Aphid- and Whitefly-Transmitted Cucurbit Viruses in Imperial County, California

J. A. Dodds, J. G. Lee, S. T. Nameth, and F. F. Laemmlen

Associate professor, laboratory assistant, and graduate research assistant, Department of Plant Pathology, University of California, Riverside 92521, and farm advisor, University of California, Cooperative Extension, El Centro 92243, respectively.
This research was supported by California Melon Research Board Award 32.
The identification of *Bemisia tabaci* (Gennadius) by T. S. Bellows, Department of Entomology, University of California, Riverside, is appreciated.
Accepted for publication 7 September 1983.

ABSTRACT

Dodds, J. A., Lee, J. G., Nameth, S. T., and Laemmlen, F. F. 1984. Aphid- and whitefly-transmitted cucurbit viruses in Imperial County, California. Phytopathology 74: 221-225.

A survey of cantaloupe virus diseases in the Imperial Valley in 1981 detected watermelon mosaic virus-2 (WMV-2) and squash mosaic virus (SqMV), but not WMV-1 or cucumber mosaic virus (CMV). A total of 920 samples were collected from 10 cantaloupe fields between May and June 1981. The most common virus detected was WMV-2, which was present in all samples from plants with mosaic symptoms in eight fields. Two fields had a low incidence of SqMV. WMV-1 and CMV were not detected. Squash leaf curl (SLC) disease, which was observed in melons and squashes and associated with the whitefly, *Bemisia tabaci*, reached epidemic levels in cucurbit crops in late summer 1981. Symptoms in cantaloupe were less severe than in watermelons and squashes. WMV-1, WMV-2, CMV, and SqMV were not detected in symptomatic plants. Geminate virus particles were associated with field-collected plants of these cucurbit crops. Geminate virus particles were also detected in squash plants experimentally infected with the SLC agent by inoculation with whiteflies. Mechanical transmission of SLC was also achieved, but efficiency was poor. A strong cross-reaction between sap from SLC-infected squash and bean golden mosaic virus antiserum was detected by ELISA. Results of this study show that whitefly-transmitted geminiviruses cause serious field diseases of vegetable crops in Imperial County, California.

The incidence of mosaic diseases has been consistently high in cantaloupes (*Cucumis melo* L.) grown in the southwestern desert areas of the USA, especially in the first cropping season (February to June) when aphid activity is greatest. Viruses previously identified include watermelon mosaic virus-1 (WMV-1), WMV-2, squash mosaic virus (SqMV), and cucumber mosaic virus (CMV) (11,18). More recently a new mosaic disease associated with whiteflies and caused by squash leaf curl virus (SLCV) has been reported in cucurbits grown in the second cropping season (July to October) (7,8,12). Because several viruses are involved, a reevaluation of the identity and incidence of those associated with these mosaics seemed timely. Previous surveys in the Imperial Valley were performed more than 10 yr ago; relatively few samples were tested and whitefly-transmitted viruses were not implicated. In addition, resistant cultivars are not being grown. Serology was previously determined to be of limited value as a survey method (17,18) because of an inability to distinguish between WMV-1 and WMV-2. Recent reports (16,26,35) show these two viruses to be serologically distinct. Therefore, a survey based on serology for WMV-1, WMV-2, SqMV, and CMV was possible, assuming that antisera with adequate specificity could be prepared and an appropriate serological test could be developed. After preliminary testing, the enzyme-linked immunosorbent assay (ELISA) was chosen.

The objectives of this study of cucurbit viruses were: to reexamine the serological distinctiveness of WMV-1 and WMV-2 for the first time by an immunodiffusion test based on local isolates; to test whether ELISA could be used in surveys for cucurbit viruses; to conduct a large-scale survey of cucurbit viruses in the Imperial Valley; and to investigate some properties of the agent (or agents) causing SLC.

MATERIALS AND METHODS

Viruses and antisera used to develop assay methods. The WMV-1 isolate used as a known positive control in serological tests and to produce an antiserum was provided by H. Johnson, Cooperative Extension, University of California, Riverside. The virus caused mosaic symptoms in squash (*Cucurbita pepo* L. 'Early Prolific') and cantaloupe (*C. melo* 'Topmark'), and infected *Luffa acutangula* Roxb. systemically, but did not infect *Phaseolus vulgaris* L. 'Black Turtle 2' or *Chenopodium amaranticolor* Coste & Reyn. It was recovered from *L. acutangula* Roxb. (30) and maintained in squash.

The WMV-2 isolate used as a positive control and to produce an antiserum was collected from yellow squash by J. A. Dodds in Connecticut. It caused mosaic symptoms in Early Prolific squash, Topmark cantaloupe, and Black Turtle 2 bean, and local lesions on *C. amaranticolor* (4). The virus was recovered from Black Turtle 2 bean (22), and maintained in squash.

WMV-1 and WMV-2 were purified from mechanically inoculated greenhouse-grown Early Prolific squash plants by the method of Purcifull and Hiebert (26). Tissue (400 g) was homogenized in a Waring blender in a solution containing 800 ml of 0.5 M potassium phosphate, pH 7.5, containing 2.0 g of Na_2SO_3, 200 ml of chloroform, and 200 ml of carbon tetrachloride. The homogenate was centrifuged at 4,000 *g* for 5 min in a Sorvall GSA rotor. The aqueous phase was collected and centrifuged at 12,000 *g* for 20 min. The supernatant was removed, and polyethylene glycol 6,000 was added at the rate of 8 g/100 ml. After it was stirred for 1 hr at 4 C, the mixture was centrifuged at 12,000 *g* for 10 min, and the pellets were resuspended in 10 ml of 0.05 M potassium phosphate, pH 7.5. The resuspended material was subjected to two cycles of centrifugation in a CsCl gradient (starting density = 1.28 g/ml, in 0.05 M potassium phosphate, pH 7.5 or 8.2) generated at 150,000 *g* (maximum) for 18 hr. The virus-containing zone was removed, diluted with an equivalent volume of buffer, and centrifuged at 12,000 *g* for 10 min. The supernatant fluid was diluted further and subjected to high-speed centrifugation at 120,000 *g* in a Beckman 40 rotor for 1 hr. The resulting pellet of purified virus was resuspended in a small volume of 0.02 M tris buffer, pH 8.2.

Rabbits were immunized intramuscularly three times at weekly intervals with a total of 2 mg of purified virus in Freund's complete adjuvant. Early bleedings from rabbits immunized with WMV-1 were collected in the first month after the final injection and pooled. Antisera from late bleedings from animals immunized with WMV-2 were collected in the sixth month after the final injection and

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

pooled. A second antiserum to WMV-2 was donated by D. E. Purcifull, University of Florida, Gainesville.

Two CMV strains, CMV-S and CMV-P (5), were maintained in Early Prolific squash for use as positive control antigens. Antiserum to CMV was donated by T. J. Morris, University of California, Berkeley. The SqMV isolate used as a positive antigen control was donated by A. Kishaba, USDA, Boyden Laboratory, Riverside, CA, and was maintained in squash. An antiserum to SqMV was donated by R. J. Shepherd, University of California, Davis.

Purified immune gamma globulin to bean golden mosaic virus (BGMV) and a lyophilized sample of BGMV in bean leaves were supplied by R. M. Goodman and S. Haber, University of Illinois, Urbana.

Serological assays. Immunodiffusion tests with sodium dodecyl sulfate (SDS)-treated viruses in sap were done according to the method of Purcifull and Batchelor (27). Test antigen was prepared by grinding 1.0 g of tissue with 1.0 ml of H_2O and 1.0 ml of 3% SDS. Immunodiffusion medium was 0.8% Noble agar, 0.5% SDS, and 1.0% sodium azide. Reactions were read within 24–48 hr after placement of test antigen and antisera in the reaction wells.

Direct ELISA tests were by the method of Clark and Adams (3). The concentration of gamma globulin in the coating antibody was 1 μg/ml (WMV-1, WMV-2, SqMV, and BGMV) and 10 μg/ml (CMV). Plant sap was diluted 1 in 10 in PBS-Tween-PVP containing 0.02 M sodium phosphate, 0.15 M NaCl, 0.02% sodium azide (pH 7.4) with 0.05% Tween-20, and 2% polyvinyl pyrrolidone (MW 40,000). One gram fresh weight of tissue was extracted in 10 ml of solution. A dry weight/fresh weight ratio of 1:6 was assumed when preparing a sample from lyophilized bean tissue. The conjugate antibody was used at a 1/500 (BGMV), 1/800 (WMV-1, WMV-2, CMV), or 1/1,200 (SqMV) dilution of gamma globulin (initially 1 mg/ml) after conjugation with alkaline phosphatase. These parameters gave $A_{405\ nm}$ values of 0.05 or less for extracts from healthy plants, without seriously reducing $A_{405\ nm}$ values for extracts from infected plants. No technical difficulties were encountered in using the ELISA to detect WMV-1, WMV-2, SqMV, CMV, or BGMV in experimentally infected positive control plants or naturally infected field plants.

Collection of samples. Ten cantaloupe fields were selected at the start of the season within a 6.2-km (10-mile) radius of El Centro (Imperial County), CA. The fields were observed six times between March and June 1981.

Samples, consisting of the growing tips and the four youngest leaves, were collected from plants between May and June 1981. One hundred samples were collected in each field, 50 from plants with obvious mosaic symptoms and 50 collected at random. Tissue was stored frozen at −20 C in plastic bags, each of which contained 50 samples. Freezing and thawing was found to have no effect on ELISA values, nor did it result in physical contamination of uninfected samples.

Samples collected from diseased plants in October 1981 and 1982 were from fields with many plants showing strong symptoms of the type described for the SLCV (8).

Purification of geminiviruses. Field-collected infected tissue (500 g) was homogenized with two parts (w/v) 0.2 M sodium citrate (pH 6.5), 0.2% 2-mercaptoethanol, and 0.25 parts (w/v) chloroform. The extract was centrifuged at 10,000 *g* for 10 min and the aqueous supernatant was collected and adjusted to 9% polyethylene glycol (MW 6,000). The suspension was centrifuged at 10,000 *g* for 20 min and the pellet was resuspended in 500 ml of 0.005 M ethylenediaminetetraacetic acid (EDTA), pH 7.0. The suspension was centrifuged at 10,000 *g* for 20 min and the supernatant was collected and further centrifuged at 85,000 *g* for 3 hr. The pellets were resuspended in a total volume of 1.0 ml of 0.005 M EDTA (pH 7.0), centrifuged at 10,000 *g* for 20 min, and the supernatant was retained for electron microscopy.

Electron microscopy. Leaf dips or partially purified preparations were negatively stained with 2% sodium phosphotungstate, pH 7.0. Some samples were fixed in 1.5% glutaraldehyde.

Mechanical inoculation. Leaf extracts were prepared in 0.02 M potassium phosphate buffer, pH 7.2, containing 1% Celite and mechanically inoculated to 7- to 10-day-old squash or cantaloupe seedlings. Reducing agents, 0.1% sodium thioglycolate and 0.3% sodium diethyldithiocarbamate, were added to the inoculum prepared from plants infected with CMV and SLCV.

RESULTS

Cross-reactions between WMV-1 and WMV-2. A cross-reaction between WMV-1 antigen and WMV-2 antiserum was detected by the immunodiffusion test (Fig. 1). The visible precipitate was closer to the antiserum well than the precipitate formed by the homologous reaction between WMV-2 and WMV-2 antiserum. It was obtained when our antiserum, but not when the Florida antiserum to WMV-2, was used, and its development could be prevented by cross absorption, when SDS-treated sap from plants infected with WMV-1 was placed in the center well for 4 hr before placing our WMV-2 antiserum in the center well. No cross-reaction between WMV-2 antigen and WMV-1 antiserum was detected. Sap

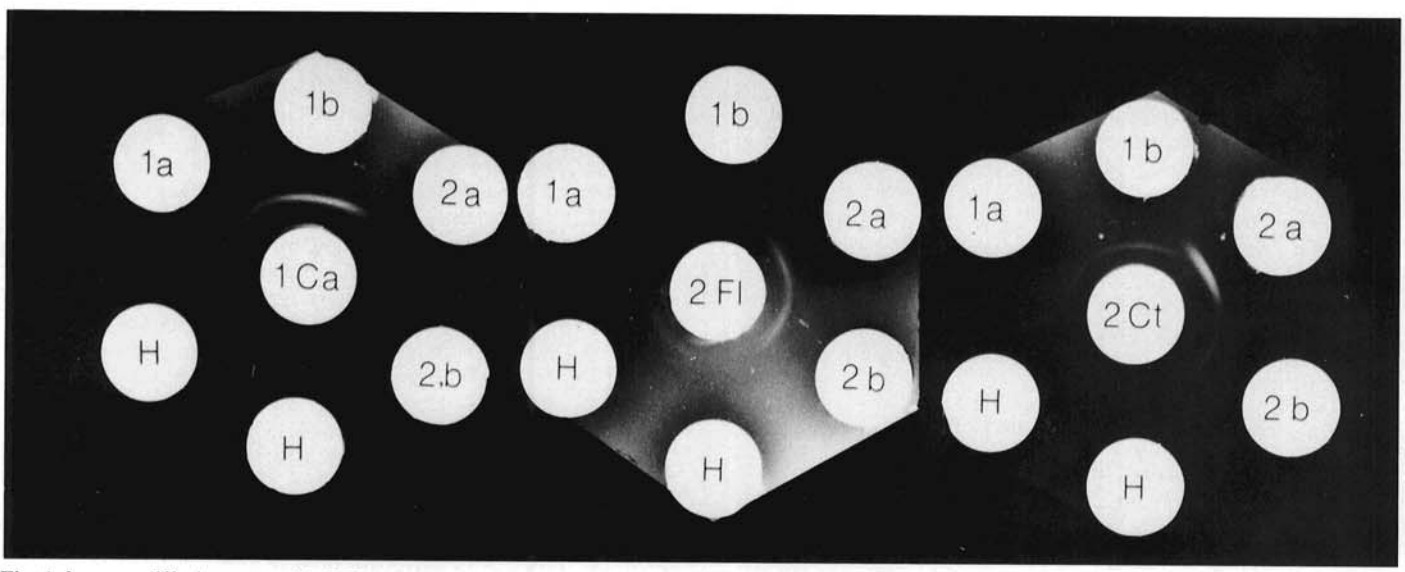

Fig. 1. Immunodiffusion tests with SDS-treated antigens. Antisera to watermelon mosaic virus (WMV)-1 prepared against a California isolate (1Ca), to WMV-2 prepared against a Florida isolate (2Fl), and to WMV-2 prepared against a Connecticut isolate (2Ct) were placed in the center wells. Extracts from squash plants infected with two isolates of WMV-1 (1a, 1b), two isolates of WMV-2 (2a, 2b), or extracts from uninoculated healthy plants (H) were placed in the outer wells. A cross-reaction between WMV-1 and WMV-2 antiserum was detected in the test shown on the right.

from healthy plants did not react with either WMV-1 or WMV-2 antiserum.

A cross-reaction between WMV-2 antigen and WMV-1 antiserum was detected by ELISA in 60 tests. The samples were from 60 different sources collected either from infected plants in the field or from greenhouse-grown plants that became infected following mechanical inoculation. The intensity of the heterologous cross-reaction was less than, but proportional to, the intensity of the homologous reaction between WMV-2 and WMV-2 antiserum. The homologous reaction was approximately 15 times stronger than the heterologous reaction in tests where the homologous reaction was less than $A_{405\ nm} = 2.7$ (Fig. 2). No cross-reaction between WMV-1 antigen and WMV-2 antiserum was detected. Sap from healthy plants gave reactions of $A_{405\ nm} = 0.05$ or less with both WMV-1 and WMV-2 antisera.

Serological survey. Four hundred cantaloupe samples from plants showing mosaic symptoms in the foliage were collected from eight fields (50 samples from each) in May and June 1981. WMV-2 was detected in all fields and in 392 of the samples. SqMV was detected in five samples, all from the same field. The identity of SqMV was confirmed by ELISA in squash plants that became infected following inoculation with extracts from the frozen field samples. WMV-1 and CMV were not detected.

An additional 50 samples were collected at random from each of the eight fields to ensure that viruses inducing mild foliar symptoms were not being overlooked due to overemphasis on sampling plants with the strongest symptom development and to obtain an estimate of the incidence of the viruses detected. WMV-2 was detected in 172 of the samples. The incidence was 1, 7, 15, 20, 28, 32, 33, or 36 positives from 50 samples in each of the eight fields. Squash mosaic virus was detected in one sample from one of the eight fields, but not from the one mentioned above. WMV-1 and CMV were not detected. Mosaic symptoms were not observed in the ninth and 10th fields selected for the survey in May and June 1981, and WMV-1, WMV-2, SqMV, and CMV were not detected by ELISA in 120 samples collected from these fields.

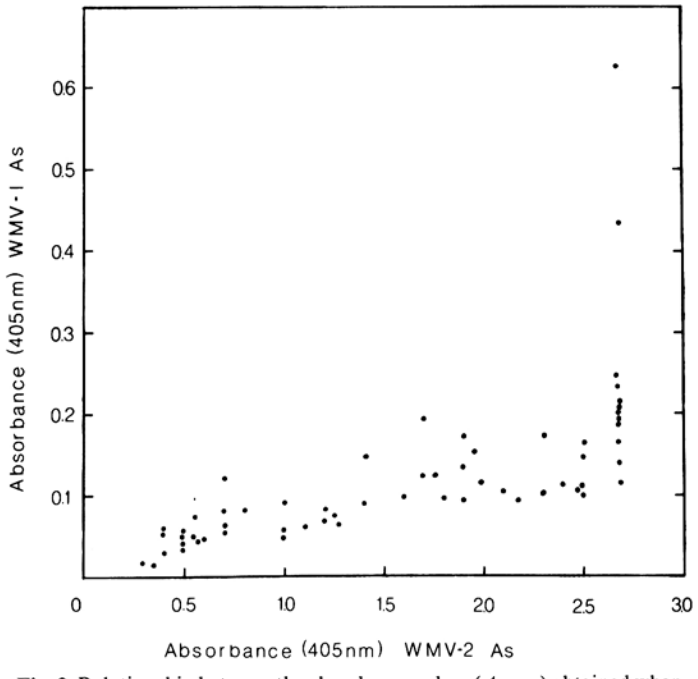

Fig. 2. Relationship between the absorbance values ($A_{405\ nm}$) obtained when watermelon mosaic virus (WMV)-2 was reacted with either homologous WMV-2 antiserum (abscissa, high values) or heterologous WMV-1 antiserum (ordinate, low values). The proportionality between the homologous and heterologous reaction indicate the positive results obtained with WMV-1 antiserum are best explained as a cross-reaction with WMV-2 antigen rather than a reaction with contaminating WMV-1 antigen. Antigen was sap, diluted 1 in 10, from field- or greenhouse-grown cantaloupe plants (60 in all) infected with WMV-2.

A total of 200 samples that had symptoms of mosaic or leaf curling were collected from fields of cantaloupe, watermelon [*Citrullus lanatus* (Thunb.) Mansfeld], and summer squash (*C. pepo*) in October 1981 and 1982. The symptoms observed resembled those described for SLCV, and were least severe in cantaloupe. WMV-1, WMV-2, SqMV, or CMV were not detected in any of the samples.

Properties of SLC disease. An agent causing symptoms of SLC was transmitted from symptomatic field plants to experimental plants both by whiteflies and by mechanical inoculation. Sources of virus, types of transmission, indicator hosts, and results are summarized in Table 1. Efficiency of transmission by whiteflies was 100% when groups of adults were transferred to individual test plants within 4 hr of collection in the field. Efficiency of mechanical transmission was low. In two attempts, 1 of 10 and 3 of 20 plants of

TABLE 1. Summary of detection of geminivirus particles in extracts from cucurbit crop plants with mosaic symptoms of squash leaf curl virus collected from the Imperial Valley, California, August to October 1981 and 1982

Source of virus	Original field plant	Greenhouse subculture	
		Mechanical[a]	Whitefly[b]
Cucumis melo L. cantaloupe	P[c]	NT[e]	NT
Citrullus lanatus (Thunb.) Mansfeld watermelon	L[d]	NT	NT
Cucurbita pepo L. summer squash	L,P	Ls	Ls,Lc
scallop squash	L	NT	NT
Mediterranean squash	L,P	NT	NT
Cucurbita maxima Duch. banana squash	L	Ls	NT

[a] Mechanical inoculation was from the original field plant to summer squash (s).
[b] Whitefly transmission was with whiteflies collected from the original field plant and caged with either summer squash (s) or cantaloupe (c).
[c] P = the negatively stained grid examined was made from a partially purified preparation and geminate virus particles were observed.
[d] L = the negatively stained grid examined was made from a leaf dip and geminate virus particles were observed.
[e] NT = not tested.

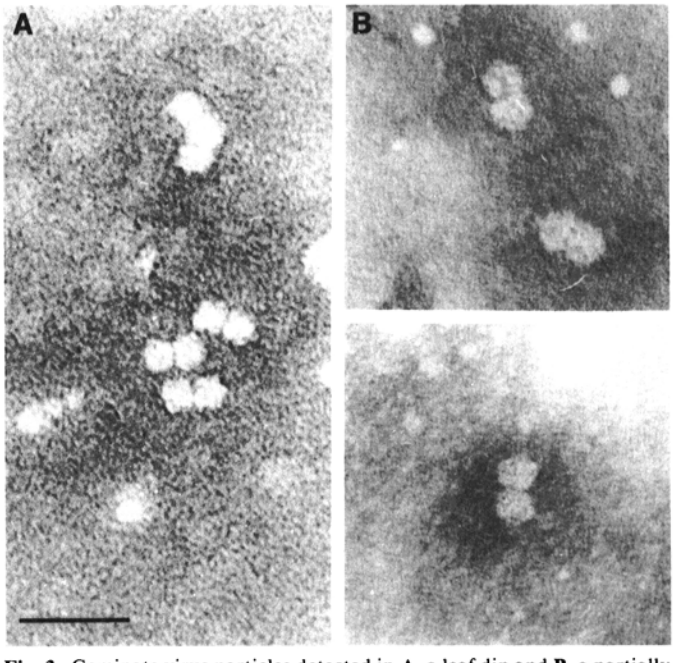

Fig. 3. Geminate virus particles detected in **A,** a leaf dip and **B,** a partially purified preparation from squash with symptoms of squash leaf curl disease. The bar represents 50 nm.

C. pepo developed symptoms of SLC disease. Inoculum was from leaves of either plants of *C. pepo* or *C. maxima* with strong symptoms collected from fields with 100% of the plants with disease symptoms. No living adult whiteflies were present on leaves used to prepare inoculum, which had been stored for 24 hr at 4 C after collection, and no whiteflies were present in the greenhouse where the mechanically inoculated plants were maintained. It was not possible to infect plants of *C. pepo* by mechanical inoculation using extracts from experimentally infected *C. pepo*.

Geminate virus particles 30 × 15 nm were consistently detected in leaf dip preparations or partially purified samples from plants with SLC symptoms (Fig. 3). Similar particles were also consistently detected in all experimentally inoculated plants of *C. pepo* and *C. melo* that developed SLC symptoms. Geminate particles were not detected in a total of eight plants of *C. pepo* and 10 plants of *C. melo* that failed to develop symptoms after inoculation.

A strong positive reaction ($A_{405\ nm} = 2.7$) was detected by ELISA between BGMV antiserum and either sap of *C. pepo* experimentally infected with SLCV or *P. vulgaris* infected with BGMV. Negative reactions were obtained ($A_{405\ nm} = 0.05$ or less) when extracts from either inoculated plants of *C. pepo*, which did not develop symptoms of SLC disease, or uninoculated plants of *C. pepo* or *P. vulgaris* were tested.

DISCUSSION

The high incidence, economic significance, and need for frequent reappraisal of mosaic diseases in cucurbits in California and elsewhere is reflected in the numerous reports on cucurbit virus. This study indicates that ELISA can be used to survey for the presence of WMV-1, WMV-2, SqMV, and CMV. Previous studies have reported conflicting results on serological relatedness between WMV-1 and WMV-2 (17,26). ELISA done with the antisera used in this study distinguish between these two cucurbit potyviruses, even though a cross-reaction was detected between WMV-2 antigen and WMV-1 antisera when antigen concentration of WMV-2 was high. The cross-reactions detected in immunodiffusion tests and ELISA suggest that a detailed comparison of WMV-1 isolates, WMV-2 isolates, and recently described new viruses (14,15) is needed. Caution should be used in the choice of WMV-1 and WMV-2 antisera used in surveys because serological cross-reactions can be detected between WMV-1 and WMV-2. The degree to which local isolates of these viruses cross-react with available antisera will determine the feasibility of using ELISA for future diagnosis and surveys of cucurbit potyviruses.

WMV-2 is the most common cause of mosaic disease in desert-grown cantaloupes in the Imperial Valley in the first growing season. There is presently no effective management practice to control this virus disease. WMV-2 also occurs in some other melon and cucurbit producing areas (1,9,13,19,32,34). In other areas, WMV-1 was the most common virus (1,16,20,25). There is a need to continue epidemiological studies on weed plant reservoirs and vectors of WMV-2 (1,21) and to select cultivars of cantaloupe for desert culture with tolerance or resistance to WMV-2. Past breeding programs have emphasized resistance to WMV-1 (2,23,24,28,29,31,33). The total of 1,000 plants that were tested is a much larger sample than those tested in previous California surveys in which fewer than 100 plants were tested. It was possible to test large numbers of samples because of the development of ELISA as a survey method for cucurbit viruses. Only three of 400 samples from plants with mosaic symptoms failed to react with antisera to any of the four viruses that were tested. No other viruses were detected in 1981.

SLCV is whitefly-transmitted and its host range and effects have been examined (7,8). We have shown that the virus can be transmitted by mechanical inoculation, has geminate particles, and reacts strongly in a serological cross-reaction with antisera to BGMV, a well characterized whitefly-transmitted geminivirus (10). These results, together with a preliminary report (6), indicate that whitefly-transmitted geminiviruses cause severe disease of squashes and melons in the USA. They also point to a need to develop methods, in addition to serology, to distinguish between the whitefly-transmitted cucurbit viruses in the Imperial Valley.

LITERATURE CITED

1. Adlerz, W. C. 1974. Spring aphid flights and incidence of watermelon mosaic viruses 1 and 2 in Florida. Phytopathology 64:350-353.
2. Bohn, G. W., Kishaba, A. N., and McCreight, J. D. 1980. WMV 29 muskmelon breeding line. HortScience 15:539-540.
3. Clark, M. F., and Adams, A. N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34:475-483.
4. Demski, J. W. 1968. Local lesion reactions of *Chenopodium* species to watermelon mosaic virus 2. Phytopathology 58:1196-1197.
5. Dodds, J. A. 1982. Cross-protection and interference between electrophoretically distinct strains of cucumber mosaic virus in tomato. Virology 118:235-240.
6. Dodds, J. A., Nameth, S. T., Lee, J. G., and Laemmlen, F. F. 1982. Aphid and whitefly transmitted cucurbit viruses in Imperial County, California. (Abstr.) Phytopathology 72:963.
7. Duffus, J. E., and Flock, R. A. 1982. Whitefly-transmitted disease complex of the desert southwest. Calif. Agric. 36:4-6.
8. Flock, R. A., and Mayhew, D. E. 1981. Squash leaf curl, a new disease of cucurbits in California. Plant Dis. 65:75-76.
9. Gates, L. F., and Bronskill, J. F. 1976. Occurrence of watermelon mosaic virus in glasshouse cucumbers in southwestern Ontario. Plant Dis. Rep. 60:964-966.
10. Goodman, R. M., and Bird, J. 1978. Bean golden mosaic virus. No. 192 in: Description of Plant Viruses. Commonw. Mycol. Inst., Assoc. Appl. Biol. Kew, Surrey, England.
11. Grogan, R. G., Hall, D. H., and Kimble, K. A. 1959. Cucurbit mosaic viruses in California. Phytopathology 49:366-376.
12. Johnson, M. W., Toscano, N. C., Reynolds, H. T., Sylvester, E. S., Kido, K., and Natwick, E. T. 1982. Whiteflies cause problems for southern California growers. Calif. Agric. 36:24-26.
13. Komm, D. A., and Agrios, G. N. 1978. Incidence and epidemiology of viruses affected cucurbit crops in Massachusetts. Plant Dis. Rep. 62:746-750.
14. Lecoq, H., Pitrat, M., and Clement, M. 1981. Identification and characterization of a potyvirus that induces muskmelon yellow stunt. Agronomie 1:827-834.
15. Lisa, V., Boccardo, G., D'Agostino, G., Dellavalle, G., and d'Aguilo, M. 1981. Characterization of a potyvirus that causes zucchini yellow mosaic. Phytopathology 71:667-672.
16. Makkouk, K. M. 1980. A severe disease of cucumbers in Lebanon caused by watermelon mosaic virus-1. Plant Disease 64:799-801.
17. Milne, K. S., and Grogan, R. G. 1969. Characterization of watermelon mosaic virus strains by serology and other properties. Phytopathology 59:801-818.
18. Milne, K. S., Grogan, R. G., and Kimble, K. S. 1969. Identification of viruses infecting cucurbits in California. Phytopathology 59:819-828.
19. Nelson, M. R., Allen, R. M., and Tuttle, D. M. 1962. Distribution prevalence and importance of some cantaloup virus diseases in southwestern Arizona. Plant Dis. Rep. 9:667-671.
20. Nelson, M. R., Laborde, J. A., and McDonald, H. H. 1966. Cucurbit viruses on the west coast of Mexico. Plant Dis. Rep. 50:947-950.
21. Nelson, M. R., and Tuttle, D. M. 1969. The epidemiology of cucumber mosaic and watermelon mosaic 2 of cantaloups in an arid climate. Phytopathology 59:849-856.
22. Provvidenti, R. 1974. Inheritance of resistance to watermelon mosaic virus 2 in *Phaseolus vulgaris*. Phytopathology 64:1448-1450.
23. Provvidenti, R., and Robinson, R. W. 1974. Resistance to squash mosaic virus and watermelon mosaic virus 1 in *Cucumis metuliferus*. Plant Dis. Rep. 58:735-738.
24. Provvidenti, R., Robinson, R. W., and Munger, H. M. 1978. Resistance in feral species to six viruses infecting *Cucurbita*. Plant Dis. Rep. 62:326-329.
25. Provvidenti, R., and Schroeder, W. T. 1970. Epiphytotic of watermelon mosaic virus among Cucurbitaceae in central New York in 1969. Plant Dis. Rep. 54:744-748.
26. Purcifull, D. E., and Hiebert, E. 1979. Serological distinction of watermelon mosaic virus isolates. Phytopathology 69:112-116.
27. Purcifull, D. E., and Batchelor, D. L. 1977. Immuno-diffusion tests with sodium dodecyl sulfate (SDS)-treated plant viruses and plant viral inclusions. Fl. Agric. Exp. Stn. Tech. Bull. 788. Inst. Food Agric. Sci., University of Florida, Gainesville. 39 pp.
28. Thomas, C. E., and Webb, R. E. 1981. W1, W3, W4, W5, and W6. Multidisease-resistant muskmelon breeding lines. HortScience 16:96.
29. Thomas, C. E., and Webb, R. E. 1982. 'Cinco' Muskmelon. HortScience 17:684-685.
30. Webb, R. E. 1965. *Luffa acutangula* for separation and maintenance of watermelon mosaic virus 1 free of watermelon mosaic virus 2.

Phytopathology 55:1379-1380.
31. Webb, R. E. 1967. Cantaloup breeding line B66-5: Highly resistant to watermelon mosaic virus 1. HortScience 2:58-59.
32. Webb, R. E. 1971. Watermelon mosaic viruses 1 and 2 in squash on the Atlantic seaboard. Plant Dis. Rep. 55:132-135.
33. Webb, R. E., and Bohn, G. W. 1962. Resistance to cucurbit viruses in *Cucumis melo*. (Abstr.) Phytopathology 52:1221.
34. Webb, R. E., Bohn, G. W., and Scott, H. A. 1965. Watermelon mosaic virus 1 and 2 in southern and western cucurbit production areas. Plant Dis. Rep. 49:532-535.
35. Webb, R. E., and Scott, H. A. 1965. Isolation and identification of watermelon mosaic viruses 1 and 2. Phytopathology 55:895-900.