Serologically and Biologically Distinct Bean Yellow Mosaic Virus Strains

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ABSTRACT

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Bean yellow mosaic virus (BYMV) isolates from various locations in the USA and Europe could be classified in three distinct subgroups on the basis of serological and biological differences. Within each major subgroup were isolates that shared common host range and antigenic characteristics but which could be distinguished on the basis of numerous minor host range differences. Two of the BYMV subgroups appear to occur primarily in different natural hosts (red or white clover). A natural basis for BYMV subgroups would enable predictions about expected occurrence and severity in different leguminous crops.

Additional key words: clover yellow vein virus, agar double diffusion, differential hosts.

Bean yellow mosaic virus (BYMV) first was described by Pierce in 1934 (4). Since then, many isolates with widely varying host ranges have been reported (4, 5, 14, 16, 20). In 1965, Hollings and Nariani (9) described a virus isolated from white clover in England and named it clover yellow vein virus (ClYVV). Pratt (15) and Barnett and Gibson (2) described similar viruses from white clover in North America. A distant serological relationship between ClYVV and BYMV was reported (2, 15).

Barnett and Gibson (2) found that 38% of the virus-infected white clover samples obtained in southeastern USA contained ClYVV. Jones and Diachun (11) found that 76% of the red clover plants assayed in Kentucky were infected with BYMV.

Strains of BYMV reportedly have been recovered from white clover (8, 14, 16), even though it normally is not considered a host for BYMV. Pratt (15) suggested that some BYMV isolates may have been misidentified and were really ClYVV isolates.

Recently Bos et al. (5) divided bean yellow mosaic virus (BYMV) isolates into three distinct groups on the basis of host range, symptoms, and reactions on cultivars of bean and pea. Using microprecipitin tests Bos et al. (5) found that isolates from different groups did not differ appreciably in serological properties. However, work done independently by others (7, 12) suggested that serological distinctions exist between members of the BYMV group.

Because of the wide host range and symptom variability reported among BYMV isolates, and the similarities between ClYVV and BYMV, studies were undertaken to determine the biological and serological relationships within the BYMV group and between isolates found infecting white or red clover.

MATERIALS AND METHODS

Virus isolates.—Various BYMV and ClYVV isolates were compared serologically and on selected differential hosts to determine their relationships. A list of the virus isolates and their sources appears in Table 1.

Differential hosts.—Inoculum for the differential host studies was a preparation made by grinding leaves of Dwarf Gray Sugar pea (*Pisum sativum* L.) or broad bean (*Vicia faba* L.) in 0.1 M potassium phosphate, pH 7.0 (1:9, w/v) with mortar and pestle. The extract was strained through two layers of cheesecloth. Inoculations were made by rubbing leaves with cotton dipped in inoculum to which Carborundum [22-μm(600-mesh)] had been added. Additional plants of each species were rubbed with buffer and Carborundum only, as controls.

Plants for virus assay were grown in the greenhouse in Jiffy Mix (Jiffy Products of America, West Chicago, IL 60185) in 10 cm diameter clay or plastic pots. Plants were given supplemental fluorescent lighting for a 14-hr day length. All plants not showing symptoms were tested 3-4 wk after inoculation by back-inoculation to Bountiful bean (*Phaseolus vulgaris* L.) or Dwarf Gray Sugar pea.

Preparation and sources of antisera.—Infected pea tissue was harvested 14-18 days after inoculation and purified using the urea-phosphate purification method (10, 11).

Virus concentrated by high-speed centrifugation was suspended in 0.5 M potassium phosphate, pH 7.0 containing 1.0 M urea, at one-thirtieth volume of the clarified extract and mechanically shaken at 1-2 C overnight. The resuspended pellets were centrifuged at 4,000 rpm for 10 min in a Sorvall Type SS34 rotor and the supernatant liquid was layered on density gradient columns. Gradient columns were prepared in 2.54 × 8.89-cm (1 × 3.5-inch) cellulose nitrate tubes by layering, respectively, 7, 10, 10, and 5 ml of 40, 30, 20, and 10% (w/v) sucrose in 0.5 M phosphate, pH 7.0. Gradients were

kept overnight at 3-4 C before use. Gradient columns were centrifuged at 27,000 rpm in a Spinco SW 27 rotor for 2.5 hr. Centrifuged gradient columns were scanned at 254 nm using an ISCO Model D density gradient fractionator and ultraviolet analyzer coupled to an external chart recorder.

Virus from density-gradients was diluted 1:3 with distilled water and centrifuged at 27,000 rpm for 90 min in a Beckman Type 30 rotor. Pellets were suspended in physiologically buffered saline (PBS: 0.85% sodium chloride and 0.01 M sodium phosphate, pH 7.0) by mechanically shaking overnight at 1-2 C. Antisera to the BYMV:204-1 and :OH-S isolates were produced in rabbits by intramuscular injections, at 1-wk intervals, of purified virus mixed with Freund's complete adjuvant (1:1, v/v). The virus concentration was determined spectrophotometrically. The amount used for each injection ranged from 1.5 to 4.5 mg protein. Monitoring of antiserum titer began after the third injection. Serum was diluted with glycerol (1:1, v/v) and stored in a freezer until used. Antiserum to the ClYVV:B isolate with an antiserum titer of 1/2,048 was provided by O. W. Barnett.

Agar double-diffusion techniques.—Ouchterlony double-diffusion tests were performed in 100 × 15-mm plastic petri dishes loaded with 15 ml of 0.6% Ionagar in 0.1 M Tris-HCl buffer, pH 9.0, containing 0.2% sodium dodecyl sulfate, 0.7% NaCl and 0.1% NaN_3 [modified from Tolin and Roane (17) by replacing water with the Tris-HCl buffer].

Virus antigens for gel diffusion were obtained by grinding infected pea or bell bean tissue in distilled water (1:2, w/v). Purified viral antigens of selected isolates suspended in water with an A_{260} of 1.0 reading also were tested.

Wells in the agar were cut with an Auto-Gel T/M punch (Garfer Corp., Detroit, MI 48238). Five-mm holes and well spacings were used.

RESULTS

Differential hosts.—On the basis of key host reactions the virus isolates studied can be classified in three distinct groups (Table 2). The first was comprised primarily of the ClYVV isolates and the BYMV isolates from white clover. They showed the following general similarities: Infection of Burley 21 tobacco; yellow mosaic, necrosis, and death in susceptible pea cultivars and bell bean (*Vicia faba* L. 'Minor'); and tip necrosis and death in susceptible bean cultivars.

Members of the second group possessed properties intermediate to the other groups. They caused mild light-dark green mosaic in pea and bell bean and moderate mosaic in susceptible bean cultivars. The BYMV type isolate (B-25) of Bos et al. (5) belongs in this group. They usually infected Red Mexican UI36 which appeared to be resistant to BYMV isolates of the other groups. On occasion, however, members of all three subgroups caused infection in Red Mexican UI36.

Members of the third group generally did not infect tobacco, but infected susceptible peas and bell bean causing either mild light-green mosaic or stronger yellow-green mosaic but no necrosis or death; they also caused

TABLE 1. Designation, original host and source of bean yellow mosaic virus (BYMV), clover yellow vein virus (ClYVV), and other virus isolates used in the comparison studies

Isolate designation	Original host[a]	Source[b]
BYMV:B-25	bean (*Phaseolus vulgaris* L.)	L. Bos
BYMV:204-1	red clover (*Trifolium pratense* L.)	S. Diachun
BYMV:OH-Sb	soybean [*Glycine max* (L.) Merr.]	A. F. Schmitthenner and D. T. Gordon
BYMV:OH-S	Sanilac bean	D. T. Gordon and A. F. Schmitthenner
BYMV:OH-M	Sanilac bean	D. T. Gordon and A. F. Schmitthenner
ClYVV:B	white clover (*Trifolium repens* L.)	O. W. Barnett
ClYVV:L	white clover	O. W. Barnett
ClYVV:F	white clover	O. W. Barnett
BYMV:Pratt		O. W. Barnett
BYMV:E-198	pea (*Pisum sativum* L.)	L. Bos
ClYVV:E-178	pea	L. Bos
BYMV:BL-BNV	white clover	R. O. Hampton
BYMV:Gil 6/RK	bean	R. O. Hampton
BYMV:Scott	USDA type-isolate	R. O. Hampton
BYMV:Y9	(unknown)	R. O. Hampton
ClYVV:H	white clover (type-isolate)	L. Bos
BCMV:NY[c]	bean	J. K. Uyemoto
PSbMV[d]	pea	R. O. Hampton
CAbMV[e]	cowpea	O. W. Barnett
SMV:Mild[f]	soybean	D. T. Gordon
SMV:Severe[g]	soybean	D. T. Gordon

[a]Original host means the plant in which the virus was initially found.
[b]Source refers to the individual who supplied the isolate for these studies.
[c]BCMV:NY = an isolate of bean common mosaic virus from New York.
[d]PSbMV = an isolate of pea seed borne mosaic virus.
[e]CAbMV = an isolate of cowpea aphid borne mosaic virus.
[f]SMV:Mild = a mild isolate of soybean mosaic virus.
[g]SMV:Severe = a severe isolate of soybean mosaic virus.

TABLE 2. Differentiation of bean yellow mosaic virus (BYMV) isolates into distinct groups on the basis of symptom response on selected differential hosts

Differential hosts	BYMV subgroups															
	Subgroup I									Subgroup II		Subgroup III				
	OH-S[a]	Sb	BL-BNV	Gil 6/RK	ClYVV[b] :B	ClYVV :L	ClYVV :F	E-178[c]	ClYVV[d] :H	B-25	Scott	204-1	OH-M	Pratt	Y9	E-198
	+[e]	+	+	+		+	+									
Cucurbita sativum L.	S,LL	L,LL	S,LL	(S,LL)	NS	S,LL	S,LL	NS	NS	NS	NS	NS	NS	NS	NS	NS
'Caserta'					NR			NR	NR	NR	NR	NR	NR	NR	NR	NR
	+	+	+	+	+	+				+	+				+	
Nicotiana tabacum L.	LL	l	LL	LL	LL	LL	LL	NS	NS	l	(l)	NS	NS	NS	l	NS
'Burley 21'								NR	NR			NR	NR	NR		NR
Phaseolus vulgaris L.	SeSM	MoSM	SeSM	SeSM	SeSM	SeSM	SeSM	CHLL	S,CHLL	MoSM	MoSM	MSM	MSM	MSM	MSM	NS
'Bountiful'	Pd	(Pd)	Pd	Pd	Pd	Pd		MSM	MSM							NR
													(MSM)			
'Red Mexican UI36'	NS	NS	NS	NS	NS	NS	NS	NS	NS	MSM	MSM	NS	NS	MSM	NS	NS
	NR	NR	NR	NR	NR	NR	NR	NR	NR	(NS NR)		NR	NR		NR	NR
Pisum sativum	SeSM	NS	SeSM	SeSM	SeSM	SeSM	SeSM	SeSM	(SeSM)	MSM	MoSM	MoSM	MSM	MoSM	MoSM	MoSM
'Dwarf Gray Sugar'		NR	Pd	Pd	Pd	Pd	Pd	Pd	(Pd)							
'Wisconsin Perfection'	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	MSM	NS	NS	NS
	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR		NR	NR	NR
Vicia faba minor L.	SeSM	SeSM	SeSM	SeSM	SeSM	SeSM	SeSM	MoSM	S,CHLL	MSM	MSM	MSM	MSM	MoSM	MSM	MSM
'Bell bean'	Pd	Pd	Pd	Pd	Pd	Pd	Pd	(SeSM)	(MSM)							

[a]The following isolates were designated as bean yellow mosaic virus (BYMV) isolates by the authors who provided them for this study. BYMV: = :OH-S, :BL-BNV, :Gil 6/RK, :204-1, :OH-M, :Pratt, Y9, :E-198, :B-25, and :Scott.

[b]The isolates :B, :L, and :F were designated as clover yellow vein virus (ClYVV) isolates by O. W. Barnett.

[c]E-178 = a pea necrosis isolate designated by Bos to be similar to clover yellow vein virus.

[d]Isolate ClYVV:H = Hollings' type-isolate of clover yellow vein virus.

[e]Symbol legend: S = visibly systemic; s = systemically latent; L = visible local infection; l = latent local infection; LL = visible local lesions; Pd = plant death; MSM = mild systemic mosaic; MoSM = moderate systemic mosaic; SeSM = severe systemic mosaic and tip necrosis; NS = no symptoms; NR = no virus recovered in back-inoculations; + = infection as shown by back-inoculation. () = symptoms produced occasionally.

mild mosaic, chlorotic spotting, or no symptoms in susceptible bean cultivars. Pea mosaic virus isolates belong in this group.

Members from all groups usually caused necrotic local lesions and systemic necrosis in the red clover clone 71-8 which was used as an indicator of BYMV. Wisconsin Perfection pea was resistant to all isolates except OH-M. The members of each group were distinguishable on the

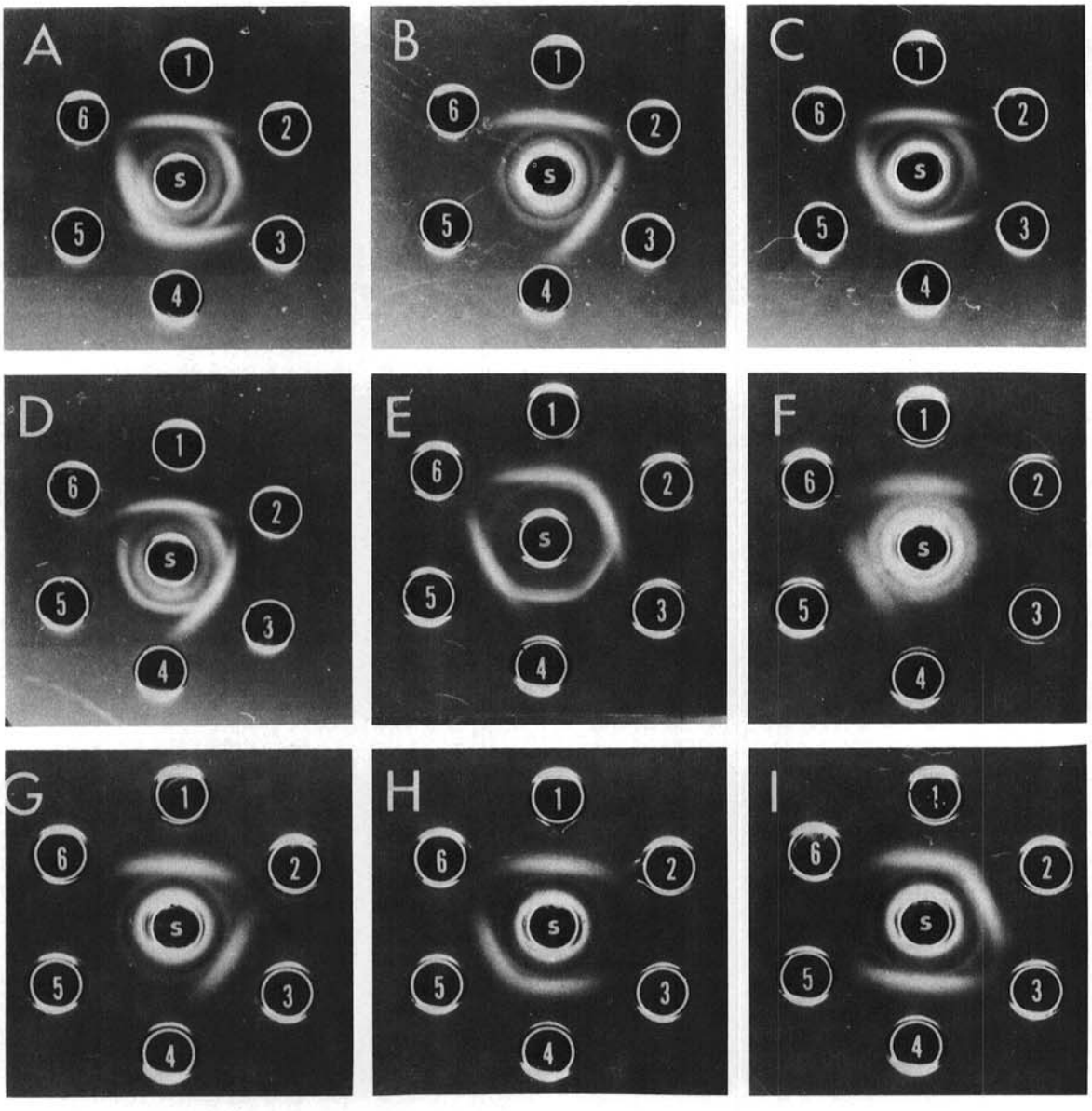

Fig. 1-(A to I). Intragel cross-absorption tests in sodium dodecyl sulfate-agar gel diffusion plates using bean yellow mosaic virus [BYMV:204-1 (A through D)] and [BYMV:OH-S (E through I)] sera (S) and crude extracts of BYMV and clover yellow vein virus (ClYVV) isolates from pea. Peripheral wells 1 and 6 contain the homologous antigen and healthy pea extract respectively, for all tests. **A)** Cross-absorption control. Well 2 contains BYMV:B-25, well 3-BYMV:OH-S, well 4-BYMV:OH-M, and well 5-BYMV:Pratt; **B)** Cross-absorption with ClYVV:B. Well 2 contains ClYVV:F, well 3-BYMV:OH-M, well 4-BYMV:OH-S, and well 5-ClYVV:B; **C)** Cross-absorption with ClYVV:B. Well 2 contains BYMV:B-25, well 3-BYMV:OH-S, well 4-BYMV:OH-M, and well 5-BYMV:Pratt; **D)** Cross-absorption control. Well 2 contains BYMV::B-25, well 3-BYMV:OH-M, well 4-ClYVV:F, and well 5-BYMV:OH-S; **E)** Cross-absorption control. Well 2 contains ClYVV:H, Well 3-BYMV:204-1, well 4-BYMV:Scott, well 5-BYMV:BL-BNV; **F)** Cross-absorption with ClYVV:H. Well 2 contains BYMV:Scott, well 3-BYMV:E-198, well 4-BYMV:204-1, well 5-ClYVV:H; **G)** Cross-absorption with BYMV:Scott. Well 2 contains BYMV:OH-M, well 3-BYMV:E-178, well 4-BYMV:204-1, well 5-BYMV:E-198; **H)** Cross-absorption with BYMV:OH-M. Well 2 contains BYMV:204-1, well 3-BYMV:B-25, well 4-ClYVV:H, well 5-BYMV:Gil 6/RK; **I)** Cross-absorption with BYMV:E-198. Well 2 contains BYMV:Gil 6/RK, well 3-BYMV:E-198, well 4-BYMV:BL-BNV, and well 5-BYMV:OH-M.

TABLE 3. Results of intragel cross absorption tests using SDS-agar gel serology with bean yellow mosaic virus (BYMV:204-1) antiserum and several BYMV antigens

BYMV strain	Cross-absorbing antigen (strain)															
	Subgroup I								Subgroup II		Subgroup III					
	OH-S[a]	ClYVV:B[b]	ClYVV:L	ClYVV:F	BL-BNV	Gil 6/RK	E-178[c]	ClYVV:H[d]	B-25	Scott	204-1	OH-M	Pratt	Y9	E-198	Control
Subgroup I:																
OH-S	–[e]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
ClYVV:B	–	–	–	0	–	–	–	–	–	–	–	–	–	–	–	+
ClYVV:L	–	–	–	–	–	–	0	–	–	–	–	–	–	–	–	+
ClYVV:F	–	–	–	–	–	–	0	–	–	0	–	–	–	0	–	+
BL-BNV	–	–	–	–	–	–	0	–	–	–	–	–	–	–	–	+
Gil 6/RK	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
E-178	–	–	0	0	0	–	–	–	–	–	–	–	–	–	–	+
ClYVV:H	–	–	0	0	0	–	–	–	0	0	0	–	0	0	0	+
Subgroup II:																
B-25	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	++
Scott	+	+	0	0	+	+	+	+	–	–	–	–	–	–	–	++
Subgroup III:																
204-1	++	++	++	++	++	++	++	++	++	++	+	+	+	+	+	+++
OH-M	++	++	++	++	++	++	++	++	++	++	+	±	+	+	+	+++
Pratt	++	++	++	++	++	++	++	0	++	+	±	+	±	±	±	+++
Y9	++	++	++	++	++	++	++	++	++	+	±	±	±	±	±	+++
E-198	++	++	++	++	++	++	++	++	++	++	+	+	+	+	±	+++

[a]The following isolates were designated as bean yellow mosaic virus (BYMV) isolates by the authors who provided them for this study. BYMV: = :OH-S, :BL-BNV, :Gil 6/RK, :204-1, :OH-M, :Pratt, Y9, :E-198, :B-25, and :Scott.

[b]The isolates :B, :L, and :F were designated as clover yellow vein virus (ClYVV) isolates by O. W. Barnett.

[c]Isolate E-178 = a pea necrosis isolate designated by Bos to be similar to clover yellow vein virus.

[d]Isolate ClYVV:H = Hollings' type-isolate of clover yellow vein virus.

[e]Symbol legend: +++ = strong precipitin line; ++ = moderate precipitin line; + = weak precipitin line; – = no visible reaction; 0 = not tested; ± = sometimes a weak precipitin line.

basis of additional host-range studies.

In general, Group I isolates had the largest host ranges; Group II isolates were intermediate, and Group III had the most restricted host ranges of the isolates tested. Although most isolates that were studied easily could be placed into one of the distinct groups, classification of isolates like BYMV:OH-Sb and ClYVV:H often was difficult when host responses were viewed individually. Their responses on the key indicators often were different than expected and tended to be variable. For example, ClYVV:H did not always infect Dwarf Gray Sugar pea and OH-Sb never did.

Agar diffusion tests.—In Ouchterlony double-diffusion tests, using BYMV:204-1 and (:OH-S) antisera, three serologically distinct BYMV groups were observed. These groups were similar to those observed on the basis of host response. With antiserum to BYMV:204-1, a Group III isolate, all Group I isolates formed lines of identity with each other but were spurred over by Group III isolates and occasionally by Group II isolates [Fig. 1-(A-D)]. All members of Group III showed lines of identity when paired in adjacent wells.

With BYMV:204-1 antiserum Group II isolates always were spurred over by Group III members, but occasionally they appeared to fuse with BYMV:Pratt. They always gave a fainter precipitin line than that produced by Pratt's isolate when 204-1 antiserum was used, but a stronger line when OH-S (a Group I isolate) serum was used. They occasionally spurred over, but often fused with, Group I isolates and always produced a stronger precipitin line when 204-1 antiserum was used and a weaker line when OH-S antiserum was used. When BYMV:OH-S, a Group I isolate, antiserum was used, only two of the three serological groups were detected. With this antiserum, Group II isolates always were spurred over by Group I isolates, but appeared to fuse with Group III isolates [Fig. 1-(E-I)]. Group III isolates also were always spurred over by Group I isolates.

That the BYMV:204-1 and OH-S antisera were quite specific for BYMV was shown by the fact they did not react with other potyviruses of legumes: a bean common mosaic virus (BCMV:NY) isolate, a pea seedborne mosaic virus (PSbMV) isolate, a cowpea aphid-borne mosaic isolate, and two soybean mosaic virus (SMV:mild, :severe) isolates. Only Group I isolates reacted with antiserum prepared against ClYVV:B (1/2,048). However, Barnett and Gibson (2) have reported spur formation between ClYVV:B and BYMV using a higher-titered antiserum.

Intragel cross absorption.—For a summary of results see Tables 3 and 4. In cross-absorption tests using the SDS agar double-diffusion technique, a 3- to 4-hr absorption with crude antigen of distantly related isolates in the serum well was sufficient to prevent visible precipitation of the cross-absorbing antigen when placed in a peripheral well. Only the central serum well was removed at the time of initial incubation.

Cross absorption did not prevent reaction by some members of the group from which the antiserum was produced. Cross absorption (using BYMV:204-1 antiserum) with any member of Group I prevented visible precipitation by all members of that group [Fig. 1-(B-C)]. Group II and Group III members still gave visible reaction, and Group III members still spurred over Group II members.

Cross absorption with Group II isolates prevented all members of Group I and Group II from forming visible precipitation lines. Cross absorption with any member of Group III prevented Group I or Group II from forming visible precipitation lines. Faint precipitation lines were

TABLE 4. Results of intragel cross absorption tests using SDS-agar gel serology with bean yellow mosaic virus (BYMV:OH-S) antiserum and several BYMV antigens

	Cross-absorbing antigen (strain)										
	Subgroup I				Subgroup II		Subgroup III				
BYMV strain	OH-S[a]	Gil 6/RK	E-178[b]	ClYVV:H[c]	B-25	Scott	204-1	OH-M	Y9	E-198	Control
Subgroup I:											
OH-S	+[d]	±	+	+	++	++	++	++	++	++	+++
BL-BNV	+	±	0	0	++	++	++	++	++	++	+++
Gil 6/RK	+	+	+	+	++	0	++	++	++	++	+++
E-178	+	±	+	0	++	++	++	++	++	0	+++
ClYVV:H	+	±	+	+	0	++	++	++	++	0	+++
Subgroup II:											
B-25	−	−	−	−	−	−	−	−	−	−	++
Scott	−	−	−	−	−	−	−	−	−	−	++
Subgroup III:											
204-1	−	−	−	−	0	−	0	−	0	0	++
OH-M	−	0	−	−	−	−	−	−	−	−	++
Y9	−	−	−	−	−	0	−	−	−	0	++
E-198	−	0	0	−	−	−	−	0	−	−	++

[a]The following isolates were designated as bean yellow mosaic virus (BYMV) isolates by the authors who served as their source for this studv. BYMV: = :OH-S, :BL-BNV, :Gil 6/RK, :B-25, :Scott, :204-1, :OH-M, :Y9, and :E-198.

[b]Isolate E-178 = a pea necrosis isolate designated by Bos to be similar to clover yellow vein virus.

[c]Isolate ClYVV:H = Hollings' type-isolate of clover yellow vein virus.

[d]Symbol legend: +++ = strong precipitin line; ++ = moderate precipitin line; + = weak precipitin line; − = no visible reaction; 0 = not tested; ± = sometimes a weak precipitin line.

still formed by members of Group III, indicating that the cross absorption was not complete. The reciprocal reactions were observed with BYMV:OH-S antiserum [Fig. 1-(F to I)] with the exception that Group II could not be distinguished from Group III.

DISCUSSION

It was concluded on the basis of biological and serological differences that at least three distinct BYMV subgroups exist (Table 5).

Distinct BYMV isolates were found in naturally infected red and white clover in Ohio and Central Kentucky (Jones and Diachun, *unpublished*). The procedures used during these surveys were described previously (11). Results showed distinct subgroups occurred primarily in one, but not in both, of the clovers. Of 421 BYMV isolates found in red clover (11), 416 were judged members of subgroup III on the basis of serology and host range. All 25 BYMV isolates from white clover were subgroup I members. They produced necrotic reactions on pea and bean and spur formation with members of subgroup III. The source of the necrotic BYMV strains from Oregon (8), the severe BYMV isolates from Ohio (10), and the ClYVV isolates of Barnett and Gibson (2) was also white clover. They as well as Bos's (E-178) pea necrosis isolate and Hollings' ClYVV:H isolate were members of subgroup I. It appears there is a natural basis for the separation of BYMV isolates into distinct subgroups.

Past (7, 12, 18) and present serological tests detected the presence of at least three BYMV serotypes. Tests using the rate-zonal serology assay of Ball and Brakke (1) showed that intact virus particles of the different BYMV isolates gave the same serological relationships as the degraded ones (12, and Jones and Diachun, *unpublished*). Differences in host reaction were based on severity of symptoms on bean, symptoms on pea and bell bean, and to a lesser extent infection of tobacco and squash. When grouping BYMV isolates on the basis of host reactions there will always be exceptions to the rule. For example, BYMV:OH-Sb did not exhibit symptoms or necrosis on pea nor did it frequently induce a necrosis in bean, but serologically it belongs with subgroup I isolates. Unlike Bos et al. (5), we were unable to distinguish BYMV subgroups on the basis of systemic infection in either *Chenopodium amaranticolor* (Coste & Reyn.) or *C. quiona* (Willd.) All isolates tested produced local lesions on *C. amaranticolor*, but systemic infection was variable. *Chenopodium quinoa* produced local lesions for all isolates except two members of the pea mosaic virus group (OH-M and E-198) which caused chlorosis. Systemic infection was again variable.

Many members of Group I produced yellow spots on caserta squash; this was not observed with members of the other subgroups. Undue emphasis should not be placed on an individual host response; instead, the host responses of an isolate should be viewed collectively when deciding in which subgroup the isolate belongs. The use of serology in conjunction with host response provides new insight into what might otherwise seem a biological continuum. In the face of host range and symptom variability - serology seems the more reliable means of subgroup classification. Despite the biological separation of BYMV into three distinct groups Bos et al. (6) still consider ClYVV (including E-178) biologically distinct from BYMV and its pea necrosis (severe) subgroup. Because of the biological variability of BYMV-like isolates it is extremely difficult to determine which distinctions are significant. How does one distinguish strain variability or variability within a strain from distinct viruses? It is hoped that serological differences can play a part in answering this question. It was serological differences which first permitted a logical interpretation of the biological differences seen within the BYMV group. Van Regenmortel and Von Wechmar (19) pointed out the pitfalls of serological distinctions based on only a few antisera prepared in a few animals. However, past tests using intact virus particles and different antisera prepared in separate laboratories to three biologically distinct BYMV isolates (:OH-S, :B-25 and :204-1) permitted distinction of the same three subgroups (12). Moreover Garnett (7) and Uyeda et al. (18) in their work with distinct BYMV isolates also detected three serologically and biologically distinct subgroups. Because essentially the same conclusion concerning three serological subgroups was reached the

TABLE 5. Differentiation of bean yellow mosaic virus (BYMV) isolates into distinct groups on the basis of serology

Bean yellow mosaic virus (BYMV) serotypes		
Group I	Group II	Group III
:OH-S[a]	:B-25	204-1
ClYVV:B[b]	:Scott	OH-M
ClYVV:L		:Pratt
ClYVV:F		Old BYMV (Y9)
:OH-Sb		E-198
BL-BNV		
Gil 6/RK		
E-178[c]		
ClYVV:H[d]		

[a]The following isolates were designated as bean yellow mosaic virus (BYMV) isolates by the authors who provided them for this study. BYMV = :OH-S, :OH-Sb, :BL-BNV, :Gil 6/RK, :204-1, :OH-M, :Pratt, :Y9, :E-198, :B-25, and :Scott.

[b]The isolates :B, :L, and :F were designated as clover yellow vein virus (ClYVV) isolates by O. W. Barnett.

[c]E-178 = a pea necrosis isolate designated by Bos to be similar to clover yellow vein virus.

[d]ClYVV:H = Hollings' type-isolate of clover yellow vein virus.

present groupings on the basis of only two antisera were made.

Apparently BYMV-C1YVV represent strains of the same virus (BYMV), with the major distinction being their occurrence primarily in either white or red clover. The red clover necrosis strain of Zaumeyer and Goth (20), however, may have represented a red clover strain which belongs in Group I. Lindsten et al. (13) also reported infection of red clover with isolates from white clover.

If the distinction on the basis of natural occurrence in red and white clover is valid, then the BYMV group is much like the sugarcane mosaic virus (SCMV) group in which maize dwarf mosaic virus is separated from SCMV by its ability to infect Johnsongrass (3).

The BYMV group is composed of several serologically and biologically distinct strains. Grouping on the basis of serology also may be correlated to key host reactions (12). White and red clover serve as potential sources of distinct BYMV strains.

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