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A Strain of Squash-Mosaic Virus and other Cucurbit Viruses Found in Puerto Rico, During 1958–62

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INTRODUCTION

Cucurbit viruses reported from Puerto Rico so far include virus "A" and virus "B", described and characterized by Adsuar and Cruz Miret (*1*)[2]. These authors found virus "B" to be a strain of cucumber virus 1 by cross-protection tests with Price's No. 6 strain in zinnia (*2*) and host-range studies. They found that virus "A" infected only cucurbitaceous hosts and that it had similar dilution and inactivation end-points as virus "B". Since that time no additional reports have been made on new virus diseases of cucurbits in Puerto Rico. Occasional reports have been made on appearance of viruslike symptoms on cucurbits, but no detailed studies of these conditions have been made.

In May 1958, observations made in an experimental plantation of muskmelon *Cucumis melo* L. var. Smith Perfect at the Solís farm led to a preliminary diagnosis of virus disease. The leaves of a large number of plants were mottled showing blisterlike areas of dark-green color and deformation of the younger leaves. Many plants were seen to be stunted (*3*).

The potential commercial value of melons as an export item to the United States during the winter season makes diseases of melons and other cucurbits economically important in Puerto Rico. Studies were therefore carried out to identify the causal agent of the above described disease. In addition, collections of mosaic-diseased plants from cucurbit plantations have been made from 1960 to 1962 in Puerto Rico. This paper reports the studies and observations made up to the present.

MATERIALS AND METHODS

VIRUS SOURCE

The virus from muskmelon was isolated and propagated in *Cucurbita pepo* L. var. Small Sugar and in squash, *Cucurbita pepo* L. var. melopepo Alef

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[2] Italic numbers in parentheses refer to Literature Cited, p. 153.

var. Grey Zucchini. It will be referred to as MMV (melon-mosaic virus) in this paper. For the tests and properties described below, it was usual to use crude sap obtained from plants which had been infected 2 to 3 weeks previously.

HOST RANGE

Forty-five species of plants belonging to fifteen families were tested for susceptibility to inoculation with the isolate. From 5 to 10 seedlings of each species were inoculated. Inoculations were performed by dusting the cotyledons or leaves with carborundum and then rubbing with cotton swabs moistened with infective juice extracted from leaves of Small Sugar pumpkin. Species of *Cucurbitaceae* were usually inoculated in the cotyledonary stage, whereas most of the noncucurbitaceous hosts were inoculated on the leaves. Attempts to recover the virus were made from the noninoculated portions of plants usually about 1 month following inoculation. The recovery tests were made with sap extracted from symptomless leaves and in some instances from leaves of plants showing symptoms. The extracted sap was inoculated to Small Sugar pumpkin and in some instances to squash var. Early Golden Summer.

DILUTION END-POINT

Infective crude juice was obtained from leaves of Small Sugar pumpkin plants which had been inoculated 3 weeks previously. The juice was diluted in previously sterilized cold distilled water to make a range of dilutions from 1/10 to 1/1,000,000.

Dilutions were tested on Zucchini squash plants in the cotyledon stage 7 to 8 days after the seed had been planted. Twenty-five to thirty test plants were used for each dilution. Inoculation was carried out by dipping a sterilized absorbent cotton swab into diluted juice and rubbing it onto the cotyledons which had been dusted previously with carborundum powder. The excess inoculum and carborundum were washed off with sterile water. Readings were made at 5-day intervals for a period of 25 days. In view of the report by Grogan, *et al.* (*4*) that squash-mosaic virus is transmitted through Zucchini squash seed, these experiments were repeated later with Small Sugar pumpkin plants, with similar results.

INACTIVATION TEST

Thermal-inactivation tests were carried out by pouring undiluted infective crude juice into a number of cotton-stoppered sterile Wassermann tubes. Juice from Small Sugar pumpkin or squash var. Early Golden Summer leaves infected 21 days previously was used for the tests. Small Sugar pumpkin was used as the test plant. A tube containing infective juice

was dipped for 10 minutes in a constant-temperature water bath and then quickly removed and plunged into ice water. Temperatures ranged from 45° C. to 80° C. at 5° intervals. After all tubes were cooled, the juice from each was separately inoculated to 50 Small Sugar pumpkin plants. The inoculated plants were observed for a 25-day period.

STABILITY IN FROZEN JUICE, AGING

Undiluted infective crude juice obtained from Small Sugar pumpkin plants as above described was stored in lusteroid tubes at about −10° C. for periods ranging from 4 months to 2 years. To test for stability, tubes were removed from the freezer, the juice was rapidly thawed and inoculated at once on Small Sugar pumpkin plants as described above.

Infective crude juice also was kept in sterile stoppered test tubes in the laboratory at room temperature and tested for infectivity after 2, 4, 7, 14, 21, 30, 45, 60, and 90 days.

SEED TRANSMISSION

Seed were collected from fruits borne by diseased Smith Perfect muskmelon plants from the same plantation where the isolate had been obtained. Seed were also collected from fruit borne by healthy-looking plants. All seedlings from these seed were observed for a period of 21 days after germination for the presence of mosaic symptoms. During the observation period the seedlings were sprayed once a week with Parathion to insure the absence of insects.

PURIFICATION AND SEROLOGY

Leaves from the diseased Smith Perfect muskmelon plants were collected from the plantation at Solís, wrapped in waxed paper, and frozen. The leaves were taken out after 3 days, allowed to thaw, and sap was then extracted by grinding in a food-chopper and squeezing through gauze. The crude sap was then clarified by centrifuging for 20 minutes at 4,000 r.p.m. in a Servall SS-2 centrifuge. The clarified sap was then centrifuged at 30,000 r.p.m. for 90 minutes in a precooled No. 30 rotor of a Model L Spinco ultracentrifuge. The supernatant liquid from this was discarded and the pellets suspended in cold distilled water and placed for a few minutes in the refrigerator; phosphate buffer pH 7.0 was then added to a final concentration of 0.1 molar, so that the final volume of suspension would be 1/20 of the original volume of clarified sap. This suspension was then centrifuged at 3,000 r.p.m., the sediment discarded, and the supernatant liquid centrifuged 2 hours at 30,000 r.p.m. The supernatant liquid was discarded, the addition of distilled water and phosphate buffer to the pellets was repeated, and the suspension centrifuged at 3,000 r.p.m. to re-

move particulate material. The supernatant from this last centrifugation was then distributed in glass vials and frozen. This is preparation MMV-1 below.

Similar preparations were made from healthy Small Sugar pumpkin plants and from plants of this same species separately inoculated with 1, the MMV isolate (preparation MMV-2) and 2, a squash-mosaic virus strain received from the University of Wisconsin[3]. The Wisconsin isolate will be referred to as SSV. All preparations were tested on Small Sugar pumpkin plants for infectivity. All were infectious except the one from healthy Small Sugar pumpkin.

Six rabbits were bled from the ear vein to obtain normal serum. Each infectious preparation was then injected intravenously into two rabbits at 3- to 4-day intervals. The rabbits were bled from time to time and the serum separated and tested to ascertain that antibodies were present. The animals were then bled out and the serum collected and frozen until needed. Precipitin tests were then set up, in which the MMV-1, MMV-2, SSV, and healthy Small Sugar preparations were mixed with the anti-MMV-1, anti-MMV-2, anti-SSV sera, and normal rabbit serum. The preparations were separately mixed with each of the sera in 6- by 50-mm. tubes and incubated at 37° C. Presence or absence of precipitation was recorded at 30-, 45-, 60-, 90-, and 120-minute intervals.

RESULTS

HOST RANGE

The plants found susceptible to MMV were 11 species belonging to the family Cucurbitaceae as follows: Summer or bush squash, *Cucurbita pepo* L. var. melopepo Alef. var. Grey Zucchini, Caserta, and Early White Bush; field pumpkin, *Cucurbita pepo* L. var. Connecticut Field, Small Sugar, and "native" pumpkin (unknown variety); pumpkin, *Cucurbita moschata* var. Kentucky Field and Green Striped Cushaw; winter squash, *Cucurbita maxima* Dutch vars. Blue Hubbard and Improved Green Hubbard; gourd, *Lagenaria siceria* Standl; citron or preserving melon, *Citrullus vulgaris* Schrad. var. *citroides* Bailey; cucumber, *Cucumis sativus* L. var. Black Diamond, Chicago Pickling, Boston Pickling, National Pickling, Marketer, Early Cluster, Davis Perfect, Straight 8, and Improved Early White Spine; West India gherkin, *Cucumis anguria* L.; *Melothria guadalupensis* L.; *Cayaponia racemosa* L.; muskmelon, *Cucumis melo* L. var. Hale's Best, Honey Dew, and Honey Rock.

[3] This strain was kindly supplied by Dr. J. C. Walker from the University of Wisconsin. It was originally isolated from wild cucumber (*Echinocystis lobata*) and found to be a strain of "severe squash mosaic" (7).

SYMPTOMS

Field pumpkin, **Cucurbita pepo** *L. var.*

As observed on several cucurbitaceous hosts the symptoms were as follows: *Cucurbita pepo* L. var. Small Sugar; vein-clearing and mild mottle were observed as early as 5 days after inoculation. After 12 days the second and third leaves showed vein-clearing and mottle, while other leaves were markedly wrinkled, cupped, and distorted. After 19 days the smaller leaves showed malformation with considerable reduction of interveinal tissue. A ringspot mottle usually developed in most of the inoculated plants.

Squash, **Cucurbita pepo** *var. Melopepo Alef. var. Grey Zucchini*

Vein-clearing and mild mottle could be observed on the first true leaf of Grey Zucchini squash as early as 5 days after inoculation. On the 12th day vein-banding and cupping could be observed on the third leaf, while the fourth and fifth leaves showed marked blistering, mottle, and cupping.

Later the leaves became severely malformed, with reduced interveinal tissue; some leaves showed a conspicuous marginal projection of veins. The symptoms on squash were therefore very similar to those produced by Freitag's squash-mosaic virus (*5*).

Winter Squash, **Cucurbita maxima** *Duchesne*

The virus produced mottle and yellow spotting on winter squash. Figure 1 shows mottle and yellow spotting as seen on the fourth leaf of this species 9 days after inoculation.

Cucumber, **Cucumis sativus**

A few days after inoculation chlorotic spots were seen on the inoculated cotyledons on cucumbers. Chlorotic spots were also seen on the second leaf 10 days after inoculation. About 12 days after inoculation, mottle, wrinkling, and cupping of the third and fourth leaves were prominent. On the subsequent leaves a faint inconspicuous mottle was observed.

Muskmelon, **Cucumis melo**

On muskmelons the virus induced vein-clearing with a mild yellow mottle. The second leaf usually appeared blistered and somewhat malformed. In more advanced stages of infection a generalized green mottle could be seen. Field symptoms, as observed in the Smith Perfect variety, were slightly more severe, especially on the younger leaves where dark green blisters on a lighter green background could be observed frequently. In the field there was also more malformation.

West India Gherkin, **Cucumis anguria**

On West India gherkin the virus induced a conspicuous blistering and cupping of the younger leaves, and dwarfing of the plant. The cupping

could first be noticed on the fourth leaf (fig. 2). This species, however, was somewhat resistant to infection by the virus and the symptoms herein reported were produced only on the third attempt at infection, after the first two trials involving a total of 18 plants had proved negative.

Sponge Cucumber, **Luffa cylindrica**

No symptoms at all were produced on the sponge cucumber in 4 different attempts involving 34 plants.

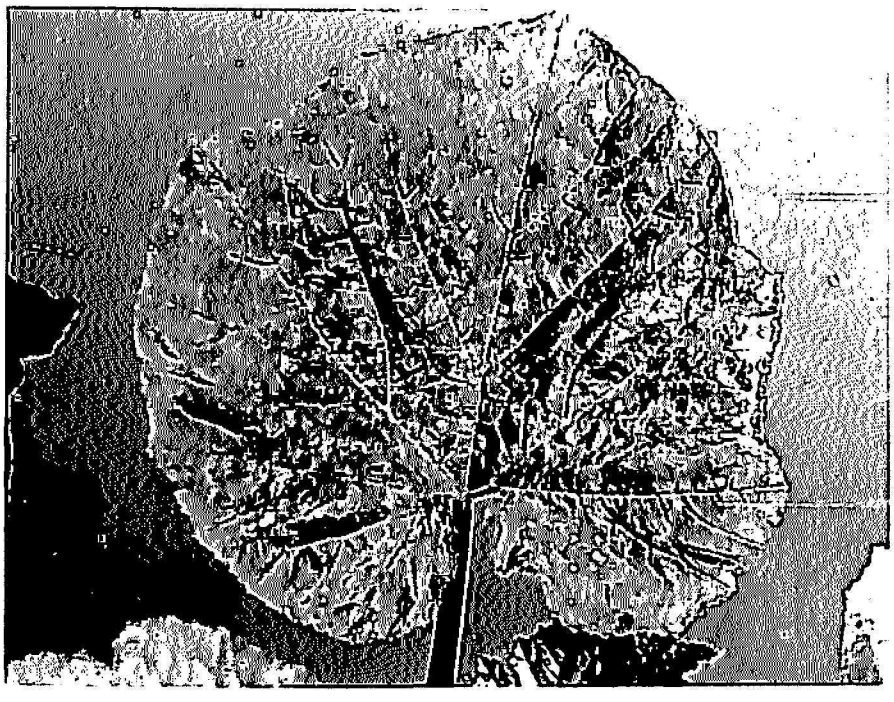

FIG. 1.—*Cucurlita maxima*, 4th leaf: Conspicuous yellow spotting 9 days after inoculation with MMV.

Gourd, **Lagenaria siceria**

Symptoms similar to those produced by Anderson's-"type" muskmelon mosaic were observed upon inoculation of *L. siceria* with MMV (*6*). Anderson's virus was later shown to be a strain of squash-mosaic virus (*7,8*). On this species the virus produced very small, brown, necrotic lesions on the cotyledons (although this reaction was inconsistent) and a severe systemic mottle with distortion and stunt. A conspicuous dark-green vein-banding and blistering was also observed.

Seven inoculation experiments were performed on *L. siceria*, involving a total of 72 plants. In order to compare reactions parallel inoculations with SSV were carried out in four of the experiments. Production of yellow spots on the cotyledons (fig. 3) was consistently observed in all four inoculation experiments with both viruses, while necrotic spots on the cotyledons

were observed in only two of the inoculation tests. Systemic necrotic spotting was obtained in only one test. A very conspicuous and outstanding symptom on *L. siceria* was cupping, blistering, and deformation of the fourth leaf (fig. 4). This was also observed after inoculation with both MMV and SSV. Marginal projection of veins as observed on squash, was also seen on this species.

FIG. 2.—*Cucumis anguria*, close-up of 4th leaf showing cupping and blistering 9 days after inoculation with MMV.

Additional Findings

MMV and SSV were also simultaneously inoculated on the following species: *Cucurbita moschata*, *Cucurbita maxima*, *Cucumis sativus*, *Cucumis anguria*, *Citrullus vulgaris* Schrad., *Luffa cylindrica* Roem. *Cucurbita pepo* var. small sugar, and *Momordica charantia*. Both viruses gave very similar symptoms in all species tested and neither produced visible symptoms or could be recovered from watermelon (*Citrullus vulgaris*), balsam-pear (*Momordica charantia*) and sponge-cucumber (*Luffa cylindrica*).

No infection resulted when MMV was mechanically inoculated in the following 34 species representing 15 families: Amyaceae—coriander,

Coriandrum sativum L.; Amaranthaceae—bachelor's button, *Gomphrena globosa* L.; Apocynaceae—Madagascar periwinkle, *Vinca rosea* L.; Caricaceae—*Carica papaya* L.; Chenopodiaceae—sugar beet, *Beta vulgaris* L. var. Crosby's Egyptian, *Chenopodium album* and *Chenopodium amaranticolor*; Compositae—sunflower, *Helianthus annus* L., zinnia, *Zinnia elegans* Jacq. var. Golden Gem and Scarlet Gem, lettuce, *Lactuca sativa* L. var. Black-seeded Simpson; Cruciferae—cabbage, *Brassica oleracea* L. var.

FIG. 3.—*Lagenaria siceria*, local chlorotic lesions on cotyledons 14 days after inoculation with SSV.

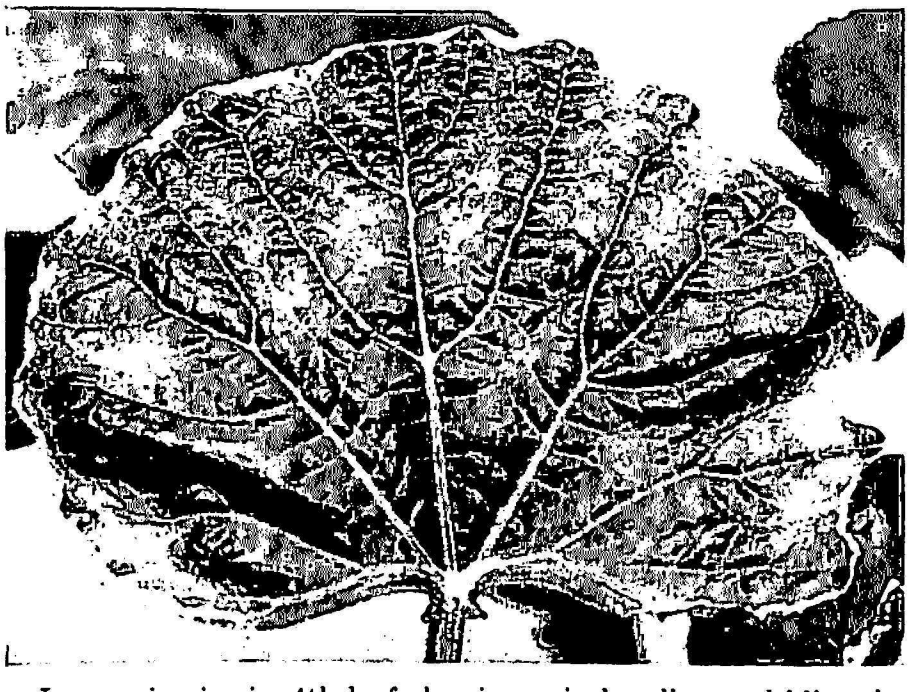

FIG. 4.—*Lagenaria siceria*, 4th leaf showing vein-banding and blistering 14 days after inoculation with MMV.

Copenhagen Market, radish *Raphanus sativus* var. Comet; Cucurbitaceae—sponge cucumber, *Luffa cylindrica*, Roem. balsam-pear, *Momordica charantia* L., watermelon, *Citrullus vulgaris* Schrad. var. Stone Mountain, Dixie Queen, Golden Honey, and Tom Watson; Gramineae—sweet corn, *Zea mays* L. var. Golden Giant Sugar and Mayorbela; Leguminosae—cowpea, *Vigna sinensis* (Torner) Savi var. Black and Blackeye, broadbean, *Vicia faba* L., kidney bean, *Phaseolus vulgaris* L. var. Bountiful and Contender, lima bean, *Phaseolus lunatus* var. Fordhook 242, garden pea, *Pisum sativum* L. var. Thomas Laxton, and Little Marvel; Malvaceae—cotton, *Gossypium hirsutum* L. var. Sea Island; Scrophulariaceae—snapdragon, *Antirrhinum majus* L.; Solanaceae—jimsonweed, *Datura stramonium* L., garden petunia, *Petunia hybrida* var. Comanche, pepper, *Capsicum frutescens* L. var. Yolo Wonder, California Wonder and Large Bell Hot, potato. *Solanum tuberosum* L., tomato, *Lycopersicon esculentum* Mill. var. Marglobe, Manalucie, and Rutgers, tobacco, *Nicotiana tabacum* L. var. Kentucky 16, Turkish, Virginia-12 and White Burley, *Nicotiana glutinosa* L., *Nicotiana rustica* L., eggplant, *Solanum melongena* L. var. Rosita; Umbelliferae—celery, *Apium graveolens* L. var. Easy Blanching and Giant Pascal, carrot, *Daucus carota* L. var. Chantenay.

Dilution End-Point and Inactivation Tests

The results of the dilution end-point tests are shown in the following tabulation:

Dilution[1]	*Plants infected/plants inoculated*
1/10	25/25
1/100	26/27
1/1,000	19/25
1/5,000	18/27
1/10,000	17/27
1/100,000	6/30
1/200,000	10/30
1/500,000	8/30
1/800,000	5/30
1/1,000,000	0/30

[1] Plants observed during a 25-day period. Dilutions made in sterile distilled water.

It may be seen that MMV was infectious up to a dilution of 1/800,000. Five out of thirty test plants were infected at this dilution. This shows that the virus occurs in high concentration in infected Small Sugar pumpkin and is in agreement with the findings of Freitag (*5*) who found his squash-mosaic infectious to a dilution of 1/1,000,000. Lindberg *et al.* (*7*) found three of the squash-mosaic virus isolates they studied active at a dilution of 1/100,000, but inactive at 1/1,000,000. Anderson's-"type" muskmelon

strain (*6*), later shown to be a strain of squash-mosaic virus (*7*) withstood only 1/10,000 dilution. Anderson found that environmental factors influenced the physical properties of his strains.

Results of the heat-inactivation tests appear in the following tabulation:

Temperature (C°)	*Plants infected/plants inoculated*
45	48/50
50	46/50
55	24/50
60	8/50
65	12/50
70	19/50
75	7/50
80	0/50

It may be seen that MMV withstood a temperature of 75° C , but not 80° C. for 10 minutes. Freitag (*5*) found his virus to withstand 70° C. but not 75° C. Anderson's viruses also withstood up to 70° C., while the results of Lindberg, *et al.* (*7*) indicate an end-point between 60 and 70° C. for squash-mosaic viruses. Our MMV isolate thus has a higher thermal inactivation point.

Stability

MMV was shown to be resistant to inactivation by freezing. Frozen crude juice kept at −10° C. was still infective 2 years later. Freitag (*5*) found his squash-mosaic virus to be active after 5 years in frozen juice. With juice stored at room temperature the virus was found still infectious after storage for 45 days, but not after 60 days. These results are similar to those obtained by other workers (*5,7*).

Seed Transmission

As described under Methods only seed from Smith Perfect muskmelons were used in the transmission tests. Seed collected from fruit borne by healthy looking plants gave rise to 512 seedlings, none of which developed symptoms during the 21-day observation period. The seed of fruit obtained from diseased plants gave the following results: In 1 experiment with 313 seedlings no symptoms were observed; in another experiment involving 849 seedlings, 3 plants were observed with definite symptoms. Thus, this experiment showed 0.35 percent of the seed to be infected. Previous reports (*4,9,10*) have definitely established that squash-mosaic virus is seed-transmitted, although at a variable rate depending on the cucurbitaceous species involved.

Serology

Precipitin tests showed that preparations MVV-1, MMV-2, and SSV cross-reacted with their respective antisera. However, the antisera for the three preparations also reacted in low titer ($\frac{1}{8}$ to $\frac{1}{16}$ dilution of antiserum) with the healthy Small Sugar pumpkin preparation. This reaction with a healthy preparation was eliminated by diluting the antisera beyond $\frac{1}{32}$. Above this dilution it was observed that the MMV-1, MMV-2, and SSV preparations still cross-reacted strongly, thus establishing a serological relationship between MMV and SSV (squash-mosaic virus). None of the preparations reacted with normal rabbit serum.

CONCLUSIONS

Results of above studies with MMV demonstrate that it belongs to the squash-mosaic virus group. MMV was similar to other strains of squash mosaic in the symptoms produced on cucurbits (*5,6,8*) especially on squash and *Lagenaria siceria*; it also was found to be closely related serologically to a typical strain of squash mosaic (SSV) (*7*) from Wisconsin. Furthermore, its physical properties and its transmission by seed lend support to its classification as a strain of squash-mosaic virus.

On the other hand, there are certain differences between MMV and other previously described strains of squash-mosaic virus. MMV had a thermal inactivation point of 75° C., while other strains have been reported to be inactivated at about 70° C. MMV also did not infect *Momordica charantia*, reported as susceptible to squash mosaic by Freitag (*5*) and also by Anderson (*6*). While Anderson reported his "type muskmelon mosaic" as producing local lesions on *Luffa cylindrica*, MMV was without effect on this host.

During the years 1960 to 1962, symptoms of mosaic disease were observed in several commercial and experimental plantings of cucurbits in Puerto Rico. Leaf samples were collected and juice extracts inoculated to *Citrullus vulgaris* Schrad. (watermelon var. Cannonball), *Cucurbita pepo* L. (pumpkin var. Small Sugar), *Vigna sinensis* (Torner), Savi (cowpea var. Black), and *Nicotiana glutinosa* L. The criteria used by Grogan, *et al.* (*4*) of the reactions on these four hosts were used in determining the virus content of each sample. Table 1 gives the results of the tests.

Although the results recorded in table 1 were obtained from a limited number of collections it may be seen that WMV was more frequently isolated than either SMV or CMV. The detection of SMV in 1961 at Lajas, shows that this virus is present in areas other than Río Piedras, as reported earlier in this paper.

The results of the studies with the muskmelon isolate of 1958 herein

reported, as well as the isolations from cucurbit plantings during the period 1960–62, show that at least three viruses have been found infecting cucurbits in Puerto Rico. These are: Watermelon-mosaic virus, cucumber-mosaic virus, and squash-mosaic virus.

In retrospect, the virus described by Adsuar and Cruz Miret as virus "A" (*1*) seems to have been a strain of watermelon-mosaic virus because of its physical properties and reactions on *Lagenaria siceria* (*8*). Virus "B" was characterized by Adsuar and Cruz Miret as a strain of cucumber-mosaic virus. The only hitherto unreported cucurbit virus in Puerto Rico is therefore squash mosaic virus.

TABLE 1.—*Detection of cucurbit viruses in 1960-62 in Puerto Rico*

Cucurbitaceous host	Locality and date of sample collection	Virus recovered
Cucumis melo var. Smith Perfect	Río Piedras, March 1960	WMV[1]
Do.	Río Piedras, May 1960	CMV[2]
Citrullis vulgaris Schrad. var. Congo	Lajas, June 1960	WMV
C. vulgaris Schrad. var. Charleston Gray	Lajas, March 1961	CMV, WMV
Cucumis melo var. Smith Perfect	Lajas, 1961	SMV[3]
Cucurbita pepo, melopepo var. Zucchini	Río Piedras, 1961	SMV
C. pepo var. ?	Río Piedras, 1961	SMV
Cucumis sativus L. var. Ashley	Ponce, 1962	WMV
C. anguria L.[4]	Ponce, 1962	WMV
C. sativus L. var. P.R.39	Río Piedras, 1962	WMV

[1] WMV, watermelon-mosaic virus.

[2] CMV, cucumber-mosaic virus.

[3] SMV, squash-mosaic virus.

[4] *C. anguria* L., was found growing profusely in the surroundings of the field planted with *C. sativus* var. Ashley.

SUMMARY

1. A virus causing mottle and blistering in leaves of *Cucumis melo* var. Smith Perfect was isolated from an experimental planting at Río Piedras, P.R., in 1958. Its host range, dilution end-point, thermal inactivation, resistance to freezing, and serological similarity with a severe strain of squash-mosaic virus from Wisconsin (SSV) indicated that it belonged in the squash-mosaic virus group.

2. The studies reported above, as well as tests performed on samples from commercial and experimental cucurbit plantings in Puerto Rico during 1960–62, show that at least three cucurbit viruses are present in Puerto Rico. These are: Cucumber-mosaic virus, squash-mosaic virus, and watermelon-mosaic virus.

RESUMEN

1. Un virus que produjo matizado y ampollamiento en las hojas de melón, *Cucumis melo* var. Smith Perfect, fue aislado en una siembra experimental en Río Piedras en 1958. La infección de hospedadoras, título de dilución, resistencia a temperatura y a los efectos de congelación y su similitud serológica frente a una cepa del mosaico severo del calabacín (SSV) mostraron que el virus pertenece al grupo del mosaico del calabacín.

2. El trabajo que se informa arriba, más las pruebas llevadas a cabo en muestras de plantas obtenidas de siembras comerciales y experimentales de cucurbitáceas durante el período 1960–62, establecen la existencia de por lo menos tres virus que afectan las cucurbitáceas en Puerto Rico. Ellos son: el mosaico del pepinillo, el mosaico del calabacín y el mosaico de la sandía.

LITERATURE CITED

1. Adsuar, J., and Cruz Miret, A., Virus Diseases of Cucumbers in Puerto Rico, Tech. Paper 6, 1950, Agri. Exp. Sta., Univ. P.R., Río Piedras, P.R.
2. Price, W. C., Acquired immunity from cucumber mosaic in Zinnia, *Phytopath.* **25** 776–89, 1935.
3. Annual Report, Agr. Exp. Sta. Univ. P.R., 1958.
4. Grogan, R. G.; Hall, D. H.; and Kimble, K. A., Cucurbit mosaic viruses in California, *Phytopath.* **49** (6) 366–75, 1959.
5. Freitag, J. H., Beetle transmission, host range, and properties of squash mosaic virus, *Phytopath.* **46** 73–81, 1956.
6. Anderson, C. W., Two muskmelon mosaic virus strains from Central Florida, *Phytopath.* **44** 371–4, 1954.
7. Lindberg, G. D.; Hall, D. H.; and Walker, J. C., A study of melon and squash mosaic viruses, *Phytopath.* **46** 489, 1956.
8. Anderson, C. W., personal communication, 1959.
9. Kendrick, J. B., Cucurbit mosaic transmitted by muskmelon seed, *Phytopath.* **24** 820–3, 1934.
10. Middleton, J. T., Seed transmission of squash mosaic virus, *Phytopath.* **34** 405–10, 1944.