Strains of Bean Yellow Mosaic Virus Compared to Clover Yellow Vein Virus in Relation to Gladiolus Production in Florida

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ABSTRACT

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In a survey, 92% of all gladioli tested from the United States and Holland were infected with bean yellow mosaic virus (BYMV). Isolates of BYMV from gladiolus were compared with BYMV isolates from *Trifolium pratense* (BYMV-204-1), *Pisum sativum* (BYMV-P), *Alpinia zerumbet* (BYMV-A), and *Freesia refracta* (BYMV-F), and a clover yellow vein virus (CYVV) isolate from *T. repens* (CYVV-P). The gladiolus isolates of BYMV were indistinguishable in host range tests, but differed from BYMV isolates from other hosts and CYVV. In immunodiffusion tests with antisera to BYMV-204-1, CYVV-P, and a gladiolus isolate (BYMV-G) all of the gladiolus isolates reacted identically but could be distinguished by spur formation from each of the other isolates. The G and 204-1 isolates of BYMV were more closely related to each other than to CYVV-P in immunodiffusion tests; however, in enzyme-linked immunosorbent assay tests, BYMV-G appeared to be only distantly related to either BYMV-204-1 or CYVV-P. The in vitro translation products of BYMV-G were more similar to those of BYMV-204-1 than to those of CYVV-P. The estimated molecular weights of at least three products of CYVV-P differed from the corresponding products of BYMV-G and BYMV-204-1. We believe that the gladiolus isolates were closely related to the isolate from *T. pratense* and distantly related to the isolate from *T. repens*.

Almost two-thirds of all the gladiolus (*Gladiolus* × *hortulanus* Bailey) cut flowers grown in the United States are produced in Florida (26). Zettler and Abo El-Nil (27) found that over 80% of the gladiolus plants surveyed in Florida were infected with bean yellow mosaic virus (BYMV), but no other viruses were detected in stock grown exclusively in Florida. In their study, all of the BYMV isolates obtained from gladioli from Florida and Oregon appeared identical, but they differed from BYMV isolates from other hosts. They also found an isolated, virus-free population of hybrids of *G. psittacinus* growing as volunteers in Florida. From such preliminary findings, it is hypothesized that virus-free gladioli can be produced in Florida provided virus-free planting stock could be obtained and grown in areas with no BYMV-infected plants.

Perenniating legumes such as red clover (*Trifolium pratense* L.) could serve as field reservoirs of BYMV inoculum, which could infect gladiolus plants (10). Although red clover does not grow as a perennial in Florida, white clover (*T. repens* L.) occasionally does and could provide a source of inoculum that could threaten virus-free gladiolus production. White clover is not susceptible to BYMV (1,17), but it is susceptible to clover yellow vein virus (12,13), which is considered by some authors to be a strain of BYMV (14).

In this study, isolates of BYMV from gladiolus are compared with BYMV isolates from other hosts, including red clover, and a white clover isolate of clover yellow vein virus (CYVV). The implications of this research in relation to establishing a virus-free certification program for gladioli in Florida are discussed.

MATERIALS AND METHODS

Sources of plants and virus isolates. One CYVV and five BYMV isolates were compared with BYMV isolates recovered from gladioli and peas in this study: CYVV-P (17), BYMV-G (27), BYMV-204-1 (14), BYMV-F (5), BYMV-A (F. W. Zettler and J. Nagel, *unpublished*), and BYMV-P (19). The hosts from which these viruses were originally obtained were, respectively, white clover, gladiolus, red clover, freesia (*Freesia refracta* (Jacq.) Klatt), alpinia (*Alpinia zerumbet* (Pers.) B. M. Burtt and R. M. Sm.), and pea (*Pisum sativum* L.). All isolates were routinely maintained in cultivar Alaska pea.

Gladioli started from seed and grown to maturity at the University of Florida Agricultural Research and Education Center at Bradenton were surveyed. Also, some gladioli were obtained from commercial sources in California, Colorado, Florida, Michigan, Minnesota, New Jersey, and Holland. Samples were received as corms and germinated in a greenhouse at Gainesville, FL, for virus indexing. Virus-free gladiolus samples from a population of *G. psittacinus* (27) and from plants of *G. psittacinus* transplanted to Archer, FL, in 1973 and maintained under field conditions in isolation from commercial gladiolus plants were reexamined for virus infection. Also, samples from a population of plants of *G. hortulanus* at Archer grown from seed sown in 1973 were tested for virus. Immune electron microscopy with protein A (20) and antiserum to BYMV-G was used to detect BYMV particles in the gladiolus leaves. Another detection method involved virus recovery by manual inoculation to Alaska pea indicator plants.

Manual inoculations. Gladiolus samples were indexed for BYMV by manual inoculation to bean (*Phaseolus vulgaris* L. 'Bountiful'), and cultivar Alaska peas. Healthy gladiolus plants were inoculated with BYMV-G and BYMV-204-1 by first prerubbing leaves with 1% Triton X-100, rinsing them with water, and then inoculating them 4 hr later (23).

Virus spread. To determine whether secondary spread of BYMV or other gladiolus viruses was occurring under field conditions in Bradenton and Archer, FL, the following "trap" crops were planted adjacent to gladioli and examined for virus symptoms: cultivar Alaska peas, cultivar Bountiful beans, and peppers (*Capsicum annuum* L. 'Cuban El'). Virus identifications were confirmed by serology, light microscopy, and/or manual inoculations of indicator plants. Light microscopic examination of epidermal cells

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of infected pea and gladiolus leaves for virus-induced inclusion bodies utilized the methods described by Christie and Edwardson (3).

Aphid collections. From 8 December 1980 to 3 February 1981, and from 22 April to 3 June 1981, aphid populations were monitored with yellow, water-pan traps in Bradenton gladiolus fields. These traps were 22 × 22 × 5-cm cake pans painted yellow (Deschler Gloss Yellow 103-5641, Sherwin Williams Co., Cleveland, OH 44101) on the inside and nongloss black on the outside. Aphids were collected weekly and stored in 70% ethanol for later identification. Aphid identifications were confirmed by H. A. Denmark (Entomology Section, Florida Dep. Agric. and Consumer Services, Division of Plant Industry, Gainesville 32602) and G. LaBonne (Centre de Recherches, Agronomiques, Guadeloupe).

Purification of viruses and cylindrical inclusions. Viruses were purified as described by Dougherty and Hiebert (6) except that chloroform and carbon tetrachloride were the organic solvents and potassium phosphate buffer was used instead of HEPES buffer. Increased yields of CYVV-P were obtained by omitting polyethylene glycol (PEG) and centrifuging 8.5-ml portions of virus preparation through sucrose cushions of 1.5 ml 20% sucrose at 92,000 *g* for 1 hr in a Type 30 Beckman rotor.

Cylindrical inclusion purifications were conducted with modifications to the procedure described by Dougherty and Hiebert (7). Systemically infected Alaska peas were harvested 4–6 wk after inoculation and cooled to 4 C. Tissues were homogenized in a chilled mixture (w/2v/v) of buffer (0.5 M potassium phosphate [pH 7.5]), 0.01 M sodium sulfite, and 0.01 M sodium diethyl dithiocarbamate and organic solvents (1:1 chloroform and carbon tetrachloride). The homogenate was centrifuged in a Sorvall GSA rotor at 1,020 *g* for 5 min. The pellet was reextracted with 0.02 M potassium phosphate buffer (pH 8.2) and centrifuged again at 1,020 *g* for 5 min. The two supernatants were combined, strained through cheesecloth, then centrifuged at 13,200 *g* for 15 min.

The pellet was resuspended with the aid of a glass tissue grinder in 100 ml of 0.02 M potassium phosphate buffer (pH 8.2), containing 0.1% mercaptoethanol and 5% Triton X-100. After stirring for 1 hr, the mixture was centrifuged in a Sorvall SS34 rotor at 27,000 *g* for 10 min. The pellet was resuspended in 50 ml of 0.02 M phosphate buffer (pH 8.2), and centrifuged at 27,000 *g* for 10 min. The final pellet was resuspended in 10 ml of 0.02 M Tris buffer (pH 8.2). Samples from purified virus and cylindrical inclusion preparations were stained with 2% uranyl acetate and viewed with a Hitachi H-600 electron microscope.

To prepare the cylindrical inclusion protein for immunization, the inclusions were electrophoresed on polyacrylamide gels. A portion of the purified inclusions were added to 2.5% SDS, 1% 2-mercaptoethanol, and a few crystals of sucrose and the mixture was boiled for 1 min. A separate portion of the purified inclusions were labeled with dansyl chloride according to Talbot and Yphantis (24), and a sample of these labeled inclusions were added to the dissociated cylindrical inclusions. This mixture was layered onto a 7.5 to 15% linear gradient polyacrylamide slab gel (3 × 140 × 150 mm) and electrophoresed for 16 hr in the discontinuous buffer system of Laemmli (15). Fluorescent bands containing the inclusion protein were cut out and triturated with a mortar and pestle in 15 ml of water. The suspension was frozen, then allowed to stand 24 hr at approximately 24 C. After filtration through cheesecloth, the filtrate was centrifuged at low speed. The supernatant was filtered through a 0.45-μm Millipore filter and then lyophilized. The residue was resuspended in a small volume of water and dialyzed for 24 hr at 24 C against three changes of water. The cylindrical inclusion protein was then lyophilized.

Serology. Immunization procedures used were as described by Purcifull and Batchelor (18). Rabbits were given four successive injections at weekly intervals. For the first injection, part of the emulsion was injected into a toe pad and the remainder was administered intramuscularly. The remaining three injections were intramuscular. Approximately 4 mg of each virus and from 2–4 mg of cylindrical inclusion protein were injected. The Ouchterlony agar double-diffusion method was employed in serological tests with sap obtained by grinding the tissue with a mortar and pestle and diluted either with water (1:1, w/v) or with 1.5% SDS. Antiserum for titering was diluted with pre-immune serum. Antigens used in titering experiments were in crude Alaska pea sap. The immunodiffusion medium routinely used contained 0.8% Noble agar, 0.5% SDS, and 1% sodium azide (18).

Isolates of BYMV and CYVV-P used in this study were submitted to O. W. Barnett and S. W. Scott (Dept. Plant Pathology and Physiology, Clemson University, Clemson, SC 29631) for enzyme-linked immunosorbent assay (ELISA) (4). The samples were reacted with BYMV-204-1 and CYVV-P antisera produced at Clemson, and all antigens were in Alaska pea.

In vitro translations. The RNAs of BYMV-G, BYMV-204-1, and CYVV-P were translated in a rabbit reticulocyte lysate system to compare the total gene products of each virus. The isolations of RNA as well as the translation procedures were as described by Dougherty and Hiebert (6–8).

TABLE 1. Incidence of bean yellow mosaic virus in gladiolus plants obtained from different geographic regions

Source	Cultivar	Infected/Total[a]
California	Friendship	4/4
	Madeline	4/4
	Minuet	3/4
	New Rose	4/4
	Peter Pears	4/4
Colorado	Foxfire	4/4
	Franconia	2/4
	Osa Mae	4/4
	Statute	4/4
	White Columbia	4/4
Florida	Florida Flame[b]	4/4
	Friendship	4/4
	Jesse M. Conner[b]	4/4
	Magie's Surprise[b]	4/4
	White Friendship	4/4
	Miscellaneous[c]	9/9
Holland	Memorial Day	4/4
	Pink Sensation	4/4
	White Friendship	4/4
Michigan	Home Run	1/4
	Oscar	3/4
	White Friendship	4/4
Minnesota	Black Walnut	4/4
	Dixie Sport	4/4
	Friendship	4/4
	Ultima II	2/4
	White Friendship	4/4
	21-73	3/4
New Jersey	Country Girl	1/1
	Friendship	1/1
	Red Majesty	1/1
	T-204	1/1

[a] Ratio is number of infected plants per total determined by immunodiffusion tests using BYMV-G antiserum and gladiolus leaf tissue as antigens.

[b] Gladiolus samples from stock grown exclusively in Florida.

[c] Miscellaneous cultivars were Mardi Gras, Ruffled Quilt, The Queen, El Toro, Nightingale, Spanish Sunset, Pink Ballet, Wind Song, and Priscilla.

RESULTS

Bean yellow mosaic virus was detected in gladiolus samples from all locations except those grown in isolation from other gladioli in Alachua and Archer, FL. Based on immunodiffusion tests, 92% of 121 commercial gladioli representing 34 cultivars were infected with this virus (Table 1). Ten gladiolus samples each from Alachua

and Archer, FL, were determined to be virus-free by immunodiffusion tests, immune electron microscopy, absence of either floral or foliar symptoms, and manual inoculations of peas and beans.

Virus spread. During the three seasons that trap crops of bean, pea, and pepper were grown in the gladiolus plantings at Bradenton, only BYMV was detected. In the spring growing season (April–June) of 1980, 11 of 324 peas showed viral symptoms. In the winter season (November–February) over 90% of the peas were infected, and in the spring of 1981 only one infected pea plant was detected. The virus isolates from these pea plants were indistinguishable from the gladiolus isolates of BYMV based on host range, immunodiffusion tests, and light microscopy. No virus infections were evident in the bean or pepper plants at Bradenton, nor in the bean, pea, or pepper plants grown May–July 1980 and 1981 beside healthy gladioli in Archer.

Aphid collections. Aphids in the subfamilies Aphidinae, Pemphiginae, and Cinerinae were collected in water-pan traps located at Bradenton. A total of 459 aphids were collected from 8 December 1980 to 3 February in 1981 and 508 aphids were collected from 22 April to 3 June in 1981. Of the total Aphidinae, 16% were *Myzus persicae* (Sulz.) and 20.9% were *Aphis citricola* v.d. Goot, both vectors of BYMV (9,21). Most of the *M. persicae* were trapped early in the winter growing season, 1–15 December (74.4% of 121 total *M. persicae* trapped), whereas *A. citricola* was most abundant from 29 April to 13 May (77.2% of 158 *A. citricola* trapped). During these two periods, 475 other members of the Aphidinae, 212 members of the Pemphiginae, and one member of the Cinerinae were also collected.

Host range. All 23 of the isolates from gladioli, as well as four isolates recovered from pea plants grown adjacent to gladiolus plantings in Bradenton, induced symptoms of BYMV in peas and beans similar to those described by Zettler and Abo El-Nil (27) (Table 2). A systemic mosaic developed on the Alaska peas and chlorotic lesions were produced on inoculated leaves of Bountiful beans. Under relatively warm (>35 C) greenhouse conditions, a mild systemic flecking was noted occasionally on some of the Bountiful beans inoculated with gladiolus BYMV isolates. However, when bean leaves with systemic flecks were used to

TABLE 2. Symptoms induced in manually inoculated cultivar Alaska peas and cultivar Bountiful beans by various bean yellow mosaic virus isolates and clover yellow vein virus

Virus source[a]	Virus isolate[b]	Alaska peas	Bountiful beans	
		Systemic	Local	Systemic
Gladiolus (FL)	G	27/28[c]	24/40	5/40
	FF1	9/11	12/12	3/12
	FF2	13/14	10/10	1/10
	FF3	10/13	7/7	3/7
	FF4	13/13	6/9	1/9
	FF5	9/13	9/11	0/11
	JC2	7/13	8/9	0/9
	JC4	10/12	5/6	1/6
Pea (FL)	TP1	11/14	8/8	0/8
	TP2	10/13	8/8	0/8
	TP3	12/12	9/9	0/9
	TP4	8/14	9/9	0/9
Gladiolus (HOL)	PS3	13/15	9/11	0/11
	PS4	9/12	11/11	0/11
	PS5	11/13	10/11	0/11
	MD2	11/13	14/14	0/14
	MD3	6/12	11/12	0/12
	MD8	7/16	11/12	0/12
	WF4	5/13	12/13	0/13
Gladiolus (MN)	FR1	6/12	10/10	0/10
	FR4	11/12	11/11	0/11
	WF1	10/13	9/9	0/9
	WF4	12/14	11/14	0/14
	WF5	5/13	11/12	0/12
	WF6	8/13	6/13	0/13
	WF7	5/12	9/11	0/11
Gladiolus (CO)	FO1	9/13	0/11	0/11
Red Clover (KY)	204-1	21/26	15/40	16/40
White Clover (C)	CYVV-P	21/27[d]	10/57	17/37
Freesia (HOL)	F	22/22	30/40	32/40
Alpinia (FL)	A	20/21	10/22	11/22

[a] Letters in parenthesis represent the following locations: FL = Florida, HOL = Holland, MN = Minnesota, CO = Colorado, KY = Kentucky, and C = Canada.

[b] Letters designate the following cultivars: FF = Florida Flame, JC = Jesse M. Conner, PS = Pink Sensation, MD = Memorial Day, WF = White Friendship, FR = Friendship, and FO = Foxfire.

[c] Ratio of number of plants infected to the total number of plants inoculated.

[d] CYVV induced a systemic necrosis on Alaska pea.

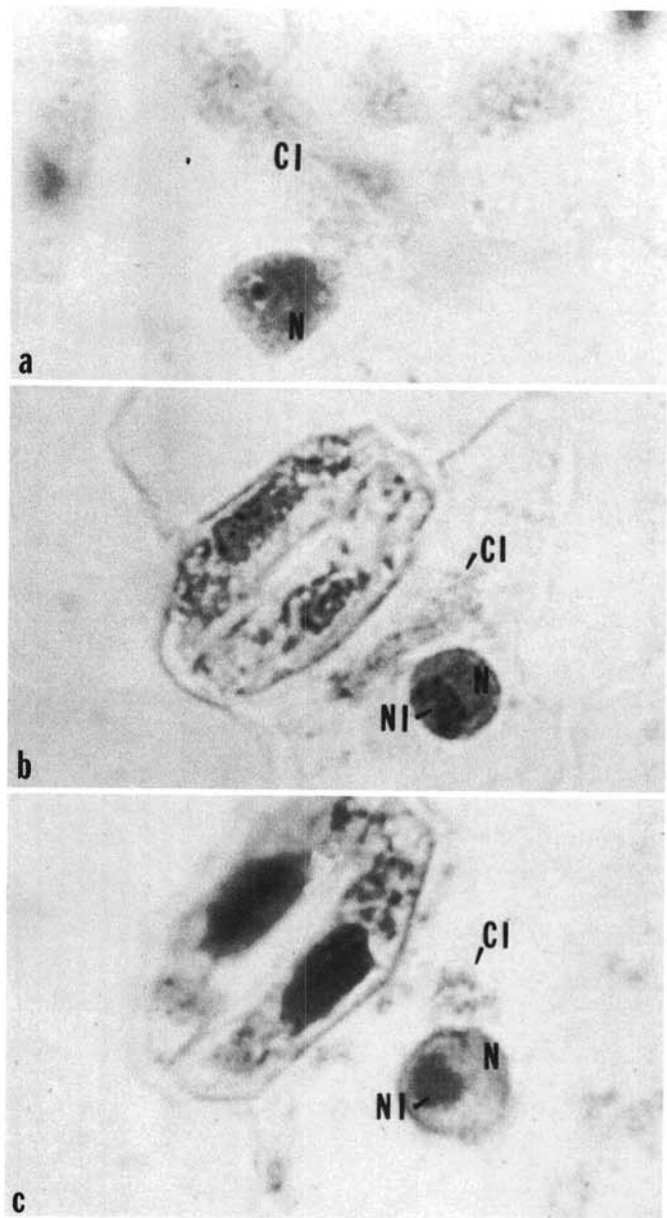

Fig. 1. Epidermal cells of cultivar Alaska peas stained with Luxol brilliant green/calcomine orange showing **a,** BYMV-G induced cytoplasmic inclusions; **b,** nuclear and cytoplasmic inclusions induced by BYMV-P; and **c,** nuclear and cytoplasmic inclusions induced by CYVV-P. CI = cytoplasmic inclusion, NI = nuclear inclusion, and N = nucleus (×1,855).

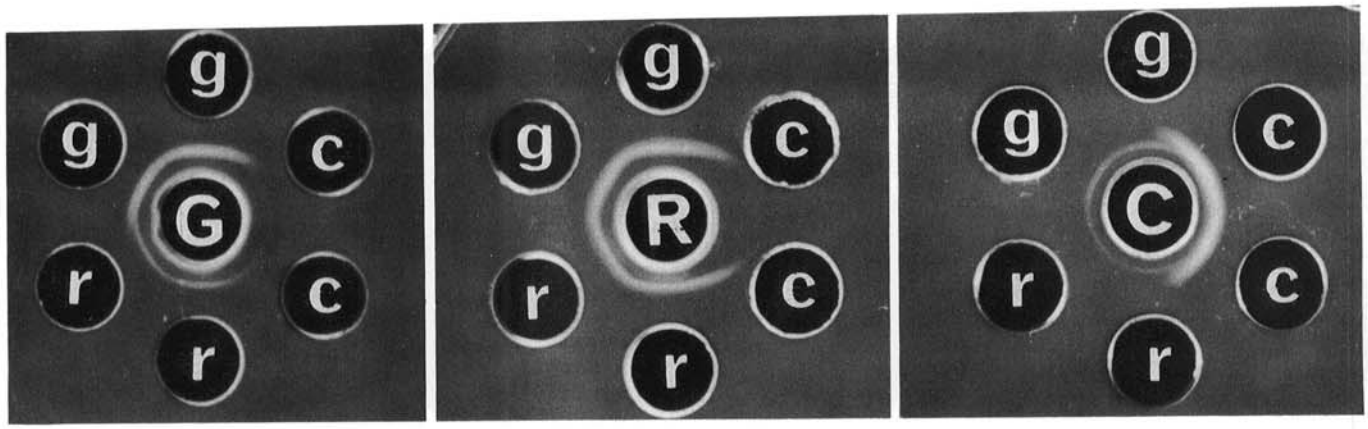

Fig. 2. Serological relationships of BYMV-G, BYMV-204-1, and CYVV-P in Ouchterlony agar-gel diffusion tests. Central wells contain: G = BYMV-G antiserum, R = BYMV-204-1 antiserum, C = CYVV-P antiserum. The peripheral wells contain sap from cultivar Alaska pea infected with the following isolates: g = BYMV-G, r = BYMV-204-1, and c = CYVV-P.

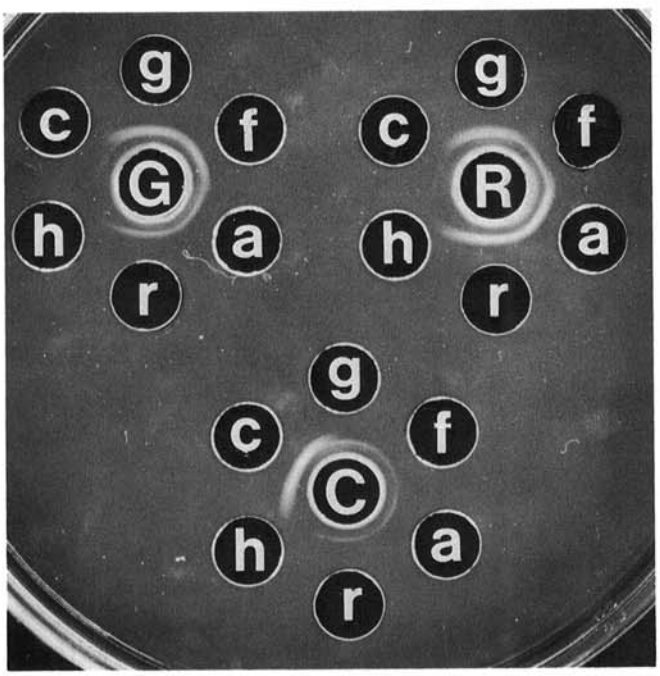

Fig. 3. Serological relationships of the G, 204-1, F, and A isolates of bean yellow mosaic virus (BYMV) and the P isolate of clover yellow vein virus originally obtained from gladiolus, red clover, freesia, alpinia, and white clover. Central wells contain: G = BYMV-G antiserum, R = BYMV-204-1 antiserum, and C = CYVV-P antiserum. The peripheral wells contain from cultivar Alaska pea sap infected with the following isolates: g = BYMV-G, f = BYMV-F, a = BYMV-A, r = BYMV-204-1, h = not infected, and c = CYVV-P.

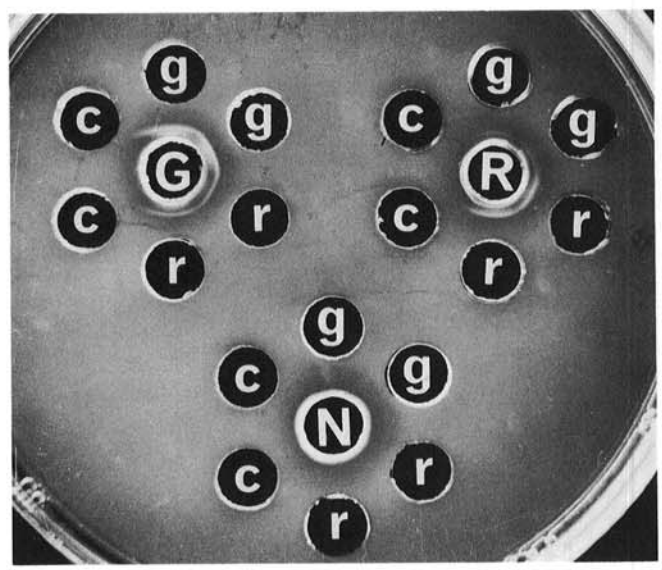

Fig. 4. Serological relationships of BYMV-G, BYMV-204-1, and CYVV-P cylindrical inclusions. Central wells contain: G = BYMV-G cylindrical inclusion antiserum, R = BYMV-204-1 cylindrical inclusion antiserum, and N = normal serum. The peripheral wells contain purified preparations of g = BYMV-G cylindrical inclusions, r = BYMV-204-1 cylindrical inclusions, and c = CYVV-P cylindrical inclusions.

inoculate beans in recovery trials, only chlorotic local lesions were produced. Similar reports of sporadic systemic infections of BYMV in beans were made by others (1,27). The BYMV-204-1 and CYVV-P isolates produced systemic mosaic symptoms and fewer chlorotic local lesions per leaf of Bountiful bean than any of the gladiolus isolates. Pronounced stunting and severe mosaic symptoms were produced by BYMV-F and BYMV-A in Bountiful beans. In Alaska peas, all of the BYMV isolates produced a systemic mosaic while CYVV-P produced a systemic mosaic and necrosis.

The BYMV-G isolate was compared to BYMV-204-1 and CYVV-P in further host range tests. In all comparisons at least 10 plants were inoculated and uninoculated controls were always included. The CYVV-P isolate infected coriander (*Coriandrum sativum* L.) while BYMV-G and BYMV-204-1 did not. Only CYVV-P produced a systemic necrosis on Alaska and Ranger peas. All three of the isolates systemically infected crimson clover (*Trifolium incarnatum* L. 'Dixie'), subterranean clover (*T. subterranean* L. 'Woogenellup'), and arrowleaf clover (*T. vesiculosum* Savi 'Amclo'). None of the isolates infected alfalfa (*Medicago sativum* L. 'Fla 66').

Light microscopy. In light microscopic examinations of epidermal cells of BYMV- and CYVV-P-infected host plants, cytoplasmic inclusions similar to those previously described (2,3) (Fig. 1) were observed. Nuclear inclusions were seen only in cells infected with BYMV-P and CYVV-P. Cytoplasmic, but not nuclear, inclusions were seen in cells of infected gladioli and in infected pea samples from gladiolus fields in Bradenton.

Purifications. When PEG was used to concentrate the virus, average yields of 40, 40, and 4 mg of virus per kilogram of Alaska pea tissue were obtained for BYMV-G, BYMV-204-1, and CYVV-P, respectively. Clover yellow vein virus particles purified with PEG appeared swollen and fragmented when viewed with an electron microscope. More intact particles and higher yields (22 mg/kg of tissue) were obtained for CYVV-P when PEG was

omitted and a high-speed centrifugation was used to concentrate the virus. The average uncorrected $A_{260/280}$ ratios for BYMV-G, BYMV-204-1, and CYVV-P were 1.13, 1.14, and 1.19, respectively.

Serology. Reactive capsid antisera to BYMV-G, BYMV-204-1, and CYVV-P were obtained 2 wk after the final injection of the rabbits. Maximum antiserum titers were realized 3–6 wk after the last injection. Homologous antiserum titers for BYMV-G, 204-1, and CYVV-P were 1/4, 1/4, and 1/8, respectively.

The CYVV-P isolate differed serologically from all the BYMV isolates. Precipitin lines resulting from the reaction of CYVV-P and its antiserum formed spurs over the lines formed by the reactions of BYMV-204-1 (Fig. 2), BYMV-G (Fig. 2), BYMV-A, BYMV-F, and BYMV-P. The CYVV-P antigen reacted only weakly with BYMV-G and BYMV-204-1 antisera (Fig. 2). The BYMV-204-1 and A isolates reacted identically with all three antisera (Fig. 3). The F and G isolates reacted differently when tested against BYMV-204-1 antiserum (Fig. 3). When BYMV-G antiserum was used, the precipitin lines of the G antigen consistently spurred over those of the 204-1 antigen; whereas when antiserum to the 204-1 isolate was used, the precipitin lines of the 204-1 antigen occasionally spurred over those of the G antigen (Fig. 2). Each of 121 BYMV isolates from gladioli and 10 BYMV isolates recovered from peas grown adjacent to gladioli in Bradenton reacted identically to BYMV-G with all these antisera.

Antisera to cylindrical inclusions of BYMV-204-1, BYMV-G, and CYVV-P proved much less reactive in immunodiffusion tests than their respective virion antisera. Homologous antiserum titers of 1:1 were recorded for BYMV-204-1 and BYMV-G, but no reaction was noted for CYVV-P. Weak precipitin reactions formed occasionally when the CYVV-P antigen consisted of a preparation of purified inclusions and CYVV-P antiserum was used. Neither BYMV-204-1 inclusion nor BYMV-G inclusion antiserum reacted with CYVV-P cylindrical inclusions (Fig. 4). All three of the inclusion antisera reacted with their homologous inclusion proteins that were formed in vitro; none of them reacted with other gene products.

In ELISA tests, antisera either to BYMV-204-1 or to CYVV-P virions reacted with all 28 gladiolus isolates tested. In all instances, the homologous reactions were stronger than the heterologous ones. Respective homologous A_{405} values for BYMV-204-1 and CYVV-P were 1.85 and 1.92, whereas average values for all of the gladiolus isolates were 0.34 (range 0.12–1.12) and 0.29 (range 0.11–0.57). The absorbance values for healthy pea antigens were 0.11 and 0.10 for BYMV-204-1 and CYVV-P antisera, respectively. The freesia isolate of BYMV reacted similarly to the gladiolus isolates, whereas BYMV-A had absorbance values of 0.67 to BYMV-204-1 and 0.11 to CYVV-P antisera, which are closer to the respective values recorded for the BYMV-204-1 isolate.

In vitro translations. The total translation products of BYMV-G were more similar to BYMV-204-1 than to CYVV-P (Fig. 5). The molecular weights of BYMV-G, BYMV-204-1, and CYVV-P capsid proteins were 35, 34, and 37 kdaltons, respectively. The two products that reacted with TEV nuclear inclusion antisera (*unpublished*) were 51 and 56 kdaltons for BYMV-G, 52 and 56 kdaltons for BYMV-204-1, and 49 and 59 kdaltons for CYVV-P.

DISCUSSION

The gladiolus isolate used in this study (BYMV-G) appears to be more closely related to BYMV-204-1 than to CYVV-P on the basis of immunodiffusion tests with virion and cylindrical inclusion antisera and the products formed during in vitro translations. In a study of the genetic maps of these isolates (11), BYMV-G and BYMV-204-1 were similar to each other and different from CYVV-P. Of these three isolates, only CYVV-P produced nuclear inclusions that could be visualized in host cells by light microscopy. The presence of nuclear inclusions alone, however, is not sufficient to distinguish CYVV strains from BYMV strains because in this study nuclear inclusions were also seen for BYMV-P. In a previous report (27), BYMV isolates infecting gladiolus were described as "pea mosaic isolates of BYMV." In the present study, gladiolus isolates resembled BYMV-P by forming local infections only in Bountiful bean. In immunodiffusion tests, however, BYMV-G differed from BYMV-P, and of the two, only BYMV-P formed nuclear inclusions in host cells (Fig. 1).

Despite the closer relationship of BYMV-G to BYMV-204-1, important differences, such as those shown in the ELISA tests, must also be considered in developing a certification program. In the ELISA, several of the gladiolus isolates, all of which were positive in immunodiffusion tests, reacted with exceptionally low absorbance values to either BYMV-204-1 or CYVV-P antisera. These results differ from those of Stein et al (22). In our tests the direct method was used, which has been described as being rather strain specific (4,25). If one strain of BYMV predominates in gladioli, as suggested in this study, then direct ELISA with BYMV-G antisera could be of value in a certification program. However, other strains could also naturally infect gladioli, as Lindsten reported (16), and these may not be detected by direct ELISA. A possible alternative may be to use indirect ELISA, which is reported to be somewhat less strain specific (4,25).

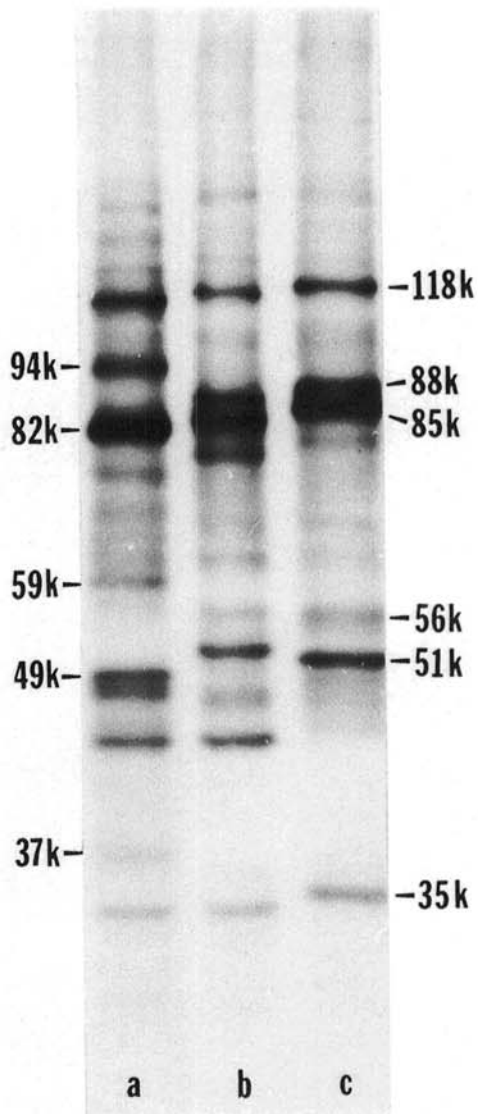

Fig. 5. Electrophoretic fractionation of the in vitro translation products of CYVV-P (lane a), BYMV-204-1 (lane b), and BYMV-G (lane c) RNAs. The products were fractioned on a sodium dodecyl sulfate-polyacrylamide slab gel (7.5 to 15%) gradient. Molecular weight markers used in electrophoretic studies were myosin, 200 kdaltons; β-galactosidase, 116 kdaltons; phosphorylase b, 94 kdaltons; bovine serum albumin, 67 kdaltons; glutamate dehydrogenase, 53 kdaltons; carbonic anhydrase, 29 kdaltons; and tobacco mosaic virus capsid monomer, 17.5 kdaltons.

Based on the observations that only one strain of BYMV appears to predominate in gladioli from the United States and Holland, the low incidence of other viruses in Florida-grown gladioli, and that healthy gladioli have remained virus-free under field conditions in Florida, we believe that the production of virus-free gladioli in Florida is feasible. Infection of virus-free stock may be avoided by planting healthy stock in isolation from inoculum sources. Red clover does not grow throughout the year in Florida and is unlikely to be a serious source of BYMV inoculum as it is in other locations (10). Although white clover can grow throughout the year in Florida, it is not susceptible to BYMV (1,17). Clover yellow vein virus is prevalent in white clover (1) but it does not necessarily constitute a threat to virus-free gladiolus production since natural infections of CYVV in gladioli have yet to be discovered. Thus, while other sources of inoculum may exist, infected commercial gladioli are likely to represent the greatest source of BYMV inoculum to infect healthy gladiolus stock.

The apparent strain/host association noted in this study for gladiolus has also been observed in other crops. For example, Jones and Diachun (14) found that 98.8% of 421 BYMV isolates from various red clover plantings in Kentucky were of one subgroup type despite the susceptibility of this host to two other BYMV subgroups. Similar observations of particular strains infecting certain crops have been made for BYMV and CYVV isolates infecting forage legumes in South Carolina (O. W. Barnett, *personal communication*). Although not clearly defined, this natural separation of virus strains into apparent pathotypes is likely to be of considerable significance in developing control strategies for these viruses. This is especially true in considering potential sources of inoculum or in selecting reliable, sensitive methods for virus detection in certification programs.

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