Southern Bean Mosaic Virus: Evidence for Seed Transmission in Bean Embryos

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ABSTRACT

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Three strains of southern bean mosaic virus (SBMV) were seed-transmitted through the embryos of three bean cultivars. Buffer extracts prepared from infected immature or mature embryos, but not from healthy embryos, were infectious. Decontaminated, infected embryos or infected intact seed also produced diseased seedlings. In all trials, however, SBMV was transmitted in higher frequency from immature seeds than from mature seeds. Attempts to increase the recovery of infectious SBMV from infected mature seeds by extraction of RNA or to detect inhibition of virus infectivity by lectins or seed extracts failed. Phenol extracts of infected seedcoats or embryos contained only a small fraction of the infectivity of buffer-extracted inoculum. Lectins, at low concentrations, tended to enhance SBMV infectivity; whereas seed extracts, but only at high concentrations, reduced lesion numbers.

Additional key words: virus degrading solutions, *Phaseolus vulgaris*.

Zaumeyer and Harter (20) described a highly stable bean virus, southern bean mosaic virus (SBMV), which was transmitted to 5% of germinated seedlings of 7-mo-old stored seeds. Extracts from immature embryos or seedcoats also were infectious. Cheo (4) confirmed these findings and obtained 50-80% transmission of SBMV in seedlings derived from immature bean seeds. However, extracts from mature embryos were not infectious when tested on indicator plants. Crowley (5) reported recovery of SBMV from immature embryos, but not from seedlings germinated from 50 mature seeds. Crowley (5) also reported that cross-pollination of healthy bean flowers with pollen from diseased plants produced infected embryos (10/47), even though leaf and pod tissues from the seed-mother plants assayed negative for SBMV. Shepherd and Fulton (17) demonstrated 3-4% transmission of the cowpea strain of SBMV to seedlings produced by planting mature seed from infected plants. More recently, however, McDonald and Hamilton (11) questioned the earlier findings of Cheo (4), and presented evidence to support the thesis that immature embryos were not infected, but were contaminated with virus-infected remnants of the seedcoat membrane. They reported that, by a 30-min tap water wash, SBMV was eliminated from immature embryos. However, buffer extracts from seedcoats, treated in a like manner, were highly infectious.

Our preliminary results agreed with Cheo's findings. This study was undertaken to determine whether SBMV is embryonically transmitted or whether transmission is due to virus contamination of embryos followed by subsequent infection of germinating seedlings by mechanical injury during germination. Also, we tested extracts from mature seeds and commercially available lectins for inhibition of virus infectivity.

MATERIALS AND METHODS

Virus strains and host plants.—Three southern bean mosaic virus strains (SBMV-type, ATCC 17; SBMV-Mexican (8); and SBMV-Davis, Calif. isolate) were inoculated onto bean (*Phaseolus vulgaris* L.) cultivars, Black Turtle Soup, Logan, and Red Kidney. The cultivars, Red Kidney (7 to 10 days old) and Kentucky Wonder (12 to 15 days old), were used for systemic and local lesion assays, respectively. Choice of bean cultivar for assay was determined by plant availability and not by differences in sensitivity to SBMV infection. Each bean cultivar was equally receptive to inoculations by SBMV. For example, inoculation with viruliferous bean sap diluted ($\log_{10}$ series in phosphate buffer) nearly to an endpoint, induced one to four lesions per four to six leaves of Kentucky Wonder plants, and at least one of three inoculated Red Kidney plants became systemically infected.

Seed source plants were inoculated mechanically with virus (infected tissues triturated in 0.05 M potassium phosphate buffer, pH 7.0) as 12-day-old seedlings and seed pods were harvested 63-78 days later. Immature and mature seeds were tested for virus content by direct assay onto the indicator plants or by serological tests of extracts from 3-wk-old seedlings produced by germination of the seeds. Seeds were considered immature if harvested

before pods and seeds were air dry; also, they were maintained under refrigeration in a plastic bag and were not allowed to become dry prior to being tested for virus. Mature seeds appeared to be dry at time of harvest, and were allowed to air dry for 2 wk or more on the laboratory bench. Both mature and immature seeds had normal pigmentation in the seedcoats.

Prior to use, all mature seeds were rehydrated by soaking in water overnight. The seedcoats were removed and all embryos (immature and mature) were washed vigorously for 30 min with flowing tap water to eliminate surface virus contamination (11). Single embryos were extracted in buffer (0.5 or 1.0 ml of 0.1 M phosphate buffer, pH 7.0, per embryo) and rubbed on assay plants dusted with corundum. All inoculated leaves were rinsed with tap water.

Some washed embryos and seeds also were germinated in flame-pasteurized or steam-sterilized soil in the greenhouse to test for transmission of virus to seedlings.

Virus purification and serology.—A modification of the Wells and Sisler procedure (19) was used; frozen infected leaf tissues of Red Kidney plants (harvested 17 days after inoculation) were extracted in 0.1 M potassium phosphate buffer, pH 7.0, containing 0.02 M each of cysteine and sodium diethyldithiocarbamate (DIECA). After filtration through cheesecloth, the extract was adjusted to pH 7.0 - 7.5 and emulsified with an equal volume of *n*-butanol and chloroform. The emulsion was broken by centrifugation (10,000 *g*, 3 min) and the clear supernatant liquid was subjected to two alternating high-speed (66,000 *g*, 2 hr) and low-speed (12,000 *g*, 10 min) cycles of centrifugation. High-speed pellets were resuspended in 0.01 M phosphate buffer, pH 7.0. Further purification was achieved by layering 2 ml of virus preparation onto a sucrose density gradient column and centrifuging at 51,000 *g* for 3 hr as previously described (18).

A series of intravenous, subcutaneous, and intramuscular injections (latter two injections emulsified with Freund's incomplete adjuvant) with density-gradient purified SBMV-type preparations were used to immunize rabbits. Serum collected 12 days after the final injection had a reactive endpoint of 2,048 and 0 (reciprocal of dilution) to infective and noninfective bean sap, respectively. Double-diffusion tests were performed in 0.75% Ionagar No. 2 gels containing 0.85% sodium chloride and 0.02 M sodium azide.

Extraction of lectin from seed.—A modification of the procedures described by Rigas and Osgood (14) and Cheo (4) were used. Briefly, mature seeds of Red Kidney and Logan were soaked overnight in distilled water. Each 100 g of hydrated seeds was homogenized and extracted in 200 ml of 0.85% saline and the homogenate was frozen for 24 hr. After being thawed at room temperature (24 hr), the extract was adjusted to pH 4.9 with 1.0 N HCl. The precipitate was collected by centrifugation (Sorvall GSA rotor, 12,000 *g* for 10 min) and discarded. The supernatant liquid was mixed with an equal volume of

TABLE 1. Transmission of two strains of southern bean mosaic virus (SBMV) in immature and mature bean seeds and embryos[a]

Infected seed host/ virus strain	Embryo assay:[b]		Germination test:[c]			
			Immature		Mature	
	Immature	Mature	Embryo	Seed	Embryo	Seed
Red Kidney/SBMV type strain	22/43[d]	2/35	8/70	16/28	...	3/127
Black Turtle Soup/ SBMV-Davis strain	11/11	0/27	91/245	23/64	0/105	0/87
Healthy bean seeds[e]	0/5	0/5	...	...	...	...

[a]Immature seeds were harvested fresh and maintained in a hydrated state under refrigeration; mature seeds were dry at time of harvest and were stored at room temperature for 2 wk before use.

[b]Mature seeds were allowed to rehydrate overnight; all seedcoats were removed; embryos were washed vigorously under tap water (30 min) and triturated in buffer and rubbed on indicator plants (Red Kidney beans).

[c]Washed embryos and intact seeds were sown in flame-pasteurized soil and allowed to germinate. Serological tests were performed on 3-wk-old seedlings to identify infected plants.

[d]Number of positive SBMV infections per number of embryos or seeds tested.

[e]Seeds harvested from healthy Red Kidney and Black Turtle Soupbean plants were used for control inoculations.

TABLE 2. Seed-borne transmissions of southern bean mosaic virus-type strain in infected Logan bean seeds

Decontamination procedure[a]	Germination tests of infected[b]			
	Immature		Mature	
	Embryo	Seed	Embryo	Seed
Nonwashed	...	4/7[c]	...	2/23
Washed	12/24	...	1/197	...
Carbonate, SDS, EDTA	6/25	...	...	...
NaClO	12/23	...	...	...

[a]Embryos were decontaminated by a wash (30 min) or a soak (15 min) in buffer mixture of 0.1 M sodium carbonate, pH 9.1, containing 0.001 M EDTA and 1% SDS and water rinse or a 1% sodium hypochlorite soak (5 min) and water rinse.

[b]Embryos and intact seeds were sown in steam-sterilized soil, germinated, and assayed by serological tests of 3-wk-old plants.

[c]Number of infected plants per number plants tested.

saturated ammonium sulfate (pH 5.0) and recentrifuged. The precipitate was discarded and another equal volume of saturated ammonium sulfate was added to the supernatant fraction and the mixture was refrigerated overnight. The precipitate was collected by centrifugation and resuspended in saline and dialyzed against saline (several changes) and against distilled water. During dialysis with saline, the preparation remained clear, but became distinctly opalescent after dialysis with distilled water. After water dialysis, the preparation was lyophilized, resuspended in saline (a clear solution resulted), adjusted to 5.0 mg protein/ml, and 5-ml aliquots were stored frozen until used. Protein concentration was determined with the folin phenol reagent procedure (10).

Other methods with specific application are described in the appropriate sections.

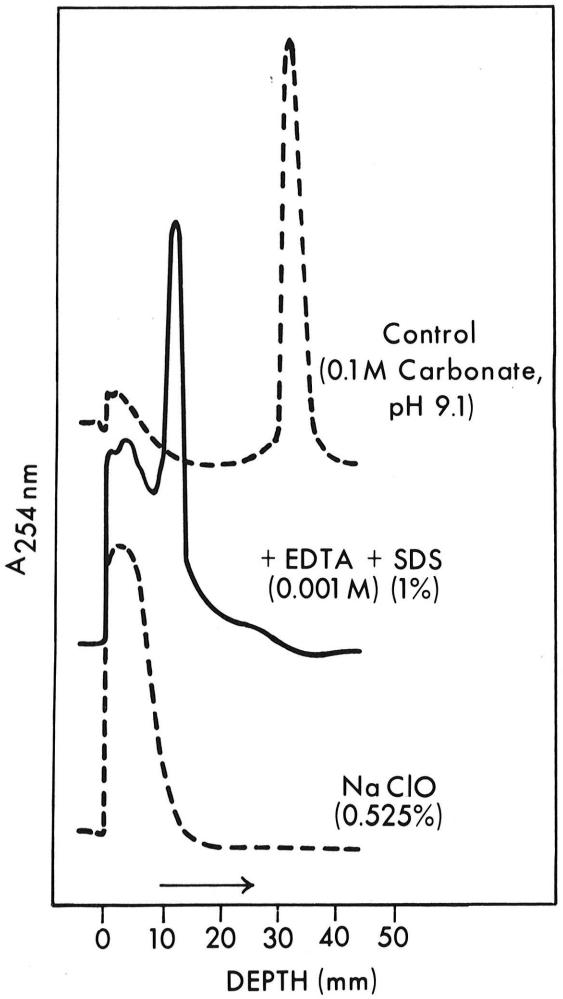

Fig. 1. Ultraviolet scanning patterns of sucrose density-gradient profiles of: top, nontreated southern bean mosaic virus (SBMV) (1 mg); middle, SBMV mixed with carbonate buffer-EDTA-SDS solutions; and bottom, mixed with NaClO. Composition of gradient was a 4, 7, 7, and 7 ml of 10 to 40% sucrose solutions in 0.01 M phosphate buffer; centrifuged at 51,000 *g* for 3 hr and sedimentation was from left to right.

RESULTS

Virus transmission through seeds and embryos.—Seeds of Red Kidney or Black Turtle Soup bean infected with SBMV-type or SBMV-Davis, respectively, were sources of infected embryos. Direct assay of washed embryos extracted in buffer and rubbed onto healthy Red Kidney plants showed a high rate of virus transmission (Table 1). Similarly, good virus transmissions were afforded among plants derived from germinating immature washed embryos and immature intact seeds. However, mature seed from infected mother plants of Red Kidney produced only three infected seedlings (Table 1). All suspected infections were verified by serological tests using leaf sap expressed from 18- to 21-day-old seedlings. Control inoculation with extracts from embryos from healthy mother plants produced no infections.

In a second trial, plants of the local-lesion assay cultivar, Kentucky Wonder, were inoculated with extracts from immature SBMV-type-infected embryos; four and six half-leaves were inoculated with extracts made in 0.5 or 1.0 ml, respectively, of buffer. The total number of local lesions from each set of inocula were 0, 1, 4, 6 (0.5 ml), and 1, 3, 5, 10 (1.0 ml). Similar inoculations with the corresponding washed seedcoat extracts incited lesions too numerous to count. Eight washed embryos from the same seed lot were sown and two of eight seedlings were infected.

Tests with virus-disassociating solutions.—Because embryos washed with water produced infectious inocula, we tested other solutions reported to degrade virus particles and thus should be more effective in elimination of infectivity due to surface contamination. Two solutions, carbonate buffer mixture (19) and dilute sodium hypochlorite (13), were tested as follows: A preparation of purified virus (1 mg/ml, $A_{260} = 5.85$) was mixed with an equal volume of 0.2 M sodium carbonate buffer, pH 9.1, containing 0.002 M EDTA and 2% sodium dodecyl sulfate (SDS) or with 1% sodium hypochlorite (NaClO). The mixtures were incubated for 10 min at room temperature, layered onto a sucrose density-gradient column, centrifuged in a Spinco 25.1 rotor, and analyzed with an ISCO density-gradient fractionator.

Figure 1 illustrates a typical density-gradient profile of treated and nontreated SBMV after centrifugation. The single peak due to intact SBMV in the control (nontreated) gradient, with an estimated sedimentation coefficient of 115S, was highly infectious and reacted with SBMV antiserum. The slower-sedimenting component (about 50-55S) evident in the preparation treated with carbonate-buffer-EDTA-SDS induced four primary lesions on three leaves, but failed to react with virus antiserum. In the NaClO-treated preparation, there was only a single peak at the meniscus; this was not tested. The carbonate-buffer mixture had been used to prepare SBMV-RNA (15, 19) and, in our tests, treatment of SBMV with this buffer readily caused dissociation into 50-55S or smaller-sedimenting components.

In a third trial, mature or immature seed pods from Logan bean plants infected with SBMV-type were harvested and assayed on Kentucky Wonder plants. Prior to extracting in buffer or sowing into soil, immature

embryos were washed for 30 min in running tap water or were soaked in the carbonate buffer mixture (15 min) or in NaClO (5 min) and rinsed in tap water (15 min). Irrespective of the surface decontamination procedure used, infected seedlings were derived from planting immature logan embryos from infected plants (Table 2). Germination of intact immature seed was very poor, but four of seven seedlings that emerged from 50 seeds were infected. In contrast, only two seedlings, each derived from planting mature intact seed and mature embryos, were infected (Table 2).

To determine the relative infectivity of extracts from different parts of embryos from SBMV-type-infected plants, carbonate-soaked immature Logan embryos were dissected into shoot-radicle and cotyledons, which were indexed separately by local-lesion assays on primary leaves of Kentucky Wonder. Two samples, each comprising the respective dissected parts from three embryos and the corresponding seedcoats, were extracted in 3 ml of 0.01 M phosphate buffer, pH 7.0. The average total number of lesions produced on four leaves were: five from shoot-radicle, 63 from cotyledons, and 288 from seedcoats. Comparable inocula prepared from whole, carbonate-treated, mature embryos (five extracts each comprising five embryos in 10 ml of buffer were tested) produced no lesions on 20 primary leaves, whereas two comparable extracts from the corresponding seedcoats induced 234 and 416 lesions on two sets of four leaves for each. Because the mature-embryo extracts indexed negative on Kentucky Wonder plants, a second set of extracts was prepared as before and these were inoculated onto Red Kidney plants. Two of five of these inocula were infectious. All control inoculations comprising tests of corresponding extracts from embryo and seedcoats from noninfected mother plants produced no lesions or systemic infections of the respective indicator bean cultivars.

Seed transmission of Mexican strain of SBMV.—In comparable tests of whole, carbonate-soaked embryos produced on Logan plants infected with SBMV-Mexican, eight of eight and two of two inocula from immature and mature embryos, respectively, were infectious. The numbers of lesions produced on four primary leaves by the respective inocula were quite variable, however, and ranged from one to 33 lesions. Furthermore, two of 61 Logan seedlings, derived from planting carbonate-soaked immature embryos from infected plants, were infected with SBMV-Mexican (virus strain verified by positive serological tests) (8).

Artificial virus contamination and decontamination of embryos.—Mature, virus-free Logan seeds were soaked overnight in water. The seedcoats were removed and embryos were washed in water before use. The embryos either were soaked in, or rubbed with purified virus suspensions. For soaking, the embryos were submerged for 15 min in a preparation of SBMV-type (0.5 mg/ml) that induced an average of 78 local lesions per half-leaf. After being soaked, the embryos were decontaminated in carbonate buffer and rinsed (each for 15 min) or were sown directly into pasteurized soil. All embryos were positioned on a bed of soil and covered with 2.5 cm of steam-sterilized sand. This was done to enhance mechanical abrasion, if any, of the germinating embryos as they emerged through the sand layer. No infection was detected in seedlings (3 wk old) derived from contaminated, unwashed (0/25) or decontaminated embryos (0/23). However, mechanical inoculation of corundum-dusted embryos with an artist brush dipped in virus inoculum resulted in infection of seedlings (9/45, radicles rubbed; and 2/49, cotyledons rubbed).

Concomitant tests were done to determine the efficacy of removal or inactivation of virus from the artificially contaminated embryos. Prewashed, virus-free embryos were contaminated by soaking in purified virus as before. Extracts from contaminated embryos were assayed for virus infectivity on half-leaves of Kentucky Wonder plants in comparison with extracts from contaminated embryos after attempted decontamination by washing in water, carbonate soak and rinse, or NaClO soak and rinse. Each inoculum comprised two embryos extracted in 4 ml of 0.1 M phosphate buffer; the comparative tests were repeated from four to seven times (Table 3). The average number of lesions produced on half-leaf assays were 214/4 (contaminated/contaminated and washed with water), 160/1 (contaminated/contaminated and carbonate soak and rinse), and 234/0.25 (contaminated/contaminated and NaClO soak and rinse). Although virus infectivity was reduced to comparatively low levels by all three procedures, none completely eliminated infectivity. Even so, this failure to completely eliminate infectivity by the decontamination treatments probably is of no significance for

TABLE 3. Efficacy of inactivation of virus from artificially contaminated Logan bean embryos. Prewashed embryos soaked (15 min) in southern bean mosaic virus (type) preparation (0.5 mg/ml)

Decontamination procedure	Experiment 1, replications:			Experiment 2, replications:			
	I	II	III	I	II	III	IV
Nonwashed/washed	205/1[b]	116/6		234/5	276/2	161/6	291/6
Nonwashed/carbonate, SDS, EDTA	116/2	75/3	78/0	211/0	365/2	98/0	174/3
Nonwashed/NaClO				238/0	153/0	347/0	199/1

[a]Unwashed inocula were comprised of two contaminated embryos triturated in 4 ml of 0.1 M phosphate buffer, pH 7.0. Other tested inocula included contaminated embryos which were decontaminated (either washed for 30 min under running tap water or soaked 15 min in 0.1 M carbonate, 1% SDS, 0.001 M EDTA buffer mixture and rinsed or soaked 5 min in 1% sodium hypochlorite and rinsed) prior to trituration in buffer.

[b]Local lesion totals per eight half-leaves for each inoculum. Numerator: number of lesions produced by nonwashed embryos; denominator: number of lesions produced by decontaminated embryos. Assayed by mechanical inoculation on Kentucky Wonder beans.

understanding the mode of SBMV seed transmission because, as reported above, planting of highly contaminated embryos did not result in virus-infected seedlings.

Viral RNA extraction and assay.—Although our data show that SBMV is transmitted embryonically in a few mature seeds, the reason for the decrease of infectivity in mature seeds is not known. Because SBMV is a stable virus with a thermal inactivation of 90-95 C and longevity in vitro of 20 to 165 days at 18-22 C (16), loss of infectivity from drying during seed maturation seems incongruous.

Extracts of SBMV-RNA were processed before testing for infectivity as described by Diener (6) and Wells and Sisler (19). Preparations of RNA from two to five washed embryos from virus-infected plants were inoculated onto the half-leaves of eight plants and compared with comparable extracts in 5 ml of phosphate buffer. In most instances, infectivity of the RNA preparations was much less than that of the buffer inocula. For example, only two of six RNA preparations from immature embryos were infectious, and only two and four lesions were produced by each; in contrast, the comparable buffer extracts from six samples produced 1, 2, 8, 9, 42, and 373 lesions, respectively. None of the five RNA preparations from mature embryos was infectious, and two of five buffer inocula produced only one lesion each. Buffer inocula from seedcoats of mature and immature seed produced more than 200 lesions per half-leaf, whereas only one comparable RNA preparation did so; the four other RNA preparations produced only about 12 lesions per half-leaf (Fig. 2).

Seed lectins and their properties.—Lectins or plant hemagglutinins commonly occur in high concentrations in legume seeds, and have long been recognized and used for determining human blood groups (1). Some lectins also are used in the extraction and purification of certain polysaccharides and glycoproteins. Other uses of lectins have been discussed in a recent review (9). The established interaction of lectins and carbohydrates (some lectins are highly specific and somewhat approximate an antibody-antigen reaction) coupled with the recent suggestion that certain seed-transmitted plant viruses possess glycoprotein on the viral capsid (12) led us to investigate the possible interaction of SBMV and partially purified seed extracts and commercially-available lectins to determine whether the inhibition of virus infectivity in mature seeds is due to lectins. Presumably the concentration of lectins would increase as the seed matures (due, in part, to desiccation), and thus enhance the chances for lectin-virus interaction, if any, to occur.

Yield and hemagglutinating properties of seed preparations.—Extracts of Red Kidney (RKE) and Logan (LE) seeds yielded, respectively, 6 and 6.3 mg protein/g of hydrated seed. A commercial source of Red Kidney lectins [Phytohemagglutinin M (PH-M) and P (PH-P) from Difco Laboratories, Detroit, MI 48232] also

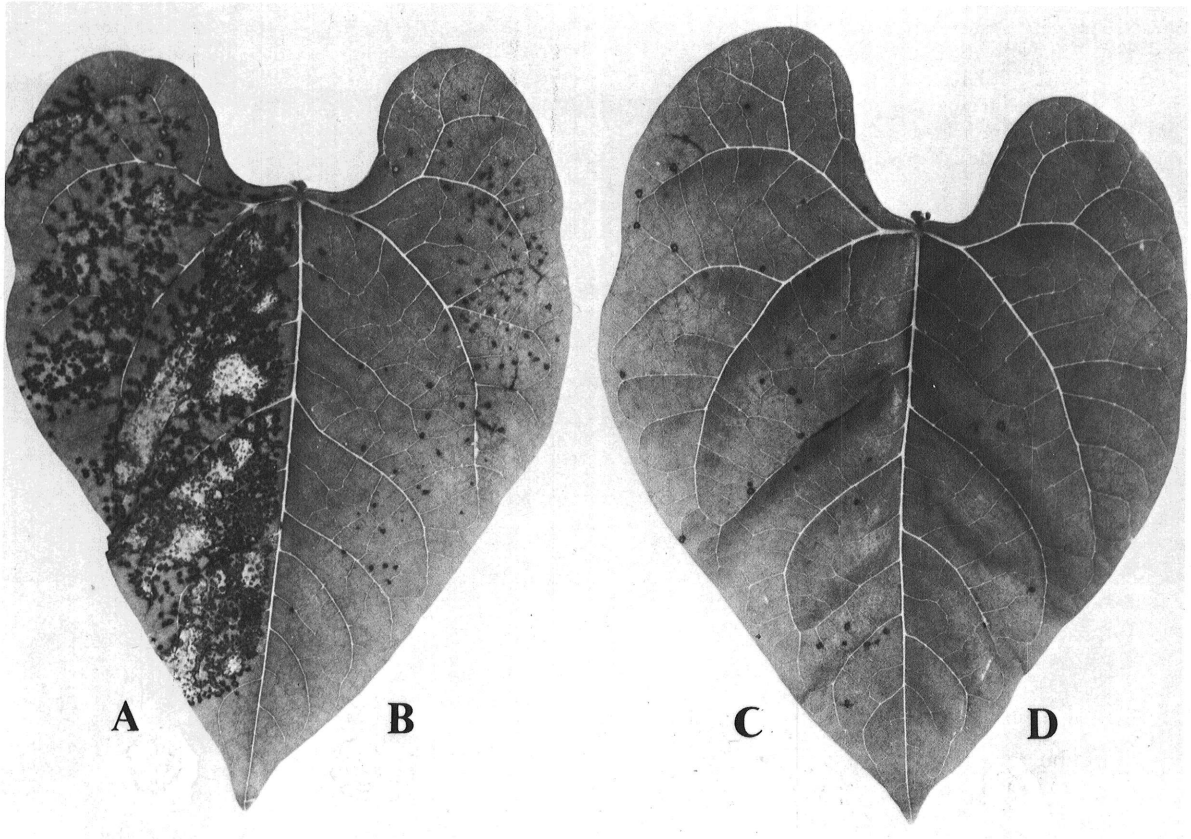

Fig. 2. Local lesions on primary half-leaves of Kentucky Wonder bean rubbed with (a) buffer extract from seedcoat, (b) phenol extract from seedcoat, (c) buffer extract from embryo, and (d) phenol extract from embryo.

was included in these tests for effect on virus infectivity.

To determine if our seed preparations possessed lectin properties other than solubility in saline, hemagglutination tests with human red blood cells were conducted as described by Campbell et al. (3). Before being mixed, preparations of bean seed and PH-P were standardized to 1 mg/ml and SBMV-type virus to 10 mg/ml. The LE preparation and PH-P lectin, but not RKE or virus preparation, reacted with each of four human blood groups tested (A, B, AB, and O), indicating that one of our seed preparations also possessed hemagglutinating properties. Titers, expressed as reciprocal of dilution, ranged from four to 32 for the Logan extract and eight to 64 for PH-P. The PH-M was not tested. Nonreactivity of the Red Kidney extract was unexpected because this seed source of lectin is reportedly active against the four blood groups (2).

Effect of lectins on SBMV infectivity.—Preparations of the seed extracts and commercial lectins were adjusted with saline to 5 or 10 mg/ml and SBMV-type to 2 mg/ml. The ratios of seed extract to virus that were tested ranged from 2.5:1.0 to 25:1.0 (w/w) and for PH-M and PH-P from 2.5:1.0 to 10:1.0 (w/w). Prior to inoculation (half-leaf assay on Kentucky Wonder primary leaves), the samples were incubated at room temperature (about 22 C) for 1 hr. Each mixture was tested two or more times and some mixtures were retested in a second trial.

Both seed extracts (RKE and LE) below a 10:1 extract to virus ratio had no apparent effect on virus infectivity. The combined average lesion numbers (from all replications and tests) per half-leaf for ratios of both 2.5:1.0 and 5:1 were 73/68 (virus control/RKE and virus) and 68/63 (control/LE and virus), respectively. At a 10:1 seed-extract to virus ratio and greater, the lesion numbers were reduced to about one-half of that of the control inocula. For example, RKE-virus ratio mixtures of 10:1, 12.5 combined with 15:1, and 22.5 combined with 25:1 incited average lesion numbers of 117/74, 84/29, and 266/141, respectively (control/RKE and virus). A similar reduction in infectivity also was noted with the comparable LE-virus mixtures.

The infectivity of commercial lectin-virus mixtures was about the same or slightly greater than that of the virus-alone controls. The lesion numbers for the 2.5:1.0, 5:1, and 10:1 ratios were similar and averaged 51/55 (control/PH-M and virus) and 42/52 (control/PH-P and virus).

To determine whether virus infectivity of lectin-virus and seed-extract and virus mixtures was decreased by drying, the PH-M-and PH-P-virus mixtures were tested at a single lectin-virus ratio of 10:1 ratios. Two replicate tests of each mixture were compared with virus-alone inocula as used in the previous tests. A final volume of 1.25 ml of each mixture or virus alone was pipetted into a mortar and allowed to air-dry overnight. Prior to assay, the residue was resuspended in distilled water and allowed to stand with occasional stirring for 1 hr before inoculation.

The combined average lesion numbers induced per half-leaf from the replicated trials were 4/10, 11/12 (control/RKE and virus) and 9/11, 6/5 (control/LE and virus), respectively, for ratios of 2.5:1.0 and 10:1, whereas the 10:1 lectin-virus mixtures were 5/29 (control/PH-M and virus) and 4/8 (control/PH-P and virus).

DISCUSSION

Although none of the decontamination procedures completely eliminated SBMV infectivity from artificially contaminated embryos, our results show that heavy surface contamination of noninjured embryos does not result in virus transmission to seedlings. Thus, the effectiveness of surface decontamination would not change our results which showed that SBMV is transmitted to seedlings via infected embryos, and is not due to fortuitous infection by surface-borne virus.

Our results agree with previously published findings (4, 5), but not with those of McDonald and Hamilton (11) who reported that SBMV was not borne internally in bean embryos. Since we used the same virus-host combination and assay hosts, we are unable to explain the differences between our results and those reported by McDonald and Hamilton (11). Perhaps our use of smaller volumes of extracting buffer per embryo and larger numbers of embryo units were critical factors. However, the high percent of virus transmission from immature embryos to seedlings obtained in our tests, and the close agreement between transmissions to seedlings and infectivity of extracts from decontaminated embryos strongly support our conclusion that seed transmission of SBMV results from infection of embryos.

The apparent inactivation of SBMV in mature seeds has been noted previously (4, 5). Our attempts to demonstrate increase in virus infectivity from immature and mature embryos or seedcoats by extraction of RNA failed. Similarly, the effect of lectins or seed extracts on virus infectivity was negligible. Even though the high seed-extract to virus ratios reduced the infectivity of inoculum, the reduction was not sufficient to account for the paucity of virus transmissions from mature seeds. Although plant lectins have been reported to affect release of virus from animal cells, this was due mainly to effects on host cells rather than on the virus (7). Cheo (4), however, reported that extracts from bean seed were highly inhibitory to SBMV infection, and that the extracts did not affect susceptibility of the host-plant cells. Our use of ammonium sulfate for fractionation of extracts from seed instead of a solution of basic lead as used by Cheo (4) might explain the differences in results. Based on data presented by Cheo (4), an inhibitor of SBMV infectivity was present in air dried immature and mature seeds, but our results indicate that this substance(s) probably was not lectin.

Possibly the loss of infectivity of SBMV in dry bean seed is due to physical stress on the virus particles from water loss in combination with increase in concentration of seed inhibitors (4), and the relatively low content of virus in infected embryos. Further work needs to be done to gain a better understanding of this phenomenon.

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