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Cucumber Mosaic Virus Isolates Seedborne in *Phaseolus vulgaris*: Serology, Host-Pathogen Relationships, and Seed Transmission

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ABSTRACT

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Selected cucumber mosaic virus (CMV) isolates seedborne in bean (*Phaseolus vulgaris*) were compared for serological relatedness, host-pathogen relationships, and seed transmissibility. CMV strains B, Le, F, B_{32}, and Pg were found to be serologically indistinguishable by gel double-diffusion tests using antisera to formaldehyde-fixed immunogens (CMV-B and CMV-Le). Detection of CMV in infected bean leaves by gel double-diffusion tests was dependent on extraction in 0.05 M citrate buffer, pH 6.5, with 0.1% sodium thioglycollate (w/v), 0.1% Triton X-100 (v/v), and 1% formaldehyde (v/v). Time of appearance and severity of symptoms induced by CMV-B, and relative virus concentration varied among 32 bean cultivars representing diverse germ plasm. Virus titer was maximal in greenhouse-grown plants 1–2 wk after inoculation with CMV-B, -Le, and -F but declined sharply after the fourth week. CMV seed transmission varied according to variety and isolate and did not occur in seeds from plants inoculated 4 or 6 wk after planting. CMV-Pg occurred in all parts of bean seedlings infected from seed. The implications of these results for detection and diagnosis of CMV in bean plants are discussed.

Seed transmission (about 7%) of cucumber mosaic virus (CMV)(9) in bean (*Phaseolus vulgaris* L.) was first reported for a Spanish isolate (CMV-B_{32}) in 1974 (5). Since that time the following isolates have been reported to be seed transmitted in beans at the given frequencies: CMV-B, New York, 0.3% (16); CMV-Pr, Puerto Rico, 1.5% (15); CMV-F, France, 9-54% (14); and CMV-Za8, Yugoslavia, 20% (2). Seedborne CMV isolates were not detected in samples of U.S. commercial and institutional breeding materials, but an isolate (CMV-Pg) was detected in one line of Spanish origin in the USDA-ARS Plant Introduction Collection of *P. vulgaris* at Pullman, WA. (7).

Seed transmission is known to be affected by many variables, notably virus, virus strain, host species and cultivar, environment during seed production, and time of plant inoculation (4,17). Detection of certain seedborne CMV isolates (2,5,15) is facilitated by clear symptom development in beans grown from CMV-infected seed. Although CMV isolates seed transmitted in beans have been reported from different parts of the world, such isolates were unknown in beans grown in the Pacific Northwest of the United States before our investigations (7).

The purpose of this paper is to describe: (1) serological detection and relatedness of certain CMV isolates, some seedborne and

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others not seedborne in beans; (2) field responses of 32 bean cultivars inoculated with the U.S. seedborne isolate, CMV-B; and (3) distribution of CMV in bean plants infected from seed and by mechanical inoculation with relation to time and symptom expression.

MATERIALS AND METHODS

Virus isolates. CMV-Pg was isolated from Plant Introduction (PI) Accession No. 271998 of *P. vulgaris* (7). Other CMV isolates used were obtained from the following individuals: CMV-B from R. Provvidenti (16); CMV-B_{32} from L. Bos (5); CMV-Pr from J. P. Meiners (15); CMV-F from J. C. Devergne (14); and CMV-Le from T. Inouye (12). Isolates were propagated in Bountiful bean, and desiccated for use as standard inoculum sources.

Purification. CMV was purified as described by Lot et al (13) with slight modifications (H. T. Hsu, *personal communication*) as follows: after the first high-speed centrifugation, virus was resuspended and maintained throughout the procedure in 20 mM disodium ethylenediaminetetraacetate, pH 7 (EDTA solution); the second high-speed pellet was resuspended and layered onto 10–40% sucrose gradients in EDTA solution and centrifuged for 2 hr at 24,000 rpm in an SW 27 rotor (Beckman Instruments, Palo Alto, CA). Purified virus in EDTA solution was stored at 4 C with the addition of 1% formaldehyde for serological work or lyophilized and stored at −10 C.

Serology. Male New Zealand White rabbits were injected with CMV-B or -Le. CMV-B was injected seven times into the marginal ear vein (IV) and three times in the hind leg muscles (IM) for a total of 10.3 mg over a 4-mo period and 8.2 mg of CMV-Le was given in three IM injections over the course of 6 wk. All virus preparations were diluted in 0.01 M phosphate, pH 7, 0.85 % NaCl (PBS) and stabilized with 1% formaldehyde before injection to optimize immunogenicity (8) and mixed with Freund's incomplete adjuvant (1:1, v/v) before to IM injections. Bleedings were done aseptically by cardiac puncture. Serum was stored at 4 C or −10 C with 0.01% sodium azide. Each antiserum preparation was tested for reaction endpoint by microprecipitin titration in 10-cm-square plastic petri dishes (3). Gel double-diffusion serology in 0.4% Ionagar made in PBS plus 0.2% sodium azide was performed using the Ouchterlony test (3). Enzyme-linked immunosorbent assay (ELISA) was performed according to Clarke and Adams (6), except as noted. Immunogammaglobulin (IgG) was purified by affinity chromatography using a 7-ml column of DEAE AFFI-Gel Blue (Bio-Rad Laboratories, Richmond, CA) in 0.02 M TRIS-Cl buffer, pH 8, with 0.028 M NaCl. Dilutions of plant sap or purified virus varied. Concentration of anti-CMV IgG and IgG-alkaline phosphatase conjugate was about 1 μg/ml and substrate was used at 1 mg/ml. Incubation times were as follows: 4 hr at 37 C for coating, overnight at 4 C for antigen binding; 4 hr at 37 C for binding of enzyme-conjugate; and 10–60 min at room temperature for substrate hydrolysis. All samples were tested in triplicate wells of 96-well polystyrene microtiter plates, using only the inner 60 wells. The dilution of tissue was generally 1:10 (w/v) but varied in some experiments as noted because of the purpose of comparing relative virus concentration rather than optimizing detection and because of the sensitivity of the test in that particular experiment. However, virus could always be detected at 10-fold or greater dilutions beyond the dilution chosen. In each experiment, the test samples were compared with healthy and infected control plants of the same age that had been stored and diluted in the same manner. A reaction was considered positive when it was greater than the mean plus two times the standard deviation of the absorbance of healthy controls. If the absorbance of the healthy tissue was greater than 0.1 or if the infected control did not react, then the experiment was repeated until these factors were corrected.

Bean cultivars. Susceptibility of 32 selected bean cultivars to CMV-B and the range of symptoms induced by CMV-B were determined in field plots near Corvallis. Plants at the primary leaf stage were dusted with 400-mesh Carborundum and inoculated with a homogenate of virus-infected plant tissue in 0.02 M phosphate buffer, pH 7. Ten plants of each cultivar were left uninoculated to serve as controls and to assess rate of virus spread by aphids. Symptom development was monitored weekly and symptomless infection was tested after 5 wk by inoculation of cowpea (*Vigna unguiculata* (L.), Walp., subsp. *unguiculata* 'Ramshorn'), cucumber (*Cucumis sativus* L. 'Lemon'), and *Chenopodium amaranticolor* L.

CMV titer versus time:field. Infected Bountiful bean plants and uninoculated plants from the above plots were sampled weekly from the upper or youngest fully expanded trifoliolate leaves. Leaf samples were immediately desiccated and stored over Anhydrone at −10 C. Samples were assayed by ELISA as previously described at a dilution of 1:100 (w/v) with buffer.

CMV titer versus time:greenhouse. Groups of 10 Bountiful bean plants were each inoculated with CMV isolates B, Le, or F when the primary leaves were just fully expanded. One week after inoculation, 1-cm leaf disks were removed from primary leaves of 10 replicate plants, combined, and desiccated. At weekly intervals thereafter for 6 wk, 1-cm leaf disks were removed from the youngest fully expanded trifoliolate leaf, combined and desiccated. All samples were then tested by ELISA at a dilution of 1:100,000 (w/v).

Seed transmission. Experiment I. One hundred seeds each of bean cultivars Tendercrop, OSU-1604, and Red Kidney were planted in 5-m rows in a field. Two weeks later, plants were inoculated with homogenates of CMV-B infected tissue as described earlier. Two weeks after that, selected plants were assayed for CMV by inoculation of cowpea, cucumber, and *C. amaranticolor*. Pods were harvested when mature, then dried and stored at room temperature. Seeds from these plants were stored for 3–6 mo (5) to ensure maturity. Seedlings were examined visually and bulk tested in groups of 10 on indicator plants 7–14 days after emergence. Plants in groups assaying positively were retested individually on indicator plants. Plants producing positive results were confirmed by another biological assay on the same hosts and tested serologically in double-diffusion gels.

Experiment II. Eleven of the 32 bean cultivars field-tested for reactions to CMV-B (Bush Blue Lake Advance (BBL), BBL 274, Early Blue, Greenpak, Harvester, Majestic, Michelite, Oregon 58, Santa Ana, Sunrise, and Sutter Pink) were selected from among the four symptom groups (Table 1) and seed was harvested for determination of seed transmission. Seedlings were assayed in groups of 10 or less by ELISA 2 wk after planting. Depending on the number of plants in the group, two to four leaf disks (1 cm) were taken from the first trifoliolate leaf using a cork borer sterilized between each plant. The fresh disks were combined and triturated in 10 volumes (w/v) of ELISA sample buffer (6). The ELISA test was repeated on individual plants in groups assaying positively for CMV.

Experiments III–VI. Beans were planted in aphid-free greenhouses under supplemental lighting (130–155 lx, 12-hr photoperiod) in September, March, May, and June for experiments III, IV, V, and VI, respectively. Temperatures were maintained at about 16–18 C at night and 21–24 C during the day. In Experiments III and IV, a group of approximately 200 Tendercrop and Topcrop bean plants, respectively, were inoculated with CMV-F and another group with CMV-Le 2 wk after planting. The effect of inoculation time on seed transmission was studied in Experiment V by inoculating CMV-F to 40, 60, and 60 Topcrop plants at 2 wk (primary leaf stage), 4.5 wk (prebloom stage), and at 6 wk (pod-forming stage), respectively, after planting. In Experiment VI, 40 and 60 Topcrop plants were inoculated with CMV-Pg 2 wk and 4 wk, respectively, after planting. Other details were as for Experiment II.

CMV distribution in infections from seed. One of two seedlings of PI Accession No. 271998 first found to be CMV-infected from seed origin of CMV-Pg (7) was aseptically dissected and tested by ELISA 3 wk after planting to determine the distribution of virus. Starting at the roots, tissue was removed from the following locations (Fig. 1): root system, hypocotyl above-root system (two sections), cotyledon (two sections), primary leaf (six sections), petiole of primary leaf (two sections), stem section of first internode, petiole of trifoliolate leaf, two leaflets of trifoliolate leaf

(four sections each), stem section in second internode, and tissue at the growing point. Tissue from each of the sampled parts was weighed and 0.03 g placed in a single well of a microtiter ELISA plate. To each well, 0.3 ml of the ELISA virus buffer was added and the fresh tissue was triturated with a glass rod. A plant from the same seedlot found by ELISA to be virus-free was processed and assayed similarly. The six Topcrop plants that were infected from seed with CMV-Pg (Experiment VI) and two virus-free plants from this group were similarly examined for CMV distribution 4 wk after planting.

RESULTS

Purification. Virus yields of up to 340 mg of virus per kilogram of tissue were obtained using Bountiful bean as a propagation host. Purified virus remained infectious for at least 6 mo when stored at −10 C in EDTA buffer or after lyophilization.

Antisera titer. The titer of CMV-B antiserum (reciprocal value of 1,024) was higher than that of CMV-Le (reciprocal values of 256). Both antisera, however, reacted in gel diffusion and ELISA tests.

Strain relationships. Purified preparations of CMV isolates B, Le, F, B_{32}, and Pg without formaldehyde were compared by gel double-diffusion tests in all possible combinations using antisera to CMV-Le and CMV-B. All precipitin bands were single, confluent bands with no spurs formed among these bands (results not shown). The serogroup (1) of these isolates could not be determined from these results.

Detection in crude sap. Extracts from infected tobacco and cucumber plants reacted typically in gel double-diffusion Ouchterlony tests. However, reactions were not normally observed with extracts from infected bean or cowpea plants. Conversely, CMV that had been purified from infected bean plants reacted in gels. It was determined that the preparation was serologically reactive after the initial extraction of tissue in the 0.5 M citrate, pH 6.5, buffer with 0.1% sodium thioglycollate and chloroform (1 g:2 ml:2 ml). Chloroform required phase separation and tended to inhibit serological reactions in trace amounts. Triton X-100 (0.1%, v/v) was an excellent substitute for chloroform. Mercaptoethanol (0.1%) was found to be interchangeable with sodium thioglycollate. The citrate buffer with 0.1% thioglycolate (or mercaptoethanol) and 0.1% Triton X-100 was adopted as a standard Ouchterlony buffer for extraction of bean and cowpea.

Because antisera were produced to formaldehyde-fixed antigen (with an unknown effect on antigenic specificity), the effect of treatment of the crude bean sap with formaldehyde was investigated. It was found that serological reactions in gels were intensified and sometimes dependent on addition of the formaldehyde to the sap (to 1%) after straining through cheesecloth. However, serological reactions in gels using unfixed purified virus were equal to or better in sensitivity and sharpness than purified virus fixed with formaldehyde. Precipitin bands

TABLE 1. Host reactions and infectivity index of field-grown bean cultivars 5 wk after mechanical inoculation with CMV-B

Symptom[a] Group	Cultivar[b]	Symptoms[c]	Infectivity Index[d]		
			Cowpea	Cucumber	Chenopodium
I	BBL Advance	−/−	2/5	3/4	−
	Vitagreen	−/−	0/4	0/4	+(1)
	Michelite	−/−	0/4	4/4	−
	Kentucky Wonder	−/−	4/4	3/4	++
II	Bountiful	−/(Chl,Mo,Stu,Vb)	2/4	2/5	+
	Gallamour	−/(Vb)	0/5	0/3	+(2)
	GV-50	−/(Mo,Stu,Vb)	0/4	1/4	+
	Royal Burgundy	−/(Stu,Vb)	0/4	0/4	−
	Charlevoix	−/(Vb)	0/5	1/4	+(1)
	Sutter Pink	−/(Mo,Stu)	0/4	0/5	+
	Dwarf Hort 1976	−/Stu,(Vb)	0/4	0/4	−
	Santa Ana	−/(Stu,Vb)	3/4	2/3	++
	Early Blue	−/(Vb)	2/4	4/4	++
	NK EXP 186	−/(Mo,Vb)	2/4	2/4	+
	Oregon 58	−/(Mo,Vb)	5/5	4/4	++
	Bush Romano	−/(Mo,Vb)	0/5	0/4	−
	Medford	−/(Ma,Mo,Vb)	4/4	4/4	++
	Astro	−/(Mo,Stu,Vb)	1/5	1/3	−
	Spartan Arrow	−/(Mo,Vb)	NT	NT	NT
III	Stretch	−/Stu	3/5	2/4	++
	Gabriella	−/Stu	2/4	2/3	++
	Sunrise	−/(Mo),Stu	3/4	2/3	−
IV	Harvester	−/Ma,Mo,Stu,Vb	NT	NT	NT
	Majestic	−/Ma,Vb	2/4	3/4	++
	BBL 274	−/Ma,Vb	0/4	0/5	−
	Eagle	−/Stu,Vb	0/5	0/4	−
	Slenderette	−/Ma,Stu,Vb	0/4	2/4	+(1)
	Checkmate	−/Stu,Vb	0/4	0/4	−
	Greenpak	−/Ma,Vb	0/4	0/4	−
	Goldrush	−/Ma,Mo,Stu,Vb	NT	NT	NT
	Green Isle	−/Ma,Mo,Vb	0/5	0/4	−
	NK EXP 519-6	−/Ma,Mo,Stu,Vb	NT	NT	NT

[a] I = Symptomless; II = Mild or infrequently expressed symptoms; III = Strong stunt; and IV = Severe foliar symptoms expressed frequently.
[b] Approximately 10 plants in a 10-ft row were inoculated, others were left noninoculated.
[c] Local/systemic symptoms. − = no symptoms, Chl = chlorosis; Ma = malformation; Mo = mosaic; Stu = stunt; and Vb = veinbanding.
[d] Results of local infection 7–14 days after inoculation onto cowpea (*Vigna unguiculata* subsp. *unguiculata* 'Ramshorn'), cucumber (*Cucumis sativus* 'Lemon'), and *Chenopodium amaranticolor* (Corvallis strain) 5 wk after inoculation of beans. Number of half-leaves infected/number inoculated for cowpea and cucumber. For *Chenopodium*, results are total lesions on three leaves where: − = 0 lesions or no infection; + = 1–10 lesions; ++ = 11–50 lesions; number of lesions in parentheses; and NT = not tested.

between homologous purified fixed and unfixed antigens were confluent (results not shown).

Bean cultivar reactions. Although all 32 cultivars tested under field conditions were susceptible to CMV-B, type and severity of symptoms varied (Table 1). Bean cultivars were categorized into four groups based on symptoms: I, symptomless infection; II, mild and transient leaf symptoms; III, marked stunting with few or no leaf symptoms; and IV, severe veinbanding, mottle, mosaic, stunt, and leaf malformation resembling 2,4-D injury. Bioassays on cowpea, cucumber, and Chenopodium using the youngest leaves collected at random or selected for symptoms if present 5 wk after CMV-B inoculation revealed that most plants contained CMV. However, as a measure of relative infectivity, sap from only 29% of the cultivars showing severe symptoms (Group IV) was infectious to at least one of these bioassay hosts, whereas 86% of the cultivars in other symptom groups (Groups I–III) infected one or more of these hosts (Table 1). Symptom development varied among cultivars in Group IV. Bush Blue Lake 274, Checkmate, and Eagle did not develop symptoms until about 4–5 wk after inoculation, whereas the other cultivars in Group IV developed symptoms within 3 wk of inoculation. Although all cultivars in Group IV displayed strong symptoms 5 wk after inoculation, Eagle, Green Isle, and Northrup King 519-6 recovered almost completely from symptoms by the end of the season.

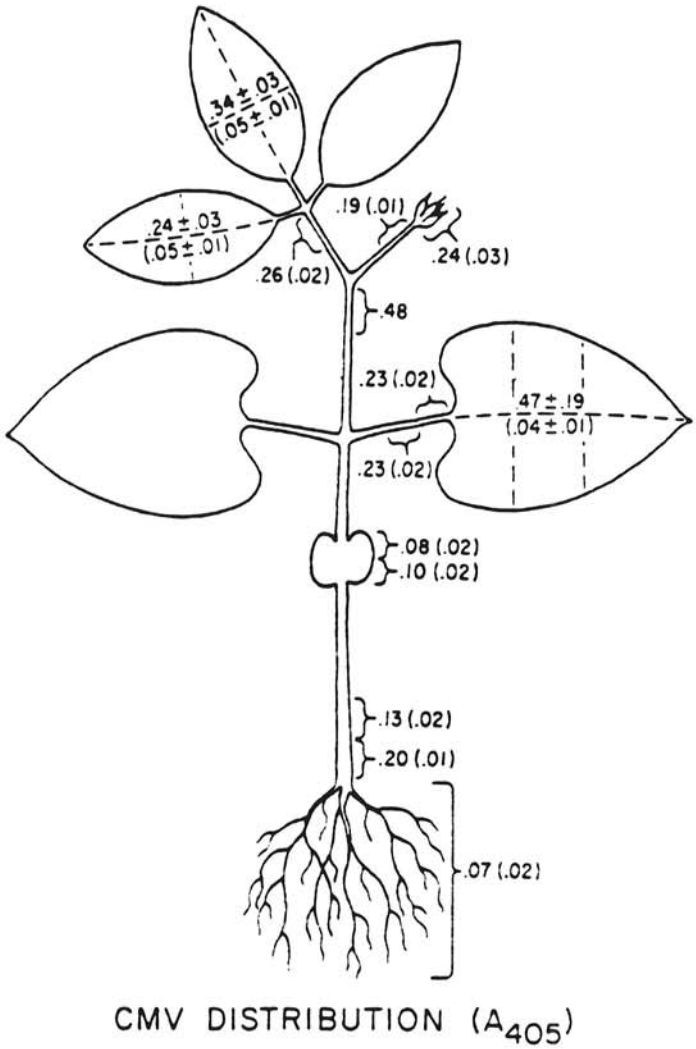

Fig. 1. ELISA (A_{405nm}) values showing cucumber mosaic virus (CMV-Pg) distribution in a 3-wk-old bean plant (PI 271998) infected from seed. Values for noninfected plant tissues in parentheses. Dotted lines show individual sections of leaves tested and averaged with standard deviations. All tissues tested at 1:10 (w/v) dilution. Endpoint of infected control tissues was beyond 1:10,000.

CMV titer in bean plants at different times after inoculation. The average absorbance values (405 nm) for the ELISA assay of desiccated samples of field-grown plants diluted 1:100 (w/v) was 0.84 for CMV-B inoculated and 0.05 for uninoculated plants collected 14 days after inoculation. The absorbance of the CMV-B inoculated plants collected at 31, 34, 41, and 48 days after inoculation was, respectively, 0.53, 0.41, 0.48, and 0.49. The 1:100 dilution of desiccated tissue was chosen because it approximates a 1:10 dilution of fresh tissue, which provided greatest sensitivity in preliminary experiments using infected and healthy bean control tissues. Virus could be detected after a further 1,000-fold dilution of infected controls.

To determine relative sensitivity and to select a tissue dilution of the greenhouse-grown plants at which samples could be compared by ELISA for relative virus concentration, CMV-Le inoculated and uninoculated Bountiful bean plants collected and desiccated 7 days after inoculation were tested in a dilution series. Absorbance decreased linearly from a dilution of 1:10,000 (A_{405nm} = 2.5) to 1:1,000,000 (A_{405nm} = 0.25). The values of the mean plus two standard deviations for uninoculated plants were below 0.1 at these dilutions. Therefore, all samples of the greenhouse-grown plants were tested at a dilution of 1:100,000, which was the midpoint of the linear portion of this dilution curve. The absorbance values for CMV-B, -F, and -Le, 7 days after inoculation were, respectively, 1.32, 1.60, and 1.46, an average of 1.46. The values for the three viruses were within 24% of each other at each sampling date and averaged 1.48, 0.51, 0.09, 0.04, 0.06, and 0.01, respectively, at 14, 21, 28, 35, 42, and 49 days after inoculation. The mean plus two standard deviations for the uninoculated controls at each date was 0.1 or less.

Seed transmission. In all seed-transmission and virus distribution tests a dilution of 1:10 was chosen for optimum sensitivity because the reaction of infected tissue was highest and the healthy reaction was below 0.1 at this dilution. Preliminary experiments to determine sensitivity demonstrated that CMV could be detected by ELISA in 1 part fresh infected control plant mixed with 100 parts healthy plant diluted at 1:100 or in 1 part infected to 10 parts healthy diluted 1:10,000. The results of the six seed-transmission experiments conducted in the field or greenhouse during a 2-yr period are shown in Table 2. CMV-B was seed transmitted in two of 14 bean cultivars tested at rates of 2.4% (Tendercrop) and 1.8% (Red Kidney). CMV-Le was not transmitted to seeds of the 2 cultivars tested. CMV-F was seed transmitted in 1 of 2 cultivars at a rate of 3.3% (Topcrop) and CMV-Pg was seed transmitted at 49% in one cultivar (Topcrop). Only 31% of the 58 plants infected through seed transmission with CMV-B, -F, or -Pg developed symptoms. These symptoms consisted of downward leaf curling and twisting and mosaic of the trifoliolate leaves, and plants recovered after about 4 wk.

Virus distribution. Distribution of CMV-Pg in a 3-wk-old bean seedling of PI Accession No. 271998 infected from seed is depicted in Figure 1. Absorption (A_{405nm}) values among matched samples from adjacent areas of the same seedling were generally uniform. The relative CMV concentration was highest in the primary leaf and upper stem tissues, intermediate in young trifoliolates, and lowest in tissues below the primary leaf.

Virus distribution was also estimated by ELISA in six 1-mo-old Topcrop bean plants infected with CMV-Pg through seed transmission (Table 3). One of the infected plants (No. 6) gave weakly positive results in this test. The other five plants (Nos. 1–5) produced strong positive results in all tissue positions with some relative-titer variation among tissue positions and among plants at the same position. The widest range (A_{405nm}) within a single plant occurred between the primary leaf and root tissues of plant No. 3 and spanned 0.64 (A_{405nm}) units. The least variation in relative CMV titer among plants occurred in tissue at the growing point (meristem), whereas root tissue varied from 0.25 to 1.01. There were no apparent differences between plants with symptoms (Nos. 1–3) and those without (Nos. 4–6), except symptomless plant No. 6, which contained barely detectable CMV concentrations. Mean absorption (405 nm) values plus two standard deviations for uninfected controls were consistently 0.04 or less.

DISCUSSION

Use of CMV indicators could be useful to plant scientists, particularly bean breeders, for monitoring the incidence of CMV in bean production areas. Observed infection of CMV-sensitive cultivars could be followed by arrangements with a plant virology laboratory for appropriate serological diagnosis. Confirmed occurrence of new CMV isolates in the agro-ecosystem would suggest the need for preventive or protective control strategies. Results from this study suggest that the amount of virus detectable by bioassay in tissues from infected indicator cultivars might be limited at the time of severe symptoms. Our results also indicate that virus concentration drops sharply 3–4 wk after inoculation of greenhouse-grown plants. Because the measured absorbance values in samples collected at 4–7 wk after inoculation and tested by ELISA decreased to insignificant levels at the high dilution tested, it is not possible to extrapolate on the shape of the relative virus concentration versus time curve beyond 4 wk. Assuming that a sharp peak in virus concentration followed by a rapid decline shortly after infection is characteristic of CMV under natural or environmental conditions, and based on the lack of good correlation between severe symptoms 5 wk after inoculation and between virus infectivity, then bean plants might be more reliably assayed for sensitive detection and diagnosis of CMV within 3–4 wk of planting.

P. vulgaris, to date, is assumed to be universally susceptible to cucumber mosaic virus infection; yet substantial diversity exists among bean cultivars in tolerance (symptomless) to disease caused by CMV-B. We found Michelite and Kentucky Wonder to be symptomless when infected by CMV-B under our conditions; however, previous workers have reported symptoms of CMV-B on these hosts (16). These discrepancies indicate the importance of conducting tests under various conditions with standard germ plasm and virus isolates. Should it become necessary to develop new cultivars resistant to the effects of new seedborne CMV isolates, it would appear feasible to use the germ plasm of cultivars tolerant to CMV-B such as Bush Blue Lake Advance, Kentucky Wonder, Michelite, and Vitagreen. Of course these cultivars would

TABLE 2. Seed transmission of cucumber mosaic virus (CMV) isolates mechanically inoculated onto selected *Phaseolus vulgaris* cultivars

Exp	Isolate	Cultivar	Location	Planting date	Inoc[a] (wk)	Assay[b] method	Results[c]	Symptoms[d]	%ST
I	CMV-B	Tendercrop	Field	7-11-78	2	B,S	6/253	1	2.4
		OSU 1604				B,S	0/126		0
		Red Kidney				B,S	6/335	0	1.8
II	CMV-B	Early Blue	Field	6-15-78	2	E	0/65		0
		Harvester				E	0/79		0
		Santa Ana				E	0/83		0
		BBL Advance				E	0/96		0
		Sunrise				E	0/49		0
		Michelite				E	0/48		0
		BBL 274				E	0/40		0
		Greenpak				E	0/37		0
		Majestic				E	0/37		0
		Oregon 58				E	0/41		0
		Sutter Pink				E	0/36		0
III	CMV-Le	Tendercrop	Green-house	8-31-79	2	E	0/144		0
IV	CMV-Le	Topcrop		3-4-80	2	E	0/136		0
III	CMV-F	Tendercrop	Green-house	8-31-79	2	E	0/148		0
IV	CMV-F	Topcrop		3-4-80	2	E	0/149		0
V	CMV-F	Topcrop	Green-house	5-27-80	2	E	2/61	1	3.3
					4½	E	0/99		0
					6	E	0/98		0
VI	CMV-Pg	Topcrop	Green-house	6-26-80	2	E	44/89	16	49.0
					4	E	0/190		0

[a] Age of plants at inoculation.
[b] B = Bioassay on cowpea, cucumber, or *Chenopodium* (see text for species). S = Gel double-diffusion serology of selected individuals. E = Enzyme-linked immunosorbent assay (ELISA). Groups of 10 seedlings were bulk assayed 2–3 wk after planting, plants of suspect groups were then assayed again individually. Tissues were assayed fresh at 1:10 (w/v) dilution. Virus could be detected in a 1:10,000 dilution of a mixture of 1 part infected tissue to 10 parts healthy tissue.
[c] No. of samples in which CMV was detected/no. of samples tested.
[d] No. of infected plants that developed symptoms.

TABLE 3. Relative absorbance (405 nm) of ELISA reactions obtained by testing 1-mo-old *Phaseolus vulgaris* 'Topcrop' plants CMV-Pg-infected from seed and healthy plants, at five tissue locations[a]

		Plant no.						
		Plants arising from infected seed						
		With symptoms			Symptomless			
Location[b]	Healthy	1	2	3	4	5	6	Average[c]
Meristem	0.04[d]	0.56[e]	0.55	0.65	0.58	0.67	0.06	0.60
Trifol 2	0.04	0.26	0.57	0.43	0.52	0.57	0.09	0.45
Trifol 1	0.01	0.33	0.42	0.44	0.54	0.59	0.11	0.45
Unifol	0.02	0.34	0.42	0.36	0.61	0.43	0.06	0.43
Roots	0.02	0.25	0.31	1.01	0.31	0.87	0.03	0.46
Average	0.04	0.31	0.42	0.54	0.50	0.59	0.08	

[a] All tissues tested fresh at 1:10 (w/v) dilution. End point of infected control tissue was beyond 1:10,000 dilution.
[b] Meristem = tissue within 1 cm of the growing point. Trifol 1 and 2 = first and second trifoliolate leaves expanding, respectively. Unifol = unifoliolate leaf. Roots = primary and secondary roots including hypocotyl.
[c] Average of the mean A_{405nm} value at each location of plants Nos. 1–5.
[d] Mean A_{405nm} values plus two standard deviations for two wells at meristem and three wells at other locations. Average of two plants.
[e] Average A_{405nm} values for all plants arising from infected seed, two wells at meristem, three wells at other locations.

need to be tested for tolerance to other seedborne isolates to establish their efficacy. The use of cultivars established to be resistant to CMV seed transmission would minimize the establishment and spread of seedborne CMV isolates in commercial seedlots of the domestic and export seed trade.

Results of Experiments V and VI indicate that time of inoculation is critical for successful seed transmission because the transmission rates decreased to 0% when plants were inoculated with CMV-F at 4.5 or 6 wk or with CMV-Pg at 4 wk after planting. This suggests that the mechanism of seed transmission involved in this system may require entry of the virus into preembryonic tissues.

This is the first report, to our knowledge, of seed transmission of CMV in beans resulting in symptomless infections. Because previous seed transmission determinations were apparently based on testing only seedlings with symptoms, published rates of seed transmission may have underestimated the actual rate.

The seed transmission experiments varied in virus isolate, cultivar, location, time, and assay method. Therefore, results are not directly comparable. However it appears that CMV-Pg, isolated from P. I. Accession No. 271998, is capable of extensive transmission (49%) through seeds of Topcrop bean. Escape of such isolates into breeding lines or commercial seedlots could be costly to breeding programs and domestic or export markets. Because symptoms of CMV infection may be mild or masked in some bean cultivars, new incidence of seedborne isolates could initially escape attention and their elimination from seedlots of some bean selections could prove difficult. Some countries are already experiencing increased CMV disease in beans. For instance, reports from Hungary indicate rapid spread of CMV in bean crops to an 80% incidence and an estimated yield loss of 38% (11).

Elimination of seedborne CMV from *P. vulgaris* germ plasm would seem to represent the least expensive control measure against CMV-Pg-like isolates. Germ plasm reclamation techniques have been suggested (10). We believe elimination of CMV from *P. vulgaris* germ plasm through reclamation techniques could be integrated with other control measures such as elimination of weed reservoir hosts to prevent virus reintroduction.

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