Transmission and Distribution of Squash Mosaic Virus in Seeds of Cantaloupe

M. Alvarez and R. N. Campbell

Former Ford Foundation Fellow and Professor, respectively, Department of Plant Pathology, University of California, Davis, CA 95616. Present address of senior author: Instituto de Investigaciones Agropecuarias, Est. Exp. La Platina, Casilla 5427, Santiago, Chile.

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ABSTRACT

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Seed transmission of squash mosaic virus (SMV) in 62 seed lots from infected cantaloupe, cultivar PMR-45, averaged 10.6% and was not correlated with flowering or seed-production factors including time from virus inoculation to fruit set, fruit weight, or seed number and weight. Different methods of assaying for embryo infection of SMV indicated the same percentage infection. For example, when the naked embryos were split into distal and germinative ends, results of tests for SMV in the distal ends agreed with tests of the seedlings or plants grown from their respective germinative ends. Infected embryos always produced infected seedlings. In studies of the tissue relationships of SMV in seeds and seedlings local lesion assays on *Cucumis metuliferus* showed that there was a low level of infectivity in cantaloupe cotyledons during the first 2 days of germination but it increased after that. The roots and hypocotyls had less SMV than the cotyledons. The SMV was not detectable in cotyledons until the 3rd or 6th day of germination with the immunodiffusion and immunofluoresence tests, respectively. When detected, SMV was uniformly distributed in the cotyledons but immunofluoresence tests of protoplasts indicated only 18% infection. This virus also was found in seed coats as well as the papery layer consisting of remnants of the nucellus and endosperm. The SMV in the papery layer occurred independently of embryonic infection and its role in seed transmission is unknown. No SMV was detected in washed, triturated pollen of pumpkin cultivar, Small Sugar. Pollen transmission was not demonstrable in cantaloupe.

RESUMEN

ALVAREZ, M., and R. N. CAMPBELL. 1978. Transmisión y distribución del virus del mosaico del zapallo en semilla de cantaloupes. Phytopathology 68:

La transmisión por semilla del virus del mosaico del zapallo (VMZ) en 62 lotes infectados de semilla de cantaloupe, PMV-45, fué en promedio de 10.6%. No se encontró correlación entre el porcentaje de transmisión del virus y factores como período desde la inoculación a la cuaja de la flor, tamaño del fruto, número y peso de semillas. Mediante diversos métodos de detección de VMZ en embriones infectados se obtuvieron porcentajes de infección similares. Al separar embriones desnudos en dos partes transversales y efectuar pruebas de VMZ en los extremos distales, hubo concordancia entre la presencia de virus en ese sector y la presencia en plántulas ó plantas obtenidas de los respectivos extremos germinativos. Embriones infectados siempre originaron plántulas infectadas. En estudios de relación histológica del VMZ en semillas y plántulas, usándose la planta indicadora de reacción local *Cucumis metuliferus*, se encontró que existía una baja proporción de virus en cotiledones de cantaloupes durante los dos primeros días, la que aumentaba pasado este período. Las raíces e hipocotilos tenian menos VMZ que los cotiledones. Usándose técnicas de inmunodifusión e inmunofluorescencia el virus no se detectó en cotiledones hasta el 3° y 6° día, respectivamente. Una vez detectado se encontró que estaba uniformemente distribuído en los cotiledones, pero por inmunofluorescencia se demostró que solo un 18% de los protoplastos estaba infectado. El virus también se encontró en la cubierta seminal y en la cutícula interna remanente del endosperma y nucelo; aquí se encontró el virus independientemente de la infección del embrión y su papel en la transmisión por semilla se desconoce. No se detectó el VMZ en polen lavado y triturado de calabaza cultivar, Small Sugar pumpkin. No se demostró la transmisión por el polen en cantaloupe.

Squash mosaic virus (SMV) was first reported in California in 1934 as being seed-borne in melons (10). Although the virus has a narrow natural and experimental host range and generally is of minor importance, it can cause serious damage in late summer or fall crops in northern California (8, 13). The seed-borne nature of SMV is of significance in the epidemiology of the disease by providing an over-wintering mechanism and widely dispersed foci of infection in fields planted with infected seed (2). Commercial and experimental seed lots of several commercial cucurbits have about 1% seed transmission (8, 13) although higher rates have been obtained with seed lots from infected plants (17, 20).

Little information is available on the mechanism of

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seed transmission or on distribution of virus in the seed. Powell and Schlegel (17) obtained higher rates of infection or transmission by a direct seedling assay (6-day-old seedlings triturated and sap-inoculated to assay plants) than by seed assay (nongerminated seeds triturated and sap-inoculated to assay plants) or by plant assays (germinated seedlings visually inspected for systemic symptoms). To account for the difference between seedling and plant assays they postulated that seed transmission was influenced by virus infection of the embryo and by unknown factors which permitted successful maintenance of the host-virus relationship only during germination of some infected seeds. The distribution of SMV within seeds is not well known owing in part to the absence of a reliable local-lesion host. The local lesion reaction of watermelon cultivar Black Diamond and the local lesion reaction preceding systemic symptom development of squash cultivar Early Yellow Summer Crookneck (6) have not been reliable with our isolates and growing conditions. *Cucumis metuliferus* has been reported to have hypersensitive resistance to SMV (19). In histological studies on the localization of SMV in seedlings using autoradiography, Powell and Schlegel (18) found localized virus increase in certain cells. They hypothesized that only a limited number of cells actually were infected with virus and that the probability of systemic invasion of the seedling was determined by the number and location of infected cells in the embryo.

Several seed-borne viruses are pollen-transmitted (2), but SMV has not been reported to be pollen-borne. Although SMV was recovered from pollen (20), these authors did not test for embryo infection via pollen transmission.

The objectives of the present paper were to compare three methods of assay for seed transmission of SMV; to investigate the virus-embryo relationship using *C. metuliferus* as a local lesion host, fluorescent antibodies, and serology; and to test for pollen transmission.

MATERIALS AND METHODS

The SMV isolate was obtained from an infected seedling of *Cucumis melo* var. *inodorus* 'Honeydew' and was stored in an infected seed lot of *C. melo* var. *reticulatus* 'PMR-45' produced in the experimental fields at Davis. It was recovered for the present study by planting infected seed in the greenhouse and maintained in PMR-45 by sap transfer. Based on its seed transmissibility, host range, host reaction, and morphology it was classified as a nonwatermelon-infecting isolate of SMV. Serological comparisons of this isolate and its antiserum with isolate IA from M. R. Nelson (Univ. Arizona, Tucson 85721) placed this isolate in serological group I (16). Members of this group are more readily seed-transmitted in *Cucumis* sp. than are those of group II (15).

Production of infected seed lots.—Cantaloupe cultivar PMR-45, widely used commercially in California, was used throughout this study. Although 'cantaloupe' is a misnomer for 'muskmelon,' we will use cantaloupe following accepted common usage in the United States (24). Seven 2-wk-old seedlings planted 2 m apart in the field at Davis were sap inoculated on 15 July. Each inoculated plant showed symptoms 2 to 3 wk after inoculation and assayed positively on cantaloupe seedlings in the greenhouse. Seven noninoculated plants left in the same field for comparison did not develop symptoms during the growing season and assayed negatively in infectivity tests. Melons were harvested at intervals during October and November. The following data were collected for each fruit: day of setting, fruit weight, and the number and weight of seeds. The seeds from each fruit were collected separately as an individual seed lot; thus 62 different lots were collected from the infected plants. The percentage germination and percentage of SMV seed transmission were determined for each seed lot by planting 120 seeds in sterilized greenhouse soil mix in trays in the greenhouse. The seeds were treated with 0.1 N HCl for 0.5 hr and rinsed in running tap water for 5-8 hr before planting to eliminate surface contamination.

Squash mosaic virus assays.—Squash mosaic virus infectivity was detected by mechanical inoculation of Carborundum-dusted plants of either of two assay species: *C. melo* var. *reticulatus* or *C. metuliferus*. The cantaloupe assay plants were cultivar PMR-45 from seed produced previously on plants that had been indexed and were free of SMV. These assay plants were inoculated on their cotyledons when 10 days old and showed systemic symptoms in 12 to 15 days if SMV was present. The *C. metuliferus* plants were locally increased from seed kindly provided by R. Provvidenti (Geneva, N.Y.). Half-leaf inoculations were done on the 3rd, 4th, and 5th true leaves of 25- to 35-day-old plants by rubbing with a stiff, camel's-hair brush. After inoculation, leaves were rinsed with tap water. On each plant one half-leaf received a standard SMV preparation and the other half of the same leaf was the noninoculated control. After inoculation, the plants were placed in a controlled environment chamber at 20-22 C and with a 12-hr photoperiod of 11,000 lux. Conspicuous chlorotic local lesions developed 96-120 hr later if SMV was present.

Several tests were used to detect seed transmission of SMV or to locate SMV in various parts of the seed. In the plant assay described above the rate of transmission was the percentage of the seedlings with symptoms on the first three true leaves. For other assays, seeds that had not been acid-treated were soaked in water for 24 hr, rinsed in running tap water for another 24 hr, and separated into seed coats, papery layer, and embryos using sterilized razor blades, tweezers, and a dissecting microscope. The papery layer is the thin membrane that envelops the embryo and that represents the remnants of the endosperm and nucellus although earlier workers have referred to it as the integument (17). The separated parts were washed in running tap water for 5-8 hr. For some experiments, the embryos were treated with 0.1 N HCl for 0.5 hr. The separated parts were mechanically inoculated to the assay plants or, in some trials, the embryos were divided transversely into the distal half and the germinative half. Individual distal halves were soaked 15-20 hr at 4 C in three drops of 0.01 M phosphate buffer, pH 7.0, in 7-mm-diameter cavities in a plexiglass board, triturated with a glass rod, and inoculated to *C. metuliferus*. This will be referred to as the half-embryo assay.

The germinative halves of these embryos were tested for SMV by either of two methods: plant assay or seedling assay. For the plant assay the embryos were germinated and the seedlings were observed for symptoms as in the plant assay of whole seeds. Special handling was needed to obtain seedlings from the divided embryos and to avoid accidental mechanical transmission of SMV. Embryos were spaced on moist paper towels in plastic boxes at approximately 30 C for 48 hr. Each seedling was transferred with a new toothpick and planted in sterilized sand in a 6-cm-diameter plastic cup. The plastic cups were placed in covered plastic boxes at approximately 30 C with 12-hr light at 4,850 lux and watered daily with nutrient solution. After 48-72 hr the box was uncovered for another 48-72 hr until the first true leaf was formed. The cups were transferred to the greenhouse and the plants observed for symptoms on the first three true leaves. For the seedling assay the embryos were germinated as above until the 6th day. They then were washed free of sand and individually triturated in buffer, and sap-inoculated to cantaloupe plants.

Virus purification and serology.—Squash mosaic virus was purified from pumpkins (*Cucurbita pepo* L. 'Small Sugar') that were inoculated at the cotyledonary stage and harvested about 4 wk later when showing severe symptoms. The purification scheme of Lastra and Munz (12) was followed using 6% polyethylene glycol (PEG 6000) with 0.2 M NaCl. Further purification was achieved by freezing overnight followed by acidification; the thawed solution was diluted to 100 ml with distilled water, 0.1 M acetic acid was added with stirring to adjust the mixture to pH 4.9, and the suspension was kept overnight at 4 C. After a low-speed centrifugation, the virus was sedimented with high-speed centrifugation, resuspended in 0.01 M phosphate buffer, pH 7.2, and clarified by a low-speed centrifugation. Centrifugation speeds and times were equivalent to those of Lastra and Munz (12). Final purification was achieved by rate-zonal centrifugation in 10-40% sucrose gradients in 0.01 M phosphate buffer for 2.5 hr at 23,000 rpm. The virus was recovered from three bands with an ISCO density gradient fractionator (Instrumentation Specialties Co., Lincoln, NE 68505) and sedimented by high-speed centrifugation. A specific extinction coefficient of $E_{260}^{0.1\%} = 7.7$ was used to quantitate the virus.

Specific antisera were produced by injection of a rabbit as follows: intramuscular injection of 2 ml of virus (1.07 mg/ml) emulsified with Freund's complete adjuvant (Difco), five intravenous injections of 1 ml at 1-wk intervals, exsanguination and separation of the serum 15 days after the last injection. The serum was stored frozen. The titer of the antiserum was 1,024 when tested against SMV at 0.08 mg/ml in standard Ochterlony gel double-diffusion plates and there was no reaction with host sap. For routine use in confirming virus infection, the antiserum was used at 1:40 dilution.

A micro double-diffusion test was used to detect virus in small sections of tissue. In this modification of the standard test, wells were 1 mm in diameter and four peripheral wells were placed about 2 mm from the central well. Each well had a capacity of 2.5 μliters and was charged by use of a fine capillary pipette and a dissecting microscope. The antiserum was used at a 1:40 dilution and reactions were observed 2-3 hr later with a dissecting microscope. As many as 160 separate reactions could be done in one petri plate.

Fluorescent antibody preparation.—The globulin fraction was precipitated from the antiserum with ammonium sulphate and residual sulphate was removed by dialysis against 0.85% NaCl as described by Spendlove (22). Conjugation of immunoglobulin with FITC was done by the dialysis method (3). The globulin concentration was determined spectrophotometrically using a $E_{280}^{0.1\%} = 1.8$. The conjugated globulin was separated from noncoupled FITC by passage through a column of Sephadex G-50 (Pharmacia Fine Chemical, Piscataway, NJ 08854) and used to locate SMV by the direct method (4). To minimize nonspecific staining, the conjugate was absorbed with acetone-extracted plant powder (5). Normal serum globulins used as control also were conjugated by the same procedure.

Separation and staining of protoplasts.—A small tip of expanded cotyledons from 6-day-old germinated seed was cut off and tested serologically. Protoplasts from infected or healthy seedlings were extracted by rubbing Carborundum-dusted cotyledons and soaking them in mannitol (1). One gram of cotyledons was further treated under vacuum in a shaking bath at 30 C for 2.5 hr with 20 ml of a mixture of 1.5% Macerozyme and 0.5% Cellulase "Onozuka" (Japan) and filtered through a double layer of cheesecloth to remove leaf material. The protoplasts were washed three times in 0.6 M mannitol, placed on glass slides coated with Mayer's adhesive (9), dried at 37 C for 15 min, treated with 95% alcohol for 10 min, and washed with phosphate-buffered saline (PBS) for 30 min. Three drops of the FITC-conjugate were added and the slides were incubated in a moist chamber at 37 C for 1 hr. Excess conjugate was washed off in PBS for 2 hr; the protoplasts were mounted in glycerine-PBS (1:1) and examined with a UV microscope.

Sections from infected seed and seedlings.—Infected embryos identified by the half-embryo test were sectioned in paraffin and stained with FITC-conjugate according to the procedure described by Nagaraj (14). Cotyledons, hypocotyls,and roots of infected embryos 1 to 8 days after germination were also prepared by this same procedure.

Comparison of ovule and pollen transmission.—In 1974 two rows (10 m apart) of 10 plants each were grown in the field. All plants of one row were inoculated with SMV at their cotyledonary stage and later showed SMV symptoms. Male and female gametes of these plants will be referred to as "infected" although individual pollen grains or ovules might have been free of the virus. The noninoculated plants remained healthy throughout the season. When the plants reached the fertilization stage, all four types of crosses were done by the standard plant breeding technique that involves emasculation of perfect flowers and covering the stigma with a gelatin capsule the day before blooming, and manual application of pollen from the desired staminate flowers on the day of blooming. To prevent mechanical transmission of virus, pollen was tapped from the staminate flowers onto the pistil and hand contact was avoided by holding healthy plants with tissue paper. All healthy plants were indexed on cantaloupes twice during the season and tested serologically. Fruits were indexed on cantaloupe at

harvest and confirmed to be virus-infected or virus-free as indicated in the experimental design.

RESULTS

Cucumis metuliferus as local lesion host.—In three trials, the number of lesions produced by different concentrations of purified virus were inversely related to the dilution, showing the reliability of *C. metuliferus* as a local lesion host of SMV (Table 1).

Seed transmission in infected seed lots.—Seed transmission of SMV in 62 seed lots from infected plants ranged from 0% to 34.6% with an average of 10.6%. There was no correlation between the amount of seed transmission and flowering or seed-production factors such as the weight of the fruits, the number and weight of the seed, or the time from virus inoculation to fruit set (Table 2). Seed germination was not affected by virus infection.

A trial was done to determine whether some symptomless seedlings in the plant assay might have had masked symptoms. Thirty-five 25-day-old seedlings from infected seeds showing conspicuous symptoms of SMV and 35 symptomless seedlings from the same seed lot each were tested serologically. None of the symptomless seedlings reacted, but all seedlings with symptoms formed strong precipitation reactions. This demonstrated that the visual observation of symptoms on seedlings was an accurate way to judge infection. The seed lots with about 30% seed transmission were selected for use in further experiments on distribution of virus in the seed.

Comparison of half-embryo, seedling, and plant assay.—To compare methods of assay utilizing the same individuals, embryos were dissected out, washed in running tap water, divided transversely and individually tested by half-embryo assay and seedling assay or by half-embryo assay and plant assay.

Half-embryo assays of 115 embryos showed that 33 (28.7%) were infected. Plant assays of the germinative ends of these same 33 embryos showed that each of the 31 that survived and produced a plant was infected. Three (4.2%) of 71 germinative ends that were negative in the half-embryo assay produced infected plants in the plant assay. Similarly, 128 embryos were used to compare the half-embryo assay and the seedling assay. Each of the 32 seeds (25%) that was positive in the half-embryo test germinated and was positive in the seedling assay. Of the 96 embryos that were negative in the half-embryo test, 88 germinated and 6 (6.8%) were infected in the seedling assay.

Distribution of SMV in the seed and seedling.—The distribution of SMV in infected seeds was determined by assaying the dissected, washed parts of seeds. Seed coats from 10 seeds were combined, finely chopped with a razor blade, triturated with a mortar and pestle, and inoculated onto cantaloupe plants. Squash mosaic virus was detected in each of three such assays. Because of the difficulty in triturating the leathery seed coats, no attempts were made to assay individual seed coats.

In another trial seed coats were removed and the decoated seeds were acid-treated before separation into papery layers and entire embryos that were washed and assayed individually. Six of the papery layers removed from 20 infected embryos and four of the papery layers removed from 20 virus-free embryos were virus-infected.

The influence of the infected papery layer on the detection of SMV in seed was tested in two trials. In the first trial, papery layers of seeds from a seed lot with approximately 30% seed transmission or from a lot with 0% seed transmission were tested individually on *C. metuliferus*. In the former lot five of 25 papery layers were infected with SMV, whereas in the latter lot three of 25 papery layers were infected. The second trial compared the amount of seed transmission from (i) embryo vs. plant assays, (ii) washed embryos vs. embryos with papery layer, and (iii) seed lots with high vs. others with no transmission. The seed lot with high transmission assayed about 30% infected regardless of the type of assay or the presence of the papery layer (Table 3). The lot with no seed transmission assayed 6% infected if the papery layer was included in the embryo assay.

TABLE 1. Effect of concentration of squash mosaic virus on number of local lesions formed on *Cucumis metuliferus* inoculated with purified virus

Virus concentration (mg/ml)	Local lesions per half leaf[a] (no.)
1.00	76
0.50	46
0.25	17
0.12	6
0.06	2
0.03	0

[a]Average of 18 replications in three trials.

TABLE 2. Lack of correlation between percentage of transmission of SMV in 62 seed lots and fruit weight, number of seeds per fruit, seed weight, time of fruit setting after inoculation, and percentage of germination

			Average values for the respective seed lots					
Percentage of seed transmission	No. seed lots	No. seeds tested	Weight/fruit (gr)	Seeds/fruit (no.)	Total seed wt/fruit (gr)	Seed wt (mg)	Time of fruit set after inoculation (days)	Germination (%)
0-5	19	2,280	808.4	331	5.5	16.7	61	84.7
5.1-10	16	1,920	833.0	403	8.8	22.0	51	86.9
10.1-15	14	1,680	826.9	363	6.7	18.5	60	87.5
15.1-35	13	1,560	833.2	334	7.1	21.2	50	86.4

To determine the location of SMV in germinating seeds, washed embryos were excised from a seed lot with a high rate of transmission. Infected embryos were detected by the half-embryo assay and their corresponding half-embryos were germinated. An 8-10 mg piece of cotyledon was removed from each seedling, triturated in buffer and assayed on *C. metuliferus*. The embryos had been timed to germinate so that on the day of assay three replicates of each age from 0 to 7 days could be tested at the same time. This was repeated five times. The virus concentration began to increase after the second day of germination (Fig. 1) and was significantly greater by the 4th day when cotyledons had changed from a pale-yellow to greenish color. Additionally, seedlings that had been timed to germinate 5, 6, or 7 days earlier were separated into cotyledons, hypocotyls, and roots. Each component from each of five replicate seedlings for each age of seedling was ground in a mortar with 5 ml of buffer per gram of tissue and the homogenate was inoculated to *C. metuliferus*. By the 5th day after germination, SMV was distributed throughout the seedling, including cotyledons, hypocotyls, and roots (Table 4). The relative concentration of virus was higher in the cotyledons than in the hypocotyls or roots.

To detect SMV in infected embryos and seedlings, protoplasts from cotyledons and microtome sections of dry embryos or seedlings were stained with FITC-labeled SMV-antibodies. The following controls were used to establish that any observed fluorescence was due to the specific reaction of SMV antigen with the conjugated antiserum: (i) SMV-infected protoplasts and sections were treated with SMV antiserum for 15 min and then with conjugated antiserum; (ii) healthy material was treated with conjugated antiserum, and (iii) infected material was treated with conjugated normal serum. No specific fluorescence was detected in sections of cotyledons from embryos germinated from 0 to 3 days, or in sections of hypocotyls or roots from 7-day-old seedlings. Only in sections of cotyledons of 6-day-old seedlings was the presence of SMV antigen detectable by the yellow-green fluorescence distributed in clusters of cells in epidermal, palisade, and spongy mesophyl tissue. The number of cells or the site of fluorescence within individual cells could not be determined. The number of infected cotyledonary cells was determined by counting the number of infected protoplasts among those liberated from the infected cotyledons of 8-day-old seedlings. Antigen was identified by the presence of discrete masses of fluorescence at randomly distributed spots in the cytoplasm. Only a small proportion of protoplasts were infected with the virus in five experiments (Table 5).

To confirm results on the distribution of virus obtained by the use of local lesion assays and immuno-fluorescence, detection of the virus was attempted using small pieces of nongerminated or germinated embryos triturated in two or three drops of buffer and tested by the micro double-diffusion method. No visible reactions were observed when nongerminated embryos or 1- to 2-day-old germinated cotyledons were tested. The presence of SMV could be consistently detected, however, in 3-day or older cotyledons. To determine the presence of the virus in other components of germinated embryos, infected hypocotyls and roots were transversely sectioned into pieces 2-mm long that were triturated in one drop of buffer and tested as above. No reactions were obtained in any case. Additionally, a test was done to determine if the virus was distributed evenly throughout the cotyledons. A

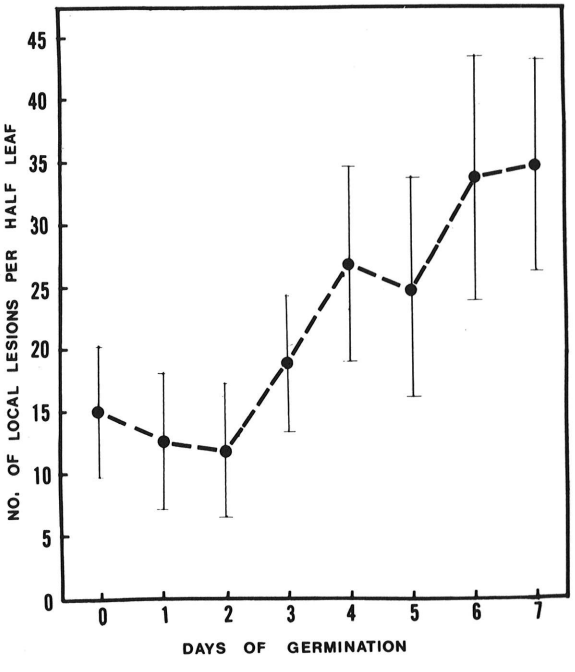

Fig. 1. Squash mosaic virus detected in cotyledons of germinating, infected cantaloupe embryos by local lesion assays on *Cucumis metuliferus*. Each point represents 15 assays from five trials; the vertical bars indicate standard deviation.

TABLE 3. Influence of the papery layer derived from endosperm and nucellus on the detection of squash mosaic virus (SMV) in cantaloupe seeds when comparing embryo and plant assays

	Ratio no. with SMV/no. tested			
	Naked embryos		Embryos with papery layer	
Rate of transmission	Embryo assay	Plant assay	Embryo assay	Plant assay
30%	17/50	13/48	15/48	13/45
0%	0/49	0/44	3/50	0/50
Healthy control	0/10	0/10	0/10	0/10

cotyledon from each of 15 infected 6-day-old seedlings was cut into three 3 × 4 mm blocks. One block from each seedling was taken at random and sliced into a least 30 sections, 30 μm thick, by means of a hand microtome. The sections were ground individually in approximately 2.5 μliter of buffer in a plexiglass composite mortar with 2-mm-diameter cavities and tested serologically. All slices from the 15 infected cotyledons reacted positively, indicating that all parts of the cotyledons were infected with the virus.

Comparison of pollen and ovule transmission.—Pollen was collected from flowers of SMV-infected Small Sugar pumpkin plants that produce more pollen than cantaloupe flowers and in which there is seed transmission of SMV (15). This pollen was externally decontaminated by sedimenting it four times by low speed centrifugation (1,200 rpm for 5 min) and resuspending it each time in buffer. The washed pollen was triturated with a pestle in a mortar to which 0.1-mm diameter glass beads were added. The supernatants and pollen slurry were assayed on cantaloupes. Although SMV was detected in some of first supernatants, it was not detected in the second, third, or fourth supernatants or the pollen in three attempts.

Each of the 35 individual seed lots from the four possible crosses between healthy and diseased cantaloupe plants was indexed by planting 100 acid-treated seeds in the greenhouse (Table 6). About 7% seed transmission resulted from ovules on infected plants regardless of the pollen source but no transmission was detected when ovules on healthy plants were pollinated with pollen from either infected or healthy plants.

TABLE 4. Number of local lesions formed on *Cucumis metuliferus* inoculated with homogenates of cotyledons, hypocotyls, or roots from 5- to 7-day-old germinated seeds of cantaloupe infected with squash mosaic virus

Seedling tissue inoculated	Number of local lesions[a] Days of germination 5	6	7
Cotyledons	17	13	23
Hypocotyls	3	2	7
Roots	5	7	3

[a]Average number of lesions produced on four half-leaves; five replicates.

TABLE 5. Frequency with which squash mosaic virus (SMV) antigen was detected in protoplasts from virus-infected cantaloupe cotyledons stained with anti-SMV-fluorescein conjugate

Experiment No.	Protoplasts Observed (no.)	Fluorescent (no.)	Protoplasts showing fluorescence (%)
1	451	101	22.4
2	343	66	19.2
3	444	57	12.8
4	268	51	19.0
5	381	61	16.0
Average	377.4	67.2	17.9

TABLE 6. Test for pollen and ovule transmission of squash mosaic virus (SMV) in controlled pollinations between healthy and SMV-infected cantaloupe plants

Crossing combination[a]	Seed lots (no.)	Seeds tested (no.)	SMV seed transmission (%)
H♀ × H♂	9	900	0.0
H♀ × I♂	12	1,200	0.0
I♀ × H♂	7	700	8.4
I♀ × I♂	7	700	6.0

[a]Abbreviations: H = healthy; I = infected; ♀ = ovule parent; ♂ = pollen parent.

DISCUSSION

The limited information regarding host-virus relationships in the seed transmission of SMV and the virus distribution and concentration in seeds and seedlings has been caused in part by the lack of a reliable local lesion host. Our results confirm that *C. metuliferus* develops local lesions about 5 days after inoculation with SMV (11, 19) except we found the reliability of the reaction was improved by keeping the inoculated plants in a growth chamber at 20-22 C rather than in the greenhouse.

There was good agreement between the three methods we used to detect SMV transmission in seeds. Embryos shown to be infected by the half-embryo test consistently gave positive results in the plant or seedling assays. A few embryos that were negative for SMV in the half-embryo test were positive in the plant or seedling assay. These are considered to represent failure of the half-embryo test to detect small amounts of virus. We did not confirm the results of Powell and Schlegel (17); those results predicted there should have been a higher rate of SMV detection in the seedling assays (triturating 6-day-old seedlings and sap-inoculating to an assay plant). Although these authors removed the seed coat and papery layer in their seed assays, it is conceivable that infected papery layers carried on the cotyledons of 6-day-old seedlings contributed to the higher rates of SMV detection in their seedling assays. Alternatively, their isolate of SMV may have differed from ours. Our conclusion,like that of Schippers (21) with bean common mosaic virus in bean and of Frosheiser (7) with alfalfa mosaic virus in alfalfa, is that SMV is in infected embryos and that the percentage of seed transmission is determined by the percentage of infected embryos. Embryonic transmission of SMV is in contrast to the situation with tobacco mosaic virus that is readily isolated from seed coats but not from mature, externally decontaminated embryos (23). The seed coat and papery layer of seeds produced by infected ovaries may contain SMV independent of that in the embryos. Although the role of infected papery layer in seed transmission is unknown, it can affect results of embryo assays for SMV if it is not completely removed.

Infectivity assays were the most useful method to detect SMV in germinating embryos and to find the organs with highest concentration of SMV. The virus titer in cotyledons began to increase after the second day of germination and was greater than the titer in the roots or hypocotyls between days 5 and 7. The other tests were not as sensitive; SMV was not detected in cotyledons of germinating seed until the 3rd day of germination by the micro double-diffusion test or until the 6th day by the immunofluoresence test. After 6-8 days, SMV had multiplied and was generally distributed throughout the cotyledons. Although only about 20% of the protoplasts extracted from such cotyledons were infected by SMV in the immunofluorescence test, the seedling should be regarded as systemically infected. These results confirm the results from the radioautography study that showed SMV increased in localized parts of the cotyledon (18). Nevertheless, our assays for seed transmission of our isolate of SMV do not support their hypothesis that the probability of an infected plant arising from an infected seed is determined by the number, location, and/or activity of infected cells in the seed.

Few of the seed-borne viruses have been demonstrated to be transmitted through pollen as well as through the ovule (2, 7). In some cases pollen transmission does not occur because infected pollen is not functional (25). This pattern was not observed with SMV-infected pollen which yielded successful crosses. We conclude that SMV is transmitted through the ovule of cantaloupe and is either not pollen-borne or only so infrequently pollen-borne that it was not detected in our tests. Rader et al. (20) detected SMV in cantaloupe pollen but it is likely that they did not wash the pollen to remove surface contamination.

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