Factors Influencing Seed Transmission of Squash Mosaic Virus in Cantaloupe

C. C. Powell, Jr., and D. E. Schlegel

Department of Plant Pathology, University of California, Berkeley, California 94720. The senior author is now a Research Plant Pathologist, ARS, USDA, Oregon State University, Corvallis 97331.

Supported in part by a National Science Foundation terminal-year graduate traineeship and USPHS Research Grant AI 03415 from the National Institute of Allergy and Infectious Diseases.

Accepted for publication 29 April 1970.

ABSTRACT

Direct seed assay of 50 seed lots from infected cantaloupe plants indicated that 12% of the seeds contained detectable amounts of squash mosaic virus (SqMV) in their embryos, but assay of seedlings indicated that 22% contained virus. In spite of this apparent increase in virus as the seedlings germinated, the observation of 30-day-old plants resulting from seeds of the diseased seed lots showed 13% virus transmission with no detectable symptomless, but infected, plants. Melon wt, size, seed number, seed wt, and percentage of germination were significantly reduced by SqMV infection of the parent plant, but no correlation was found between these reductions and the variable infection percentages in each seed lot. The percentage of virus transmission was about the same in all fractions of seed lots separated on the basis of seed wt. The proportion infected declined from 23 to 5% during 2-year seed storage. Such results indicate that the seed transmission of this virus is determined not only by viral invasion of the seed, but also by the successful maintenance of the host-pathogen relationship during seed formation, storage, and germination. Phytopathology 60:1466-1469.

Squash mosaic virus (SqMV) is found throughout California (13), despite the fact that it has a narrow host range (9). The continued use of virus-infected seed is no doubt responsible for this distribution (14). Details of the virus-host interactions which result in the production and survival of virus-infected seeds are generally unknown. Such knowledge is, however, essential for studies on the control of seed-borne virus diseases. The present study details conditions contributing to the incidence, appearance, and behavior of infected seeds, and was undertaken as a prerequisite to a histological study of the nature of seed infection by SqMV.

MATERIALS AND METHODS.—The virus isolate used in these studies was obtained from a cantaloupe field near Yuba City, Calif. It produces distinctive ringspot symptoms on zucchini squash (*Cucurbita pepo* L.), and is probably closely related to a virus originally termed "muskmelon mosaic virus" or "cucurbit ring mosaic virus" (8) and later determined to be a strain of SqMV (15). These symptoms on squash are similar to those caused by strains reported to cause stunting of watermelon (17). Repeated inoculations of watermelon (*Citrullus vulgaris* Schrad. 'Market Midget'), however, produced only a localized necrotic reaction similar to that reported by Demski (4) and Grogan et al. (13).

Production of infected seed.—Cantaloupe (*Cucumis melo* L. 'Rocky Ford', furnished by Ferry-Morse Seed Co.) was used throughout this study. Virus-infected seed was produced by inoculating 10-day-old greenhouse-grown cantaloupe seedlings and transplanting them to field areas in San Jose and Davis, Calif. The seeds from each melon were harvested separately 90 days after inoculation and are referred to hereafter as one seed lot.

Assay of percentage seed infection and disease transmission.—The percentage of virus-infected seed in individual seed lots was determined using 25 seeds/sample. The seeds were soaked in water overnight and the seed coats and integuments removed. Single embryos were ground in a mortar with approximately 10 volumes of a 0.1%-K_2SO_3 solution, and the resulting suspension was used to inoculate Carborundum-dusted cotyledons of 7-day-old zucchini squash. Entire 5-day-old seedlings were homogenized in 10 volumes of 0.1% K_2SO_3 and used to inoculate zucchini squash plants as an additional assay.

The actual percentages of virus transmission were determined by random planting of 90 seeds from each seed lot in greenhouse flats. The resulting plants were examined for symptoms of SqMV infection 30 days after planting. In early experiments all the plants were assayed on squash to check for latent transmission of SqMV, but no latent infections were detected.

Germination of the seeds.—Germination of the seeds was carried out by placing 30 seeds between paper germination discs in a petri plate and adding 8 ml of water. Three such plates were prepared for each seed lot tested, and placed in an incubator at 27 C for varying periods of time.

Correlation and regression analysis.—Relative melon wt, diam at greatest girth, position on the vine, and seed number were determined for each seed lot at time of harvest. Average wt per seed, percentage germination, average hypocotyl-root length at the time the max germination occurred, the variability of hypocotyl-root lengths within any one seed lot at the time of max germination, and the days required to reach max germination were determined after drying and cleaning the seeds. A two-dimensional correlation and regression study of the percentage of virus infection vs. the above factors were undertaken. The seed lots from virus-infected plants were categorized so that analyses could be done on seed lots from a single plant as well as the over-all data. Melons from five plants were thus analyzed. Four data transformations (arithmetic-arithmetic, arithmetic-log, log-arithmetic, and log-log) were analyzed for each data set. A CDC 6400 computer

(Univ. Calif. Computer Center, Berkeley) was used for these computations.

RESULTS.—The importance of surface contamination in the seed transmission of SqMV was assessed by treatment of seeds from infected plants with 10% trisodium phosphate for 1 hr (11). In two experiments involving 35 seed lots, the average level of virus transmission was 7.0% for the treated seed and 7.6% for the untreated seed. When homogenate prepared from SqMV-infected cantaloupe leaves was poured over a sample of healthy seed and allowed to air-dry, 1.5 to 5.5% seed transmission resulted. There was, however, no virus transmission following treatment of these seeds with trisodium phosphate. Thus, seed transmission of SqMV was not due to simple surface contamination.

Percentage of virus transmission and percentage of virus-infected seed of individual seed lots.—The proportion of the seeds transmitting virus in the above 35 seed lots varied from 0 to 50%. Therefore, the relationship between the percentage of virus-infected seed and the percentage of virus transmission was investigated in an attempt to determine if this variability were a result of the assay method. A group of 50 seed lots from diseased plants was assayed for virus in three ways: (i) mechanical assay of seed samples; (ii) mechanical assay of seedling samples; and (iii) observation of the numbers of diseased plants arising from samples of planted seed. The average reading obtained from all the seed lots assayed by either the mechanical seed assay (i) above, or the planted seed assay (iii) above, are equivalent at 12 and 13%, respectively (Fig. 1). Such a result could lead to the conclusion that seeds originally infected with some critical level of virus are those that result in infected progeny. But the fact that mechanical assay of seedlings (ii) above, exhibited almost twice the average infection (Fig. 1), as well as the fact that the averages for all three assays are derived from significantly different sample distributions (above 0.95 by F-tests), suggests that seed transmission is only indirectly related to the amount of virus in individual seeds. In addition, the wide range of assay percentages occurred regardless of the assay method used.

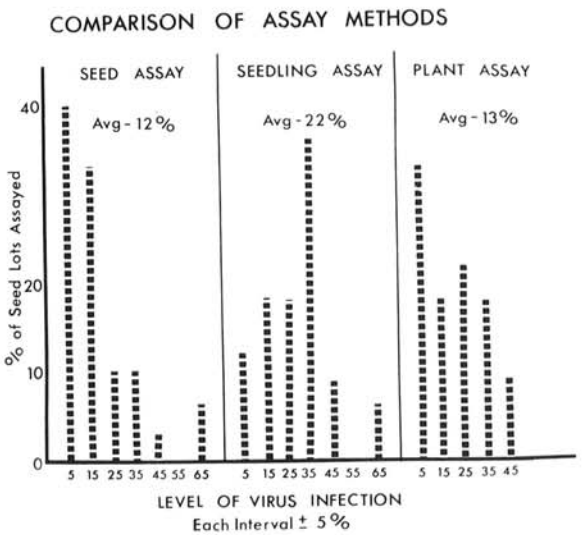

Fig. 1. Grouped frequency distributions of the percentage of squash mosaic virus infection in 50 cantaloupe seed lots assayed in three different ways: (i) *Seed assay:* ungerminated seed ground in buffer and used to inoculate zucchini squash; (ii) *Seedling assay:* 6-day-old seedlings ground in buffer and used to inoculate zucchini squash; (iii) *Plant assay:* seeds from each seed lot planted, and the level of virus infection determined by the production of symptoms on the resulting plants. Each vertical bar represents the group of seed lots showing virus infection at the level indicated on the horizontal scale. The exact virus assay levels obtained were grouped into 10% intervals prior to tabulation.

The relationship of virus infection to melon size, seed yield, and various seed germination properties.—Melon diam, seed number, wt per seed, and percentage of germination were significantly reduced in fruits and seeds from virus-infected plants (Table 1); however, root growth and germination rates were not affected.

TABLE 1. Melon size, seed yield, and various seed germination properties from squash mosaic virus-infected and healthy cantaloupe[a]

Factor	Infected plants		Healthy plants		% Reduction	Significance[b] level
	Avg	*SD*[d]	*Avg*	*SD*		
Melon wt	329.00 g	124.00	385.00 g	106.00	15	.995
Melon diam	7.80 cm	.96	8.89	.72	13	.999
Melon seed no.	342.00	103.00	371.00	77.00	9	.950
Avg wt/seed	14.10 mg	4.2	17.10 mg	5.70	18	.999
% Germination	67.00	31.00	81.00	26.00	17	.995
Avg length of seedling root at max germination	2.61 cm	.77	2.51 cm	.71		
Days to reach max germination	4.00 days	1.50	4.10 days	1.30		
Variation of root lengths at max germination[c]	1.10 cm	.62	.88 cm	.49		

[a] Fifty melons from 10 diseased plants and 50 melons from 10 healthy plants included in the average readings.
[b] F-test ratios.
[c] Expressed as the average standard deviation of the average lengths for each sample of seedlings analyzed.
[d] Standard deviation.

Since the observed reductions could have resulted from the generally diseased condition of the plants with no relationship to actual virus invasion of the seeds, each seed lot was subjected to a correlation and regression study to relate each of the eight parameters listed in Table 1 with the levels of virus-infected seed (as determined by each of the three assay methods given above). No consistently significant correlations were detected.

Analysis of seedling assays of seed lots from all plants indicated that the melons closer to the crown of the plant may contain fewer virus-infected seeds (Table 2). This difference was significant at the 0.9 level from the proximally positioned melons only, and did not appear when the data from individual plants was analyzed separately. Melon position would seem to have little effect on the seed, since seed germination did not differ among the groups.

Seed wt and transmission of SqMV.—A further study was undertaken to see if higher rates of seed transmission could be demonstrated in the lighter-wt fractions of seed from any one seed lot. Thirty-five infected seed lots were separated into thirds on a wind tube seed fractionator (E. L. Erickson Products, Brookings, S. D.). Although the percentage of germination was 99% in the heaviest third, 96% in the middle third, and 69% in the lightest third, the percentage of seed transmission was practically unchanged at 4.6, 6.2, and 6.9% for each third, respectively. Correlation studies of these data on an individual seed-lot basis further substantiated this lack of significance.

Seed storage and virus transmission.—That the relationship of SqMV to the seed could be altered by manipulation of the seed environment after harvest but prior to germination was illustrated by the fact that seed transmission generally declined with storage of the seed. In 1967, the average germination of 13 randomly selected seed lots was 81% (range 47-100%), with 23% transmission of virus (range 11-43%). After 2 years' storage in paper bags under laboratory conditions (temp range: 21-27 C), the seed lots again averaged 81% germination (range 50-99%), but with only 5% virus transmission (range 0-17%). The percentage of virus transmission in only one seed lot remained essentially unchanged, 15% in 1967 and 17% in 1969. Seven seed lots which showed 11-31% transmission in 1967 showed no transmission in 1969.

TABLE 2. The average percentage of virus and percentage of germination assayed in seedlings from seed lots grouped according to the position of the melons on the vine

Position of melon on vine[a]	% Virus assayed[c]		% Germination	
	Avg	*SD*[d]	*Avg*	*SD*
Distal[b]	21.4	18.8	78.0	14.8
Central	26.1	19.4	76.5	21.4
Proximal	14.5	11.3	75.1	21.4

[a] Distal = melons on distal portion of vine; central = melons on central portion of vine; proximal = melons nearest crown of plant.
[b] Each group consisted of 13 melons.
[c] 300 seeds were assayed in each group.
[d] Standard deviation.

Fruit maturity at the time of inoculation of the parent plant and the resulting percentages of seed transmission.—Melons continue to grow and produce fruit throughout the summer. By inoculating at one point on a vine and recording the stage of development of the fruit and flowers along the vine, it was possible to determine whether virus could invade seeds of fruit that had already begun development. Seed lots exhibiting 1-4% virus transmission were obtained from fruit formed but not yet fully matured at the time of inoculation. Such seed lot infection only occurred when inoculations of the parent plants were made on the older leaves in the position on the vine which bore opened female flowers or very small fruit. Assays of leaf preparations from all of the parent plants shortly after inoculation and again at harvest indicated that the plants were initially healthy and did become infected, but detailed studies on the time of viral invasion of seeds were not made.

DISCUSSION.—Previous studies on the seed transmission of SqMV in melons (16, 18) have indicated a great variability in percentage of virus transmission from seed lot to seed lot. Such variability was also found in this study and was exhibited both in actual virus transmission measurements and in assays to determine percentages of infected seed and seedlings. The reasons for such variability remain unknown. The failure to correlate virus transmission levels with such factors as percentage of germination or seed wt may be because virus-infected seeds are able to mature, germinate, and survive just as well as the virus-free seeds of a seed lot from a diseased plant. Indeed, Diaz-Polanco et al. (5) have recently reported that SqMV-infected squash plants survived longer than virus-free plants when they were inoculated with *Fusarium solani* f. sp. *cucurbitae* race 1. In any case, control measures involving rouging melons or seed based on these factors would not seem possible.

The present study indicates that although the percentage of virus transmission declined with storage of the seed, the magnitude of this decline varied from seed lot to seed lot. Such a variable inactivation or destruction of virus could account for some of the variability in seed transmission mentioned above, if one can assume that it also occurs as the seed matures. Several viruses normally not seed-transmitted have been shown to be inactivated in the seed as it matures (1, 7, 10). Furthermore, a low percentage of the seed of very young melons can apparently be invaded by SqMV after fruit set. This phenomenon, which is analogous to findings of Eslick & Afanasiev (6) and Crowley (3) for barley stripe mosaic virus, could contribute to variable levels of infection from seed lot to seed lot.

Aside from studies with nonseed-transmitted viruses which can be recovered from immature but not mature seeds (1, 7, 10), only Schippers (19) has compared assayable virus in the seed with the level of infection obtained by planting out the seeds. He reported that the percentage of bean seed with assayable bean common mosaic virus was about equal to the percentage of virus transmission through seedlings, indicating

that the main factor determining the percentage of virus transmission was whether or not the seed was infected. Whereas such a conclusion may be true for that virus, the present results indicate that the interactions of host and virus as the seed germinates and the resulting plant matures may be of equal or even more importance in determining the success or failure of SqMV transmission in melon seed.

An obvious hypothesis to explain the increase in percentage of infected seedlings from infected seed is that the virus is multiplying as the seed germinates. Gilmer & Wilks (12) suggested this possibility in a recent study of tobacco mosaic virus in apple seed. Another hypothesis is that an inhibitor to mechanical inoculation may have been present in the seed but not in the seedling (2). Likewise, the presence of in vivo inhibitors or inactivators in older plants could explain why the seedling assay indicated higher viral infection levels than the actual levels of virus transmission obtained. It is also possible that the virus was restricted to certain tissues or cells in some seedlings and was unable to spread into the developing plant, in spite of apparent multiplication in seedling cells. Further histological studies are underway on the mechanisms responsible for the apparent virus increase which takes place in many more seedlings than eventually produce infected plants.

LITERATURE CITED

1. Cheo, P. C. 1955. Effect of seed maturation on inhibition of southern bean mosaic virus in bean. Phytopathology 45:17-21.
2. Crowley, N. C. 1955. The effect of seed extracts on the infectivity of plant viruses and its bearing on seed transmission. Australian J. Biol. Sci. 8:56-67.
3. Crowley, N. C. 1959. Studies on the time of embryo infection by seed transmitted viruses. Virology 8:116-123.
4. Demski, J. W. 1969. Local reaction and cross protection for strains of squash mosaic virus. Phytopathology 59:251-252.
5. Diaz-Polanco, C., S. M. Smith, & J. G. Hancock. 1969. Effect of virus infection on stem rot of squash caused by *Fusarium solani* f. sp. *cucurbitae*. Phytopathology 59:18-22.
6. Eslick, R. F., & M. N. Afanasiev. 1955. Influence of time of infection with barley stripe mosaic on symptoms, plant yield, and seed infection of barley. Plant Dis. Reptr. 39:722.
7. Ford, R. E. 1966. Recovery of pea streak virus from pea seed parts and its transmission by immature seed. Phytopathology 56:858-859.
8. Freitag, J. H. 1952. Seven virus diseases of cucurbits in California. Phytopathology 42:8 (Abstr.).
9. Freitag, J. H. 1956. Beetle transmission, host range, and properties of squash mosaic virus. Phytopathology 46:73-81.
10. Gay, J. D. 1969. Effect of seed maturation on the infectivity of cowpea chlorotic mottle virus. Phytopathology 59:802-804.
11. Gilmer, R. M., & L. J. Kelts. 1968. Transmission of tobacco mosaic virus in grape seeds. Phytopathology 58:277-284.
12. Gilmer, R. M., & J. M. Wilks. 1967. Seed transmission of tobacco mosaic virus in apple and pear. Phytopathology 57:214-217.
13. Grogan, R. G., D. H. Hall, & K. A. Kimble. 1959. Cucurbit mosaic viruses in California. Phytopathology 49:366-376.
14. Kendrick, J. B. 1934. Cucurbit mosaic transmitted by muskmelon seed. Phytopathology 24:820-823.
15. Lindberg, G. D., D. H. Hall, & J. C. Walker. 1956. A study of melon and squash mosaic viruses. Phytopathology 46:489-495.
16. Mahoney, C. H. 1935. Seed transmission of mosaic in inbred lines of muskmelons. Amer. Soc. Hort. Sci. Proc. 32:477-480.
17. Nelson, M. R., J. C. Matejka, & H. H. McDonald. 1965. Systemic infection of watermelon by a strain of squash mosaic virus. Phytopathology 55:1362-1364.
18. Rader, W. E., H. F. Fitzpatrick, & E. M. Hildebrand. 1947. A seed-borne virus of muskmelon. Phytopathology 37:809-816.
19. Schippers, B. 1963. Transmission of bean common mosaic virus by seed of *Phaseolus vulgaris* L. cultivar Beka. Acta Bot. Neerlandica 12:433-497.