic lesion)	Zaumeyer & Fisher 1953	LL	−	+[(b)]	−	−	+	−	−	LL
Bean yellow mosaic*	Fry 1953	+	+	+[(c)]	+	−				
Bean yellow mosaic*	Chaudhuri 1950	+	+	+	−	+		−	−	−
Pea mosaic*	Doolittle & James 1925	+	+	−	+					
Pea mosaic*	Zaumeyer & Jones 1936	+	+	−[(a)]	+		−	−	−	−
Pea mosaic*	Chamberlain 1936	+	+	−	+			−	−	−
(Pea 2A, 2B, 2C)	Stubbs 1937	+	+	−[(a)]	−	+	−	−	−	−
(Pea 3)	Murphy & Pierce 1937	+	+	−[(a)]	+	+	−	−	−	−
(Winconsin pea stunt) (Red clover vein mosaic)	Hagedorn & Hanson 1951	+	+	−	+	+	−			−

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TABLE 1 (*Continued*)

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Pea streak and stunt*	Chamberlain 1938	–	+	LL	+	LL	LL			
(Pea streak)	Zaumeyer 1938	+	+(*p*)	–	+	+	+		–	–
Pea streak and stunt*	Ainsworth 1940	LL	+	+						
Pea streak and stunt*	Hagedorn & Walker 1949	–	+(*p*)	–	+	–	–	–		–
(Pea wilt)	Johnson 1942	+N	W(*p*)	+	+	+	–	–	–	–
Pea enation mosaic*	Osborn 1938	+	+	–	–	+	+		–	–
Alfalfa mosaic	Zaumeyer 1938	LL	+	LL(*a*)	+	–	+	LL		+
Alfalfa mosaic	Krietlow & Price 1949	LL	+	LL	+	–	+	LL	+	+
(Alfalfa yellows)	Zaumeyer 1953	–	LL(*p*)	LL(*b*)	–	+	+	+	LL	LL
Broad bean mottle	Bawden *et al.* 1952	+	W	+(*c*)	+	+	LL	–	–	–
Cucumber mosaic*	Whipple & Walker 1941	–	+	+			+	+	+	+
Pea mottle*§	Johnson 1942	+	+(*p*)	+	+	+	–	–	–	–

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† (*p*) = Perfection variety used in addition to Greenfeast.

‡ (*a*) = Corbett's Refugee; (*b*) = Blue Lake; (*c*) = Canadian Wonder.

§ Pea mottle differs from viruses of the legume group by having many hosts outside the family Leguminosae.

The present investigation was commenced in 1953 with the initial object of determining the viruses prevalent in pea and bean crops, their host ranges, and possible potential. It was later extended to include pasture legumes and, in particular, subterranean clover.

In a survey of this type it seemed desirable to select an appropriate range of indicator plants whose reactions would enable the sap-transmissible virus diseases of peas and beans to be determined. An attempt was made to utilize the smallest host range possible which would differentiate these viruses. Weiss (1939) had proposed a scheme, but new viruses necessitated changes being made and, from a review of the literature, the differential hosts shown in Table 1 were selected.

Considerable confusion exists between different authors regarding the naming of P.M.V. and B.Y.M.V. and thus, for example, Osborn (1937), Pierce (1935), and Fry (1953) record P.M.V. as capable of infecting beans. With the system adopted in Table 1, these viruses are considered as B.Y.M.V. The table thus presents a convenient scheme for grouping, as well as differentiating, many of the sap-transmissible legume viruses, as the groupings become apparent when reactions on the hosts used are considered. Here, species differences have been utilized in establishing the groupings rather than differences in symptom expression or differences in varietal response within species. Most viruses affecting peas and beans are listed, together with the authority, and where a name differs from the group name this has been included in brackets.

II. Materials and Methods

(*a*) *General*

All investigations were made in the greenhouse where temperature variation was unfortunately large, varying from 11–33°C with the largest mean daily variation in winter of 14·1°C. In these studies consideration was given to previous investigations and an attempt to simulate the general methods of inoculation of previous investigators was made. Isolates obtained from field-infected plants were inoculated on to six *Phaseolus vulgaris*, var. Canadian Wonder, and six *Vicia faba* L., var. Windsor Long Pod, plants, but subsequent inoculations were made with sap expressed from infected broad beans. Commercial seed was used in all studies. Carborundum (180 mesh) was the abrasive, and plants were inoculated in the early stages of growth either with a pestle, or in the case of small-leaved plants, a ground-glass spatula. In all cases leaves were washed with water 1–2 min after inoculation.

Plants were grown in steam-sterilized soil in 4-in. pots, three plants per pot, and inoculated plants were retained for 6 weeks. In cases where symptoms were not visible, or were indistinct, an attempt was made to recover the virus inoculated, by sap inoculation from these plants to six bean and six broad bean plants of the above varieties.

The viruses used were isolated from the field in New South Wales with the exception of Phaseolus virus 2 Pierce (Hutton and Peak 1954), which was obtained from Dr. E. M. Hutton, Division of Plant Industry, C.S.I.R.O., Canberra, A.C.T. Subsequently, isolates of this virus were obtained from field-infected legumes.

Inoculum for physical tests was obtained from broad bean plants which had been showing symptoms for 1–2 weeks only. For each test, sap was obtained from a single plant and filtered through a cotton wool pad before treatment. When testing P.M.V. strains, broad beans were used as the indicator plants, but for strains of B.Y.M.V., beans were used as indicator plants. All plants were carefully selected for uniformity, and two expanded leaves of each broad bean and the two primary leaves of each bean inoculated respectively. In each test made, 24 plants were used as indicators, having regard to previous investigations, and the tests made as follows:

(i) *Dilution Tolerance.*—Sap from a single plant was used to prepare an appropriate dilution series in water.

TABLE 2

GROUPED REACTIONS OF ALL ISOLATES ON DIFFERENTIAL HOSTS

− = No symptoms; + = mottle symptoms

Species Inoculated	Bean Yellow Mosaic Virus		Pea Mosaic Virus	
	Strain 1	Strain 2	Strain 1	Strain 2
Vicia faba	+	+	+	+
Pisum sativum	+	+	+	+
Phaseolus vulgaris	+	+	−	−
Trifolium pratense	−	+	−	+
Trifolium incarnatum	+	+	+	+
Glycine max	+	+	−	−
Datura stramonium	−	−	−	−
Nicotiana glutinosa	−	−	−	−
Nicotiana tabacum	−	−	−	−

(ii) *Aging* in vitro.—In each time-interval test, 2 ml of sap were used and stored at $20 \pm 0{\cdot}2$°C for the required period.

(iii) *Thermal Inactivation.*—In each test, 2 ml of sap were placed in a thin-walled glass capsule and held in a water-bath for 10 min, which was agitated for the first minute. After 10 min the capsule was removed and immediately plunged into an ice-bath for rapid cooling, and the contents used to inoculate the indicator plants.

(*b*) *Species Trials*

These were carried out in the greenhouse using commercially available seed. In most cases 21 plants were inoculated with each isolate, three plants per 4-in. pot, and three uninoculated control plants retained. In all cases inoculum was obtained from broad bean plants showing symptoms for not longer than 3 weeks.

III. RESULTS

(*a*) *Presence of Different Strains of B.Y.M.V. and P.M.V.*

The grouped results of reactions on the differential hosts, and varieties of those outlined in Table 1, are given in Table 2. Reference to Table 1 also shows, from the

reactions outlined in Table 2, that strains of B.Y.M.V. and P.M.V. are responsible. All tests were duplicated and 12 plants were used in each test.

Descriptions of the symptoms caused by the B.Y.M.V. strains 1 and 2 and P.M.V. strains 1 and 2 on differential hosts are set out in Table 3. Physical tests, incorporating thermal inactivation, dilution tolerance, and aging *in vitro* of both strains of B.Y.M.V. and P.M.V., are given in Table 4, and host-range studies of these strains on leguminous hosts are shown in Table 5.

(*b*) *Symptom Expression of B.Y.M.V. Strain* 1 *on Various Hosts*

(i) *Beans.*—The varieties of bean tested can be grouped according to their symptom expression:

(1) *Top Necrosis and Severe Mottle.*—The second or third trifoliate leaves of beans in this group develop a severe yellow-green leaf mottle, which, in the case of the variety Epicure, is followed by veinal necrosis extending backward to the petiole (Plate 1, Fig. 2). Some necrosis of the growing point follows, but a percentage of recovery is noted and such plants have distorted leaves much reduced in size, and with a severe yellow-green leaf mottle. The varieties Epicure and Corbett's Refugee belong to this group.

(2) *Severe Mottle.*—Symptoms are similar to those already given for Canadian Wonder and the following varieties also react in a similar manner: Blue Lake, Bountiful, Brown Beauty, Burpee's Stringless Greenpod, Goward's Special, Kentucky Wonder, Michelite, Pinto, Refugee No. 5, Wellington Wonder, Windsor Long Pod, and the Wax variety of butterbean.

(3) *Mild Mottle.*—Sympton expression is characterized by a mild yellow-green leaf mottle and yellow-green leaf spots, but with no reduction in leaflet size (Plate 1, Fig. 3). Stringless Greenpod and Westralia varieties belong to this group and both tend to grow away from the virus under glasshouse conditions.

(ii) *Peas.*—Symptom expression is essentially similar for all varieties and, in general, is less severe than the symptom expression to the P.M.V. Vein yellowing is the first symptom, followed by either chlorotic spots or a general yellow-green leaf mottle with dark green vein-banded flecks in a few cases only.

(iii) *Clovers and Medics.*—Symptoms developed following inoculation by the *two* strains of B.Y.M.V. are essentially similar to those described for crimson clover (*Trifolium incarnatum* L.), and in almost all cases result in a reduction in leaf size, a severe yellow-green leaf mottle, and often distortion of the leaflets. Reactions of the varieties of subterranean clover are discussed later (see Section III (*e*)).

(iv) *Field Pea* (Pisum arvense *L.*), *Common Vetch* (Vicia sativa *L.*), *and Bokhara Clover* (Melilotus alba *Desr.*).—In each case a yellow-green leaf mottle with some dark green vein-banded flecks and chlorotic spots resulted.

(v) *New Zealand Blue Lupin* (Lupinus angustifolius *L.*).—With this species severe top necrosis without leaf symptom resulted 12–14 days after inoculation, but a small number of plants recovered. The new leaves of these plants had a brilliant yellow-green mottle, were reduced in size, and unfolded slowly or had recurved margins.

(vi) *Sweet Pea* (Lathyrus odoratus *L.*).—A mild mottle only resulted with this species and there was no reduction in leaf size or stunting of the plants.

TABLE 3

DESCRIPTION OF SYMPTOMS ON DIFFERENTIAL HOSTS

A. Bean Yellow Mosaic Virus Group

Host	Strain 1	Strain 2
Vicia faba	Diffuse mottle of 7th leaf 10–12 days after inoculation; 8th leaf with severe vein yellowing, and younger leaves with a yellow-green mottle, chlorotic spots, and dark green vein-banded flecks (Plate 1, Figs. 4, 5, and 6)	Similar to strain 1, not as marked, but mottle of yellow-green type with dark green vein-banded flecks
Pisum sativum	Vein yellowing followed by yellow-green mottle of leaves 12–15 days after inoculation. Younger leaves are slow to expand and have mild chlorotic spots and a mild mottle	Severe vein yellowing 10–14 days after inoculation, followed by a brilliant yellow-green leaf mottle and dark green vein-banded flecks. Leaves and stipules are slow to unfold
Phaseolus vulgaris	Faint chlorotic spots and vein clearing of second trifoliate leaf 6–10 days after inoculation. Younger leaves develop a severe yellow-green mottle, have extensive chlorotic areas, are relatively thick and brittle, and have recurved margins (Plate 1, Fig. 1)	Vein yellowing of the 2nd and 3rd trifoliate leaves 10–12 days after inoculation, followed by a yellow-green mottle or irregular chlorotic spots only in some cases (Plate 1, Fig. 4)
Trifolium pratense	No symptoms on 45 inoculated plants in three trials	Yellow-green leaf streaks on youngest leaves 10–12 days after inoculation. These become more pronounced in younger leaves and dark green vein-banded flecks may also appear (Plate 2, Fig. 11)
Trifolium incarnatum	Slight vein yellowing of youngest leaves 10–12 days after inoculation; subsequently leaves develop a brilliant yellow-green mottle with dark green vein-banded flecks (Plate 2, Fig. 10). Leaves reduced in size and plants stunted	Pronounced vein yellowing of youngest leaves 10–12 days after inoculation. A brilliant yellow-green leaf mottle follows and leaflets may be yellow-green with a few dark green vein-banded flecks. Leaves reduced in size, distorted, and plants stunted
Glycine max	Yellow-green mottle and irregular chlorotic spots but no reduction in leaf size	Similar to strain 1

TABLE 3 (*Continued*)
B. Pea Mosaic Virus Group

Host	Strain 1	Strain 2
Vicia faba	Vein yellowing of the 6th leaf followed by a general yellow-green leaf mottle which is not as severe as that of the B.Y.M.V. group	Vein yellowing and chlorotic spots on 6th leaf 10–12 days after inoculation, followed by a general yellow-green leaf mottle, more pronounced towards the leaf margins
Pisum sativum	Pronounced vein yellowing 12 days after inoculation followed by yellow-green streaks and dark green vein-banded flecks on youngest leaves	Vein yellowing followed by a brilliant yellow-green leaf mottle with dark green vein-banded flecks. Leaves and stipules slow to unfold
Trifolium pratense	No symptoms	Slight vein yellowing 10–12 days after inoculation followed by a mild mottle and no marked reduction in leaf size (Plate 2, Fig. 12)
Trifolium incarnatum	Pronounced vein yellowing followed by a brilliant yellow-green leaf mottle, dark green vein-banded flecks, and a reduction in leaf and plant size	Severe vein yellowing of youngest leaves 8–10 days after inoculation followed by a severe yellow-green leaf mottle, vein banded-flecks, leaf distortion, and marked stunting of infected plants

(*c*) *Symptom Expression of B.Y.M.V. Strain* 2 *on Various Hosts*

(i) *Beans.*—On beans symptoms were generally less severe than those recorded for strain 1, and the varieties tested showed general yellow-green leaf mottle symptoms with no reduction in leaf size or necrosis.

(ii) *Other Hosts.*—Symptom expression on the other hosts tested (Table 3) was similar to strain 1, and significant differences in symptoms could not be detected between the two strains except in reactions on subterranean clover (see Section III (*e*)).

(*d*) *Symptom Expression of P.M.V. Strains* 1 *and* 2 *on Various Hosts*

(i) *Peas.*—The mottles produced by viruses of this group are more severe than those produced by B.Y.M.V. in the greenhouse.

(ii) *Clovers and Medics.*—Symptom expression is similar to that described for B.Y.M.V. on crimson clover.

(iii) Cassia corymbosa *Lam.*—Following inoculation with P.M.V. strain 1, the young leaflets developed a yellow-green leaf mottle and dark green vein-banding, as described by Norris (1943).

(iv) *Field Pea and Common Vetch.*—Strain 2 of P.M.V. produced a distinct yellow-green leaf mottle in both species, but strain 1 could not be transmitted to the common vetch on three separate occasions involving 45 plants.

(v) *New Zealand Blue Lupin.*—Symptoms similar to those described by Norris (1943) and Chamberlain (1936) could not be produced on this host, nor was it a symptomless carrier for B.Y.M.V. as shown by Hagedorn and Walker (1949).

(vi) *Bokhara Clover.*—A very mild mottle resulted, together with the production of little-leaf symptoms, following inoculation with either strain of P.M.V. in the greenhouse, though this host is virtually a symptomless carrier.

TABLE 4

THERMAL INACTIVATION, DILUTION TOLERANCE, AND AGING IN VITRO OF BEAN YELLOW MOSAIC AND PEA MOSAIC VIRUS STRAINS

Results given as number infected/number inoculated

Test	Bean Yellow Mosaic Virus		Pea Mosaic Virus	
	Strain 1	Strain 2	Strain 1	Strain 2
Thermal inactivation				
50°C	19/23	—	—	—
55°C	17/23	9/23	18/24	3/24
58°C	0/24	6/23	17/24	1/24
60°C	0/24	3/24	13/24	1/24
62°C	—	0/24	14/24	0/24
64°C	0/24	0/24	0/24	0/24
Dilution tolerance				
1 in 10	14/24	—	15/24	13/24
1 in 100	4/24	13/24	12/24	3/24
1 in 1000	1/24	2/24	7/24	0/24
1 in 2000	0/24	1/24	2/24	0/24
1 in 5000	0/24	0/24	0/24	0/24
Aging *in vitro* (hr)				
24	8/24	19/24	2/20	5/24
36	9/24	—	—	2/24
48	6/24	18/24	2/24	6/24
60	2/24	13/24	7/24	1/24
72	1/24	9/24	0/24	0/24
84	1/24	—	—	—
96	0/24	0/24	—	—

(*e*) *Reactions of Subterranean Clover to B.Y.M.V. and P.M.V.*

The varieties shown in Table 5 were all tested in the greenhouse with both strains of B.Y.M.V. and P.M.V. Hutton and Peak (1954) report a lethal necrosis of the varieties Northam First Early, Pink Flowered, and Dwalganup when inoculated with Phaseolus virus 2 Pierce or strain 2 of B.Y.M.V. Plants of these varieties, of similar age, were inoculated with sap from broad beans infected with either strain of B.Y.M.V. and P.M.V. Only the B.Y.M.V. strain 2 of Hutton produced lethal necrosis on these varieties. The other strains of P.M.V. and B.Y.M.V. produced general yellow-green leaf mottles with some vein banding.

Variations in symptom expression between individual plants within varieties of subterranean clover made it impossible to detect differences in symptom expression produced by the different strains. In general, the varieties Clare and Yarloop responded with the most brilliant yellow-green leaf mottles and the greatest reduction in leaf size and distortion of leaflets.

A severe field infection of a varietal trial of subterranean clover was observed in the spring of 1954. Four isolates, from among the 21 varieties, indicated that P.M.V. strain 2 was responsible. In the field the varieties Yarloop and Clare showed severest infection, having a marked reduction in leaf size and consequent stunting of plants.

A severe field infection was also observed in a subterranean clover field trial at Dickson Experiment Farm, Canberra, A.C.T. B.Y.M.V. strain 2 was isolated from three varieties, and from red clover bordering the field. A notable feature of the symptom expression of some introduced varieties was a lack of distinct mottle symptoms but a definite reduction in leaf size, resulting in a "little-leaf" condition of infected plants, usually accompanied by a marked reddening of the petiole and lamina.

(*f*) *Field Isolations of P.M.V. and B.Y.M.V. Strains* 1 *and* 2

Viruses of both groups were isolated from 17 different areas. On five occasions B.Y.M.V. strain 1 was isolated, and B.Y.M.V. strain 2 was isolated on four occasions. P.M.V. strain 1 was isolated on two occasions only, but P.M.V. strain 2 was isolated on six occasions. From the limited nature of such a survey it is not possible to map accurately the distribution, nor determine the hosts most usually affected and the percentage of infection.

Symptom expression on field-infected hosts usually differed from that observed in the greenhouse. Except in the case of subterranean clover already mentioned, plants in the field were less severely affected by the viruses studied.

B.Y.M.V. strain 1 was isolated from field-infected beans, variety Canadian Wonder, having typical yellow-green leaf symptoms, but the reduction in leaf size was not severe so that plants were not unduly stunted. Peas affected by this virus also, although with clearly-defined mottle symptoms, were not markedly stunted. Symptom expression on a field-infected gladiolus (*Gladiolus cuspidatus* Jacq.) was characterized by a diffuse yellow-green leaf mottle and irregular yellow-green patches.

P.M.V. strain 1 was obtained from pea, variety Greenfeast, showing a general yellow-green leaf mottle and some reduction in leaf size, but with no distortion of leaflets or stipules. Field-infected broad beans, however, show only diffuse chlorotic spots. P.M.V. strain 2 was isolated from pea, variety Greenfeast, having a pronounced yellow-green leaf mottle with some vein banding and dark green vein-banded flecks towards the margins, but there was little reduction in leaf size. Broad bean, field-infected with this strain, showed marked vein banding.

(*g*) *Differences in Severity of Isolates of B.Y.M.V. Strain* 1

An isolate of this strain was obtained from pea, variety Greenfeast, infected in the field, which produced a mild mottle only in bean, variety Canadian Wonder.

Expanded leaves developed distinct chlorotic spots 4–6 weeks after inoculation and leaves developed later were reduced in size and had a severe yellow-green mottle. When transfers were made from these beans to others, symptoms appeared after 12–14 days. This isolate also produced more severe symptoms in pea varieties than did B.Y.M.V. strain 1 already described. This is shown in Plate 2, Figures 7 and 8,

TABLE 5

REACTIONS OF STRAINS OF BEAN YELLOW MOSAIC VIRUS AND PEA MOSAIC VIRUS ON LEGUMINOUS HOSTS

+ = Mottle symptoms; N = necrotic symptoms; − = no symptoms

Species	Variety	Bean Yellow Mosaic Virus		Pea Mosaic Virus	
		Strain 1	Strain 2	Strain 1	Strain 2
Cassia corymbosa Lam.	—	−	−	+	−
Lathyrus odoratus L.	—	+			
Lupinus angustifolius L.	—	+	+	−	−
Medicago denticulata Willd.	—	+	+	−	+
M. laciniata (L.) Mill.	—	+	+	+	+
M. sativa L.	—	−	−	−	−
M. tribuloides Desr.	—	+	+	−	−
Melilotus alba Desr.	—	+	+	+	+
Phaseolus lunatus L.	—	−	−	−	−
Phaseolus vulgaris L.	Blue Lake	+	+	−	−
	Bountiful	+			
	Brown Beauty	+	+	−	−
	Burpee's Stringless Greenpod	+			
	Corbett's Refugee	+	+	−	−
	Epicure	+	+	−	
	Goward's Special	+			−
	Gt. Northern U.1 No. 1	−	−		
	Kentucky Wonder	+	+	−	−
	Michelite	+			
	Pinto	+		−	−
	Refugee No. 5	+		−	−
	Startler Wax	+			
	Stringless Greenpod	+	+	−	−
	Wellington Wonder	+			
	Westralia	+	+	−	−
	Windsor Long Pod	+	+	−	−
Pisum arvense L.	Dun, Grey	+	+	+	+
Pisum sativum L.	Wm. Massey (Gem)	−	−	−	−
	Canner's Perfection 53	−		−	−
	Canner's Perfection 75	+		+	+
	Telephone	+	+	+	+
	Witham Wonder	−	−	−	−
	Yorkshire Hero	+	+	+	+

TABLE 5 (*Continued*)

Species	Variety	Bean Yellow Mosaic Virus		Pea Mosaic Virus	
		Strain 1	Strain 2	Strain 1	Strain 2
Trifolium repens L.	—	−	−	−	−
Trifolium subterraneum L.	Bacchus Marsh	+	+	+	+
	Clare	+	+	+	+
	Dwalganup	+	+N	+	+
	Macarthur			+	+
	Mt. Barker	+	+	+	+
	Mulwala	+	+	+	+
	Northam First Early	+	+N	+	+
	Pink Flowered	+	+N	+	+
	Ruakura			+	+
	Tallarook	+	+	+	+
	Wenigup	+	+	+	+
	Yarloop	+	+	+	+
Vicia sativa L.	—	+	+	−	+
Vigna sinensis (L.) Endl. ex Nassk.	—	−	−	−	−

where differences in the symptom expression are shown on the pea variety Telephone. There was also a difference in thermal inactivation and dilution tolerance:

Physical Test	B.Y.M.V. Strain 1	
	Severe Isolate	Mild Isolate
Thermal inactivation	55–58°C	60–64°C
Dilution tolerance	1 in 1000–1 in 2000	1 in 2000–1 in 5000

The host ranges of both isolates were similar with respect to 46 hosts.

IV. DISCUSSION

Difficulties in deciding the criteria to be adopted when classifying and distinguishing viruses of the legume group presents a major problem. This seems partially due to the failure of earlier investigators to appreciate the very wide host range capacity of viruses of the group. Thus descriptions appeared of bean mosaics, pea mosaics, and sweet clover mosaic viruses as these viruses were described on new host plants. Later workers tended to describe, in detail, differences between strains of these viruses, utilizing as criteria differences in symptom expression on selected varieties of peas and beans. While in some cases these may have been justified from differences in host ranges, in others the differences were not of sufficient magnitude to warrant naming of new strains. Further confusion has been created between the pea and bean mosaic viruses by descriptions of pea mosaics which attack beans. Thus Fry (1953) describes a B.Y.M.V. which he considers similar to the P.M.V. of Chamberlain (1936) but which now attacks beans.

In Table 1 an attempt has been made to provide a means of grouping the sap-transmissible virus diseases of peas and beans by the utilization of a series of differential host plants. This is not intended as a method of classification, but it is suggested that such groupings may provide a basis for the development of a system of classification for viruses of this group. Use has been made of the fact that species differences in host range would seem *a priori* to be a better criterion for differentiation than varietal differences within species or symptom expression within varieties, which can vary a great deal with environmental factors. Thomas and Zaumeyer (1953) have introduced a similar scheme but with a larger number of differential hosts for differentiating viruses of the B.Y.M.V. group, and recognize six strains of the virus.

Physical tests have been used as criteria when describing viruses of the legume group, and particularly when differentiating strains of B.Y.M.V. and P.M.V. The success of such tests depends upon the concentration of virus in the treated inoculum, the technique of inoculating indicator plants, and the number of plants inoculated. Only small differences between virus strains are reported in some cases and this is particularly evident with thermal inactivation values. It is doubtful if 2°C differences in thermal inactivation can be considered to be real when the number of variable factors associated with the technique are considered.

Zaumeyer (1940) has tabulated results of physical tests on 14 legume viruses and it is readily seen that overlapping in the physical properties occurs, particularly between strains of B.Y.M.V. and P.M.V. It seems, then, that these tests have doubtful value even as an adjunct to host-range studies, and that it is essential to derive a suitable set of indicator hosts similar to that proposed in Table 1.

It is at once apparent, however, that different workers have not been consistent in the use of similar varieties of the differential hosts, and particularly is this the case for peas and beans. It is thus impossible to fit all described legume viruses to one scheme. A large number have, however, utilized Corbett's Refugee as the bean variety, this being immune to B.Y.M.V. With pea varieties, Greenfeast types have been selected as a rule, together with the resistant Perfection type as a check.

Major differences in host range and host reaction are apparent between several of the viruses, viz. bean mosaic, bean southern mosaic, bean yellow stipple, bean red node, alfalfa mosaic, and broad bean mottle virus. However, greatest confusion exists between the P.M.V. and B.Y.M.V. groups, which differ significantly from those listed above but which do not differ greatly from each other in host range or host reaction. The basic difference lies in the inability of P.M.V. to infect beans. Within each group several strains can be recognized, chiefly by the different reactions on red and crimson clovers, and on soybeans. The pea mosaic viruses reported by Murphy and Pierce (1937) and Hagedorn and Hanson (1951) would appear to be similar from the reactions on the nine hosts in Table 1 and they differ chiefly in the severity of reaction on peas.

The B.Y.M.V. group presents a more complex problem, but it is possible to differentiate most of the components; however, in some cases, insufficient information is available for adequate comparison. Those viruses whose host ranges correspond over the nine indicator hosts are found to be closely related and differentiated

only by differences in bean varietal reactions. Thus the host range described by Johnson and Jones (1937) for severe pea mosaic (which must be considered a strain of B.Y.M.V. on host reactions) is similar to that described by Zaumeyer (1940) for pea mosaic virus 4 (also a strain of B.Y.M.V.). The difference lies in the latter's inability to infect Corbett's Refugee, Wisconsin Refugee, or Great Northern U.1 No. 1 beans, and the former's ability to infect Perfection peas.

The B.Y.M.V. strain 1 reported in this investigation has a host range similar to that recorded by Grogan and Walker (1948) for their "type strain". Symptom expression and host range on the bean varieties was similar to that described, and included the inability to infect the Great Northern U.1 No. 1 bean variety. Similar symptoms were also observed on field pea, crimson clover, common vetch, broad bean, and soybean and no symptoms on lima bean and white clover; burr medic, however, was susceptible. The physical properties differ slightly, as Grogan and Walker record a thermal inactivation of 58–60°C compared with 55–58°C, but the aging *in vitro* differed markedly, being 84–96 hr compared with 24–48 hr.

Evidence has been presented to show the existence of a less severe isolate of this strain, which is similar in all respects except in the severity of symptoms and a slight difference in physical properties. Symptom expression on beans is similar to that recorded by Zaumeyer and Wade (1936) for pea virus 2 (a strain of B.Y.M.V.), but host range and physical properties are not similar and the isolate cannot be considered as being similar to this strain of B.Y.M.V. These results do show, however, the variation occurring between isolates of apparently distinct strains of B.Y.M.V. and the probable existence of a range of strains within and between existing strains of these viruses, possibly due to a process of continuous variation in the virus.

The second strain of B.Y.M.V. has been described by Hutton and Peak (1954) and, as suggested by them, is similar to that described by Pierce (1934). The subterranean clover mosaic of Aitken and Grieve (1943) also appears to belong to this group. The physical properties differ from those reported by Pierce. Aging *in vitro* is 72–96 hr compared with 28–32 hr, dilution tolerance is 1 in 2000–1 in 5000 compared with 1 in 800–1 in 1000, and the thermal inactivation is 60–62°C compared with 56–58°C. This second strain is somewhat similar in host range to strain 1 but differs in the ability to infect red clover and to cause necrosis in early varieties of subterranean clover.

Strain 1 of P.M.V. is similar in host range to pea viruses 2A, 2B, and 2C described by Stubbs (1937). Differences are apparent in the physical properties as Stubbs' strains have a dilution tolerance of 1 in 1000–1 in 1500 compared with 1 in 2000–1 in 5000, and an aging *in vitro* of 24 hr compared with 60–72 hr for the virus reported here. This strain is also similar in most respects to the P.M.V. described by Norris (1943) in Western Australia and has in common the ability to infect the shrub *Cassia corymbosa*, which is not shared by the other viruses studied.

The remaining strain of P.M.V. is similar in host range to pea virus 3 of Murphy and Pierce (1937) and the physical properties also bear a close relationship. Murphy and Pierce record thermal inactivation between 58 and 60°C compared with 60–62°C, and aging *in vitro* of 2–3 days compared with 2½–3 days reported here. The only difference in host range was an inability to cause necrosis of *Lupinus angustifolius*

although several tests were carried out. This strain is also similar to that described by Chamberlain (1936) in New Zealand and Zaumeyer and Wade (1936) in the United States of America.

Similarity in symptom expression for the different viruses on their common differential hosts is evident from the symptom descriptions given, and the viruses could not be satisfactorily separated by this means. Clonal and environmental variations are probably largely responsible, and in an experiment with broad beans inoculated and grown under controlled conditions of light and temperature, variation in symptom expressions among plants was so great that no differences between the viruses could be detected.

The wide host ranges of the viruses reported indicates the ability of species of legumes present in natural and established pastures in Australia to provide an adequate reservoir of virus. This fact could be of importance in the establishment and maintenance of subterranean clover pastures as all varieties of this species tested are particularly susceptible to both B.Y.M.V. and P.M.V. The relatively low incidence of virus in pastures and in commercial bean crops may be a reflection upon the efficacy of insect transmission, but the potential, nevertheless, is present. With peas, however, the wide use of the variety Gem or William Massey for market consumption, and the use of Canner's Perfection 75 in the canning industry has virtually eliminated virus as a disease of this crop.

V. Acknowledgments

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EXPLANATION OF PLATES 1 AND 2

Symptoms developed on various hosts

PLATE 1

Fig. 1.—Leaves of bean, var. Canadian Wonder, showing a yellow-green leaf mottle, vein clearing, and a marked reduction in size following inoculation with B.Y.M.V. strain 1.

Fig. 2.—Ventral surface of a leaf of bean, var. Epicure, showing a systemic necrosis of the veins which extends to the petiole, following inoculation with B.Y.M.V. strain 1.

Fig. 3.—Mild yellow-green chlorotic spots are developed on bean, var. Stringless Greenpod, following inoculation with B.Y.M.V. strain 1.

Fig. 4.—Leaf of bean, var. Canadian Wonder, showing vein yellowing and irregular chlorotic spots which appear on young leaves approx. 2 weeks after inoculation with B.Y.M.V. strain 2.

Fig. 5.—Mottle developed on the 7th leaf of broad bean, var. Windsor Long Pod, following inoculation with B.Y.M.V. strain 1. This is characterized by large yellow-green areas and is followed by the general mottle symptoms shown in Plate 1, Figure 6.

Fig. 6.—Leaves of broad bean, var. Windsor Long Pod, showing the general yellow-green leaf mottle with irregular chlorotic areas developed 2–3 weeks after inoculation with B.Y.M.V. or P.M.V. strains.

PLATE 2

Fig. 7.—Pea, var. Telephone, showing mild chlorotic mottle symptoms, with distinct chlorotic spots, developed on leaves following inoculation with the mild isolate of B.Y.M.V. strain 1.

Fig. 8.—Pea, var. Telephone, showing distinct chlorotic spots, dark green vein-banded flecks, and yellow-green leaf streaks; symptoms developed after inoculation with the severe isolate of B.Y.M.V. strain 1.

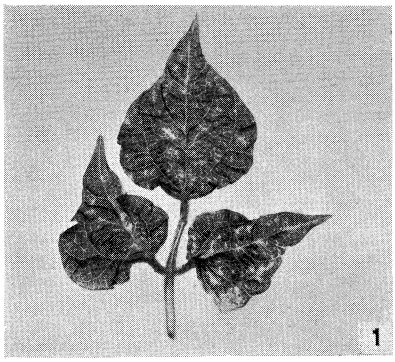
Fig. 9.—Pea, var. Telephone, with general yellow-green leaf mottle and vein-banding of leaves developed after inoculation with P.M.V. Compare with Plate 1, Figure 5, and Plate 2, Figures 7 and 8.

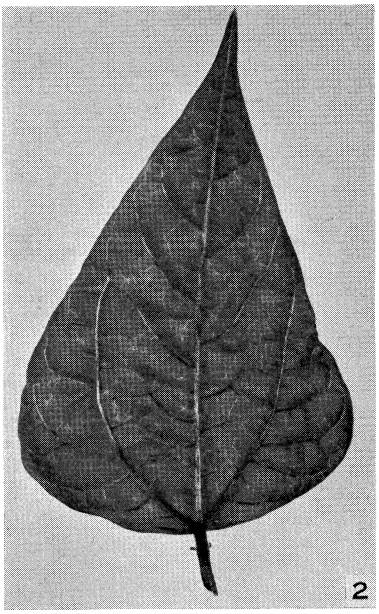
Fig. 10.—A range of mottle symptoms developed on crimson clover (*Trifolium incarnatum*) after inoculation with B.Y.M.V. strain 1. The leaf on the left shows the most severe symptoms with a general yellowing of the leaflets and pronounced dark green vein-banded flecks. The other leaves show chlorotic spots and vein yellowing and all are reduced in size.

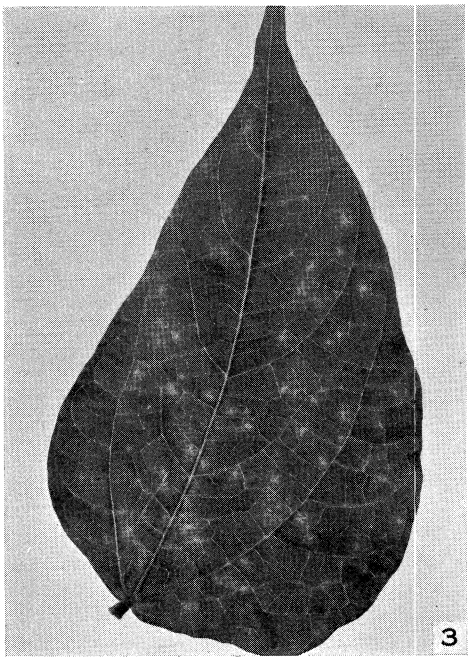
Fig. 11.—Red clover (*Trifolium incarnatum*) showing yellow-green leaf streaks and vein-banded flecks developed following inoculation with B.Y.M.V. strain 2.

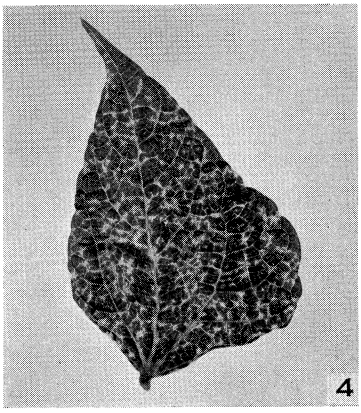
Fig. 12.—Red clover (*Trifolium incarnatum*) with mild yellow-green leaf mottle, and some vein-banded flecks, which develop after inoculation with P.M.V. strain 2.

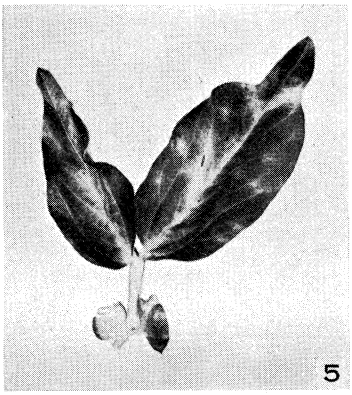
RELATIONSHIPS OF LEGUME VIRUSES IN AUSTRALIA. I

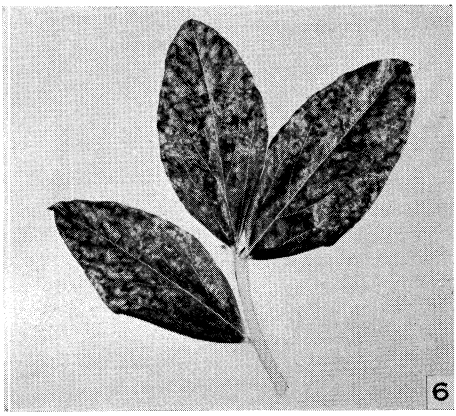












RELATIONSHIPS OF LEGUME VIRUSES IN AUSTRALIA. I