Serological Relationships and Partial Characterization of Zucchini Yellow Mosaic Virus Isolated from Squash in Florida

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ABSTRACT

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Thirty-nine plants with foliar mosaic symptoms were collected in Alachua, Lake, and Sumter counties in the fall of 1981 and tested by sodium dodecyl sulfate (SDS)-immunodiffusion tests against antisera to watermelon mosaic viruses 1 and 2 (WMV-1 and WMV-2), a Moroccan isolate (WMV-M), squash mosaic virus (SqMV), and cucumber mosaic virus (CMV). Seven plants were infected with WMV-1 only, 21 were infected with WMV-2 only, 8 were infected with both WMV-1 and WMV-2, and 3 were infected with a virus serologically related to WMV-2 but distinct from it. None of the samples was infected with WMV-M, SqMV, or CMV. One of the WMV-2-related isolates (1119) was mechanically transmitted to zucchini squash in the greenhouse, where it caused systemic mosaic, distortion, veinbanding, and blistering of leaves. This isolate also induced systemic infections of cucumber, watermelon, cantaloup, and *Luffa acutangula* and local infections of *Chenopodium amaranticolor*, *C. quinoa*, *Phaseolus vulgaris*, and *Pisum sativum* 'Alaska.' The virus was transmitted in a styletborne manner by *Myzus persicae*. Striated inclusions and filamentous particles (about 760 nm long) were found in negatively stained leaf extracts. Isolate 1119 was found to be closely related serologically to zucchini yellow mosaic virus (ZYMV) from Italy in SDS-immunodiffusion tests. Virus isolates serologically and symptomatologically similar to ZYMV were found in six counties representing the northern, central, and southern portions of Florida in 1982.

More than 25 viruses, including at least seven potyviruses, are known to infect cucurbits naturally (14). In Florida, watermelon mosaic virus (2) types 1 (WMV-1) and 2 (WMV-2) are potyviruses that occur commonly in cucurbits (1,21,22). These viruses also occur elsewhere in the United States and in many other areas of the world (14,15,21). Zucchini yellow mosaic virus (ZYMV), another potyvirus, causes severe diseases of squash and muskmelon in Europe (11,13) and has also been reported in the northeastern United States (11).

Sodium dodecyl sulfate (SDS)-immunodiffusion techniques are useful for detecting and distinguishing WMV-1 and WMV-2 (19; D. E. Purcifull, *unpublished*). The SDS-immunodiffusion techniques have also been used to distinguish WMV-1 and WMV-2 from a Moroccan isolate of WMV (WMV-M) (3,19) and from two isometric viruses that infect squash (squash mosaic and cucumber mosaic viruses (18) and to detect ZYMV in cucurbits (11,13). We report results of surveys conducted in 1981, 1982, and 1983 to determine the identity and incidence of viruses in squash in Florida.

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MATERIALS AND METHODS

In 1982, a naturally infected zucchini squash plant with prominent mosaic symptoms was collected in central Florida. This isolate (1119) served as the source of virus for preparation of antiserum, most of the serological tests, electron microscopy, and for some of the host-range and aphid-transmission trials reported in this paper. Other isolates, including one from yellow squash (81-25), were used for some of the host-range, aphid-transmission, and serological assays. The WMV-1, WMV-2, and WMV-M isolates were as described previously (8,19). The ZYMV isolate was provided by V. Lisa (13). Squash mosaic virus (SqMV) was the American Type Culture Collection isolate PV-36 and cucumber mosaic virus (CMV) was an isolate from winged bean (*Psophocarpus tetragonolobus*) (10).

All viruses were transmitted mechanically for host-range trials and routine maintenance of cultures. Inocula were prepared by triturating leaf tissue in 0.02 M potassium phosphate, pH 7.5, followed by addition of Carborundum. Cheesecloth pads were dipped in inocula and rubbed on leaves of test plants. At least four plants of each species were inoculated.

Aphid transmission trials were conducted with *Myzus persicae* and *Aphis citricola*. With *M. persicae*, individual aphids were allowed access probes of

15–60 sec on a zucchini squash leaf infected with isolate 1119. Single aphids were transferred to each of 25 zucchini plants. After an inoculation access period of 2–3 hr, aphids were removed and plants placed in a greenhouse for observation. With *A. citricola*, individuals were given an access period of 10–60 sec on a pumpkin leaf infected with isolate 81-25. After an inoculation access period of at least 1 hr, the plants were placed in a greenhouse.

Extracts for electron microscopic examination were prepared as follows: A drop of extract was placed on a carbon-coated, Formvar membrane mounted on a grid (75 × 300 mesh). After 1 min, the sample was removed with filter paper and the grid was immediately and extensively washed with 0.1 M potassium phosphate, pH 7.0, and subsequently with deionized water. The sample was stained with 2% uranyl acetate containing 125 μg/ml bacitracin and excess stain removed by blotting with filter paper. After drying, the sample was examined in a Hitachi H-600 electron microscope. Particle measurements were made by comparing enlarged micrographs with micrographs of a diffraction grating (462.9-nm spacing) photographed at the same magnification.

For immunosorbent electron microscopy (IEM) (12,16), the technique was modified as follows: After the samples were washed with buffer, droplets of antisera (diluted 1:20 with buffer) were placed on the grids and allowed to stand 5 min. The grids were then washed with buffer and with water and stained with uranyl acetate as before.

An antiserum against isolate 1119 was prepared as follows: The virus was partially purified from systemically infected leaves of zucchini squash collected 10–12 days after inoculation at the cotyledonary stage. For the first injection, the virus was purified by chloroform-carbon tetrachloride clarification as described for WMV-1 (19), except the phosphate buffer used for homogenization contained 0.01 M EDTA and the virus was fixed with 1.8% formaldehyde (9) before centrifugation in CsCl. After removal of a virus-containing zone from CsCl gradients, the virus was reconcentrated by precipitation with polyethylene glycol (PEG, mol wt 6,000). The virus (about 3 mg in 1 ml) was emulsified 1:1 with Freund's complete adjuvant (17) and injected in a toe pad (0.15 ml) and thigh muscle of rabbit no. 1028. Two additional attempts to purify the virus by this method were unsuccessful. Consequently, the purification procedure was modified by substituting 0.02 M HEPES buffer (6), pH 7.5, for phosphate buffer and the formaldehyde fixation step was omitted. Virus (about 2.3 mg in 1 ml) was emulsified with Freund's incomplete adjuvant and injected 2 mo after the initial injection. The rabbit was bled every 7–12 days for several weeks, starting 1 wk after the second injection.

Antisera to isolate 1119 collected 1–5 wk after the second immunization were used. Antisera to the following viruses were as described: WMV-1 (19), WMV-2 (19), WMV-M (3), SDS-treated SqMV (18), and SDS-treated CMV (10). Antisera to ZYMV isolates were obtained from V. Lisa (10) and H. Lecoq (11).

SDS-immunodiffusion tests were conducted in media containing 0.8% Noble agar, 0.5% SDS, and 1.0% sodium azide as described previously (17). Crude extracts treated with SDS were usually heated in a boiling water bath for 4 min before use (18).

Enzyme-linked immunosorbent assays (ELISA) were conducted as described by Clark and Adams (5). Alkaline-phosphatase-conjugated IgG from serum collected 5 wk after the second injection of rabbit no. 1028 with isolate 1119 was used for assays. Results were recorded with an EL307 Bio-Tek reader (Bio-Tek Instruments, Burlington, VT 05401).

RESULTS AND DISCUSSION

Thirty-nine squash plants with mosaic symptoms were collected in field plots and commercial fields in the fall of 1981 in north central and central Florida and tested initially in SDS-immunodiffusion tests. None of the 39 samples was infected with WMV-M, SqMV, or CMV. Seven plants were infected with WMV-1 only, 21 were infected with WMV-2 only, 8 were infected with both WMV-1 and WMV-2, and 3 were infected with a virus related to WMV-2 but distinct from it. Isolates from the latter three plants were serologically and symptomatologically similar. One isolate (1119) was used for most of the following tests.

Antiserum to WMV-2 collected 7–9 wk after immunization did not react with isolates of the 1119 type (Fig. 1A), although WMV-2 antiserum collected

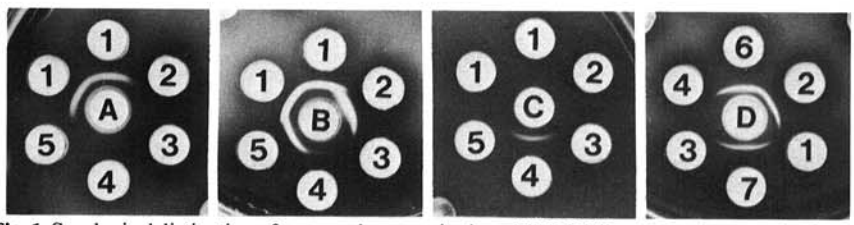

Fig. 1. Serological distinction of watermelon mosaic virus-1 (WMV-1), watermelon mosaic virus-2 (WMV-2), and isolate 1119 obtained from squash in Florida. Contents of center wells: A = WMV-2 antiserum collected 7 wk after immunization, B = WMV-2 antiserum collected 7 mo after immunization, C = WMV-1 antiserum, and D = antiserum to isolate 1119. Peripheral wells contained sodium dodecyl sulfate immunodiffusion-treated antigens from squash leaves: 1 = infected with WMV-2, 2 = infected with isolate 1119, 3 = healthy, 4 = infected with WMV-1, 5 = infected with field isolate 1129 (serologically and symptomatologically similar to isolate 1119), and 6 = zucchini yellow mosaic virus (ZYMV) from Italy. Well 7 contained extract from ZYMV-infected cucumber leaves.

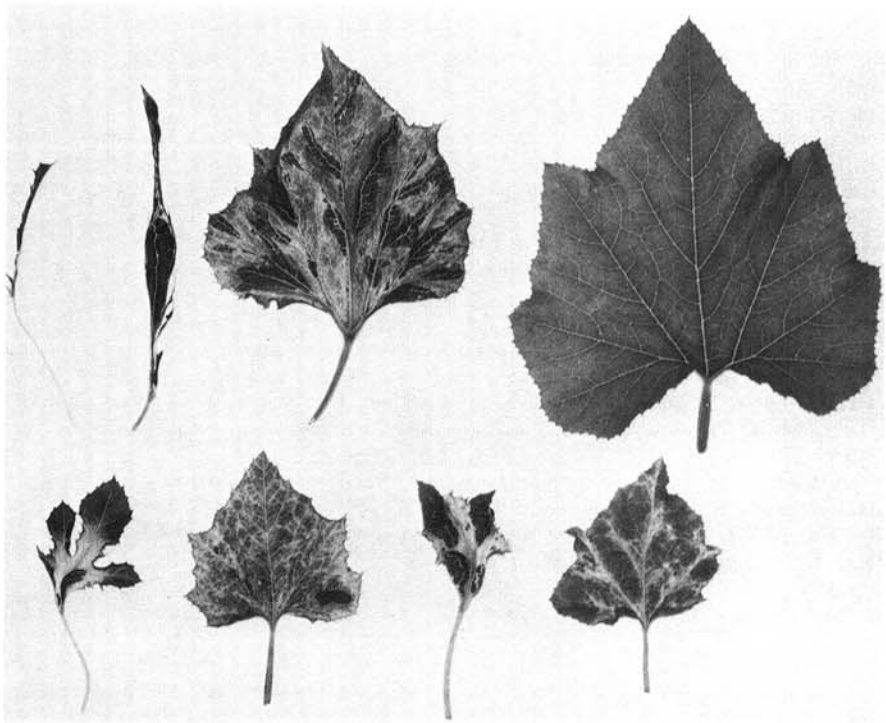

Fig. 2. Symptoms induced by isolate 1119 in zucchini squash. (Upper right) Healthy leaf.

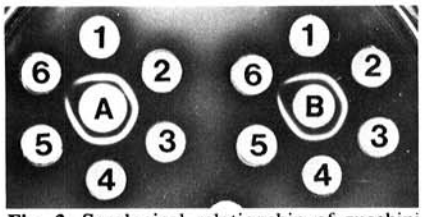

Fig. 3. Serological relationship of zucchini yellow mosaic virus (ZYMV) from Italy to isolates from squash in Florida. In both plates, healthy sap was added to the center well 21 hr before addition of antigens to peripheral wells and antisera to center well. A = antiserum to the Italian isolate of ZYMV, provided by V. Lisa. B = antiserum to Florida isolate 1119. Peripheral wells were filled with sodium dodecyl sulfate-treated leaf sap from healthy squash leaves (well 6) or from leaves infected with isolate 1119 (well 1), ZYMV (wells 2 and 5) and two additional isolates serologically and symptomatologically similar to isolate 1119 (wells 3 and 4).

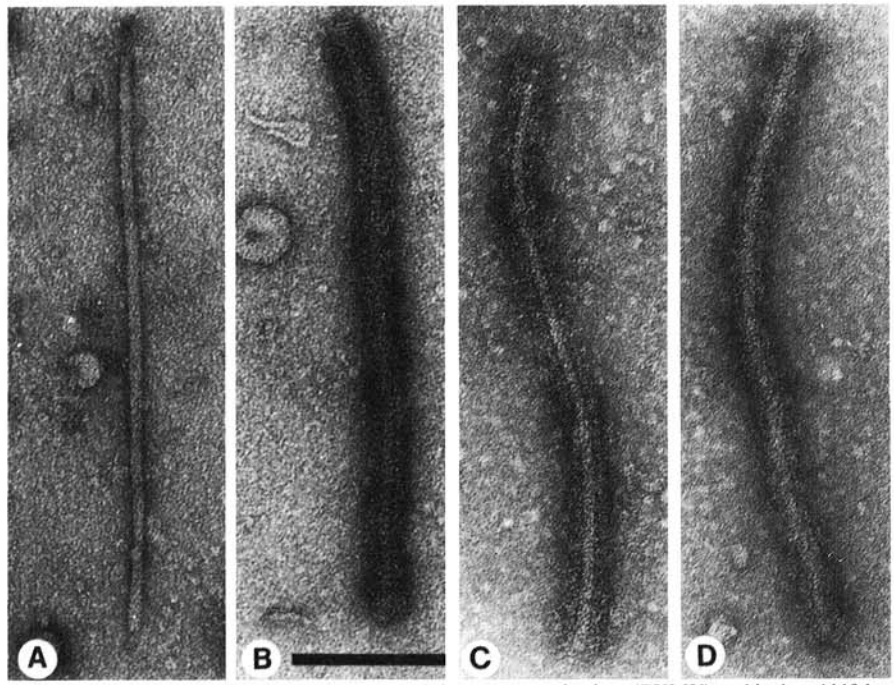

Fig. 4. Serological relationship between zucchini yellow mosaic virus (ZYMV) and isolate 1119 by the immunoelectron microscopic decoration of filamentous particles in leaf extracts. **(A)** Isolate 1119 treated with normal serum, **(B)** isolate 1119 treated with homologous antiserum, **(C)** isolate 1119 treated with antiserum to zucchini yellow mosaic virus from Italy, and **(D)** zucchini yellow mosaic virus treated with antiserum to isolate 1119. Scale bar = 200 nm.

5–8 mo later from the same rabbit did (Fig. 1B). However, the resulting precipitin bands with the 1119 type isolates formed definite spur reactions with the precipitin bands formed by WMV-2 (Fig. 1B).

Zucchini squash infected with isolate 1119 showed prominent veinal chlorosis symptoms followed by mottling, blister mottle, veinbanding, and leaf distortion (Fig. 2). Isolate 1119 also caused systemic infections and mosaic symptoms in *Cucumis melo* (cantaloup), *Citrullus lanatus* (watermelon), and *Cucumis sativus*. It infected *L. acutangula* systemically; this species is systemically infected by WMV-1 although it is not susceptible to many isolates of WMV-2 (19,20). Isolate 1119 was recovered by back-assays from inoculated leaves of *Chenopodium quinoa*, *Pisum sativum* 'Alaska,' and *Phaseolus vulgaris* 'Black Turtle Soup 2.' Immunodiffusion tests with extracts from squash infected by back-assays from the previously mentioned hosts were used to confirm infection with isolate 1119. This isolate also induced chlorotic local lesions in *Chenopodium amaranticolor*.

Filamentous particles were seen in leaf-dip preparations from zucchini squash infected with isolate 1119. Ninety-two of 110 particles measured were 729–806 nm long (the modal class was 760 nm). Striated inclusion bodies typical for viruses that induce scroll and pinwheel inclusions (4,7) were detected in leaf extracts.

Antiserum prepared to isolate 1119 had a titer of 1/8 in SDS-immunodiffusion tests and gave strong reactions against sap from leaves infected with the homologous isolate (Fig. 1D). Weak reactions with extracts from healthy sap and undiluted 1119 antiserum were sometimes noted.

Isolate 1119 were detected by SDS-immunodiffusion tests in leaf extracts from systemically infected plants of the following species inoculated in the greenhouse: *Cucurbita pepo* 'Small Sugar Pumpkin,' 'Hybrid Zucchini,' 'Table King,' 'Early Prolific Straightneck,' 'Early Summer Crookneck,' and 'Gourmet Globe '; *Cucumis sativus* 'Table Treat,' 'Pickling SMR-18,' 'Chicago Pickling,' 'Poinsett,' 'Victory,' and 'Pic O' Pickle'; *Cucumis melo* 'Bit O' Honey'; *Citrullus lanatus* 'Congo' and 'Sugar Baby'; and *Melothria pendula*. Virus was detected in the inoculated leaves of *Phaseolus vulgaris* 'Black Turtle Soup 2.'

Isolate 1119 and ZYMV appeared serologically identical in SDS-immunodiffusion tests. The two antigens gave fused precipitin bands without spur formation when tested against either ZYMV antiserum (Fig. 3A) or antiserum to isolate 1119 (Figs. 1D and 3B). Likewise, in intragel absorption tests, the reactivity of the antiserum to isolate 1119 was neutralized by cross-absorption with ZYMV. Antiserum against isolate 1119 reacted weakly with WMV-2 antigen but the band formed by 1119 antigen formed spurs over the band formed by WMV-2. There were no cross-reactions observed between WMV-1 and isolate 1119 (Fig. 1C,D).

Serological relationships between ZYMV and isolate 1119 were also demonstrated by ELISA and IEM. In double-antibody sandwich ELISA with antiserum to the 1119 isolate, the average A_{405} values in two tests were 1.23, 0.62, and 0.01 for comparably diluted extracts from isolate 1119-infected squash, ZYMV-infected squash, and healthy squash, respectively. In reciprocal IEM decoration tests, the ZYMV antiserum and 1119 antiserum decorated particles in extracts from plants infected either with isolate 1119 (Fig. 4) or with ZYMV.

Isolate 1119 was transmitted in a styletborne manner by 5 of 25 individuals of *M. persicae* and isolate 81-25 was transmitted by 7 of 25 individuals of *A. citricola*. Symptoms induced in plants infected by aphid transmission were similar to those induced by mechanical transmission of these isolates. Transmissibility of each isolate was confirmed by demonstrating reactions of identity between mechanically transmitted and aphid-transmitted forms in SDS-immunodiffusion tests.

The SDS-immunodiffusion tests were used in 1982 to detect isolates in Alachua, Collier, Lake, Palm Beach, Pasco, and Sumter counties that were serologically identical to isolate 1119. Such isolates were associated with strong mosaic symptoms, fruit abnormalities (malformation and disruption of color patterns in yellow squash), and apparent loss of production.

Based on particle morphology, induction of striated cytoplasmic inclusions, styletborne aphid transmissibility, and serological relationships, isolate 1119 is a potyvirus closely related to ZYMV. The reactions of squash, *Chenopodium amaranticolor*, *L. acutangula*, and Alaska pea to isolate 1119 are similar to those induced by ZYMV (13; D. E. Purcifull, *unpublished*). The ZYMV types of isolates were found in 87 of 294 squash plants collected in Florida from November 1981 to June 1983. Diagnoses by county were as follows (number of samples positive for ZYMV-isolates/total

number of samples studied): Alachua (2/76), Collier (2/34), Dade (0/34), Lake and Sumter combined (59/103), Madison (2/2), Palm Beach (15/35), and Pasco (7/10). We conclude that at least three potyviruses, WMV-1, WMV-2, and a virus closely related to ZYMV, are associated with the mosaic disease complex of squash in Florida and that the latter virus represents a significant threat to squash and probably other cucurbits.

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