Zucchini Yellow Mosaic Virus Associated with Severe Diseases of Melon and Watermelon in Southern California Desert Valleys

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ABSTRACT

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A severe mosaic disease affected melon production in certain fields in Imperial County, CA, every spring during 1982–1984. Incidence of the disease has increased annually since 1982. The effects were especially severe in 1984, when diseased plants showed severe foliar mosaic and had small, deformed melon fruit with star-shaped cracking. Long, flexuous rod-shaped virus particles were associated with the disease. The virus was vectored by the green peach aphid (*Myzus persicae*). The host range was different from that of either watermelon mosaic virus-1 (WMV-1) or WMV-2 and was similar to that of zucchini yellow mosaic virus (ZYMV). Sap extracts from infected cantaloupes and purified virus reacted with a ZYMV antiserum from Italy. Antisera made against the California strain (ZYMV-Ca) did not react with WMV-1 or WMV-2. No resistance or tolerance to ZYMV-Ca was detected in seedlings of 30 cultivars of melon and watermelon grown in a greenhouse. Presently grown cultivars, selected in part for their tolerance to WMV-2, were included. Seed transmission of ZYMV-Ca was not detected in squash. Virus isolates from cucurbits grown in Oregon and Mexico also reacted with antiserum to ZYMV-Italy. ZYMV is a newly recognized threat to melon production in California.

The Imperial and Riverside counties of California are major melon-producing areas in the state. Watermelon mosaic virus-2 (WMV-2), WMV-1, squash mosaic virus, and cucumber mosaic virus have previously been isolated from diseased melons in California (4,7). A 1980–1981 survey of 1,000 plants from 10 cantaloupe fields in the Imperial Valley indicated that WMV-2 was the most common virus associated with mosaic diseases in the spring growing season (3). In the spring of 1982 and of 1983, isolated cantaloupe and mixed melon fields were found in the Imperial and Coachella valleys where many plants showed severe foliar mosaic and severe fruit deformation. One field in the Niland area was a complete loss in 1982. The explanation for these severe symptoms was unclear at the time because WMV-2, which is normally found in cantaloupe fields during the spring, does not cause severe symptoms on cantaloupe, especially on Topmark, the principal cultivar grown in the Imperial Valley. Topmark is somewhat tolerant to infection by WMV-2. By 1984, the unidentified disease caused major fruit losses in cantaloupe and watermelon fields in both the Coachella and Imperial valleys. Preliminary testing of field-collected tissue in 1982 and 1983 suggested that this disease was associated with a potyvirus distinct from WMV-2 and WMV-1 (8). This paper reports that the virus causing the unidentified disease is related to zucchini yellow mosaic virus (ZYMV), which causes severe diseases of cucurbits in Europe (5,6) and the United States (1,10,11). The virus is designated ZYMV-Ca in this report. The incidence and extreme severity of ZYMV-Ca, its effects on southern California desert melon production, and varietal susceptibility, cross-protection, and its lack of transmission by seed are discussed.

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MATERIALS AND METHODS

Viruses. Sap from field-grown tissue infected with ZYMV-Ca was diluted (1:1, w/v) in 0.02 M potassium phosphate buffer, pH 7.0, containing 1% Celite (w/v) and rub-inoculated onto 10-day-old seedlings of yellow squash (*Cucurbita pepo* L. 'Early Prolific') and cantaloupe (*Cucumis melo* L. 'Topmark'). The most severe isolate taken from cantaloupe in the Niland field was designated isolate 459. This isolate was sap-inoculated into Early Prolific squash, and these plants were used as sources of inoculum for virus purification, host range, cross-protection, and serological tests.

The WMV-1 isolate used was provided by H. Johnson, Cooperative Extension, University of California, Riverside. The virus systematically infected Early Prolific squash, Topmark cantaloupe, and *Luffa acutangula* Roxb. but not *Phaseolus vulgaris* L. 'Black Turtle 2' or *Chenopodium amaranticolor* Coste & Reyn. Sap from *L. acutangula* infected with WMV-1 was inoculated to squash. The virus was kept in squash until needed. The WMV-2 isolate used was collected by J. A. Dodds from yellow squash grown in Connecticut. It caused mosaic in Early Prolific squash, Topmark cantaloupe, and Black Turtle 2 bean and local lesions on *C. amaranticolor*. Sap from Black Turtle 2 bean tissue infected with WMV-2 was used to inoculate squash. Infected squash plants were used as a source of inoculum for the experiments.

Virus purification. Virus was purified by the method of Purcifull and Hiebert (13). Tissue (400 g) was homogenized with a Waring Blendor in a solution containing 800 ml of 0.5 M potassium phosphate, pH 7.5, 2 g of Na_2SO_3, 200 ml of chloroform, and 200 ml of carbon tetrachloride. The extract 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 (PEG 6000) was added at the rate of 8 g/100 ml. The mixture was stirred for 1 hr at 4 C and centrifuged at 12,000 *g* for 10 min. 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 of 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 equal volume of buffer, and centrifuged at 12,000 *g* for 10 min. The supernatant was diluted further and subjected to high-speed centrifugation at 120,000 *g* for 1 hr. The pellet was resuspended in 0.02 M Tris buffer, pH 8.2.

Host range. Purified virus was diluted in 0.02 M phosphate buffer, pH 7.5, containing 1% Celite (w/v) and rub-inoculated onto seedlings of *Cucurbita pepo* 'Early Prolific,' *Chenopodium amaranticolor, L. acutangula, Cucumis melo* 'Topmark,' and *P. vulgaris* 'Black Turtle 2.' Inoculated plants were kept in a greenhouse and observed for symptom expression. Sap from inoculated host range plants was inoculated to squash to determine which of these plants were infected.

Electron microscopy. Leaf dip of

infected tissue were prepared on 100-mesh Formvar-coated copper grids that were then coated with heavy carbon. All preparations were negatively stained with 2% potassium phosphotungstic acid, pH 7.0. Purified virus was also negatively stained. Stained preparations were examined with a Hitachi model HU12 transmission electron microscope.

Serology. Antisera to ZYMV-Ca were prepared by injecting 6-mo-old New Zealand white rabbits intramuscularly with purified virus emulsified with Freund's complete adjuvant (1:1, v/v). Each rabbit was injected initially with 0.5 mg of purified virus. The rabbit was subsequently injected with 0.5 mg of virus 10 days after the first injection, then with 0.2 mg of virus 14 days after the first injection. The first bleeding was conducted 10 days after the final injection. Antiserum to WMV-1 and ZYMV-Ca was a pool of early bleedings collected during the first month after the final injection. Antisera to WMV-2 were pooled late bleedings collected during the sixth month after the final injection. A second antiserum to WMV-2 was donated by D. E. Purcifull, University of Florida, Gainesville. An antiserum against ZYMV was provided by V. Lisa (Istituto di Fitovirologia Applicata CNR, Torino, Italy) (6). Squash tissue from Oregon was supplied by J. P. McMorren (Oregon State University). All isolates were tested for relatedness to ZYMV by direct enzyme-linked immunosorbant assay (ELISA) (2). The Ouchterlony double-diffusion (9) test was used to test the interrelatedness among isolates of ZYMV. The plates contained 0.9% Nobel agar and 0.3% sodium dodecyl sulfate (SDS) (12). Sap was diluted 1:1:1 (w/v/v) in water and 3% SDS.

Aphid transmission. Aphid transmission studies were conducted using the green peach aphid (*Myzus persicae* Sulz.). Aphids (both adult and nymphs) from colonies known to be nonviruliferous were placed in a petri dish containing moist filter paper and starved for 1–1.5 hr. Virus-infected leaf pieces were added to the petri dish, and the aphids were given a 20-min access to this tissue. They were then removed and placed on healthy Small Sugar pumpkin test plants (*Cucurbita pepo*). A small piece of wax paper was placed between the infected leaf piece and the healthy plant to avoid the possibility of mechanical inoculation. About 100–200 aphids per plant were moved from the infected leaf pieces and allowed to feed on the healthy plants. To determine the presence or absence of virus in the test plants, 0.5-g samples of leaf tissue were removed from the Small Sugar pumpkin plants 14 days after inoculation and processed for ELISA. All ELISA values ($A_{405\ nm}$) with an absorbance reading equal to or greater than twice that obtained with sap from healthy plants were considered positive results.

Cultivar trials. Ten-day-old seedlings of 30 cultivars of melon and watermelon were mechanically inoculated with sap from a squash plant infected with ZYMV-Ca. Four weeks after inoculation, the plants were evaluated for severity of mosaic and stunting, and the presence of ZYMV-Ca was confirmed by ELISA.

Cross-protection. For cross-protection experiments, 10-day-old Early Prolific squash seedlings (10 seedlings per virus tested) were mechanically inoculated on the cotyledons and the first true leaf with one of the following protecting viruses: WMV-1, WMV-2, or ZYMV-Ca. Ten days after inoculation, the second and third true leaves were inoculated with a challenge virus, either WMV-1, WMV-2, or ZYMV, that differed from the protecting virus. The presence of the protecting virus in the second and third true leaves was then confirmed by ELISA. Ten days after inoculation with the challenge virus, the fourth and fifth true leaves were assayed for the presence of the challenge virus by ELISA.

Seed transmission. About 1,400 seeds were harvested from several cultivars of squash plants that had been naturally infected with ZYMV-Ca. Infection by ZYMV-Ca and absence of either WMV-1 or WMV-2 in the mother plants was confirmed by ELISA. The naturally infected squash plants were grown commercially for seed production by a local seed company.

Seeds were planted in 4-in. fiber pots (five seeds per pot). When the seedlings reached the third true leaf stage, the third leaf from each plant was harvested. Single leaves from 10 plants were placed one on top of another, and a no. 14 cork borer was used to remove a 0.5-g core sample. The presence of ZYMV-Ca in the composite sample was determined by ELISA. Previous experiments indicated that a strong positive ELISA result ($A_{450\ nm} > 0.5$) could be obtained when only one leaf in 10 was infected with ZYMV-Ca. A value greater than twice that from the healthy control was interpreted as a positive reaction. Healthy control is defined in this case as a composite sample of 10 leaves taken from healthy squash plants. Plants that showed any abnormalities were tested as individual samples.

Field incidence. Symptomatic tissue collected from affected melon and watermelon fields during 1982–1984 were analyzed for the presence of WMV-1, WMV-2, and ZYMV-Ca by ELISA and double-diffusion tests. Field samples were obtained from California, Oregon, and Mexico.

RESULTS

Long, flexuous rod-shaped virus particles were observed in electron micrographs of both leaf dips and purified preparations of infected tissue from both field and experimentally inoculated plants. Of the 100 particles measured, 86 were between 730 and 800 nm long. The model length for all particles was 755 nm. Particles with these dimensions were not seen in negatively stained preparations of uninoculated plants.

ZYMV-Ca isolate incited a systemic mosaic on *Cucumis melo* 'Topmark,' *Cucurbita pepo* 'Early Prolific,' and *L. acutangula*, and caused local lesions on *Chenopodium amaranticolor* 14 days after inoculation. No symptoms were observed on *P. vulgaris* 'Black Turtle 2.' The presence or absence of virus was verified by ELISA (Table 1). Samples from cantaloupe, squash, *Luffa*, and *C. amaranticolor* infected with ZYMV-Ca gave ELISA values more than twice those obtained from sap of uninoculated plants. Viral antigen was not detected in *P. vulgaris* 'Black Turtle 2' in either inoculated or later-formed leaves. The plants selected are able to distinguish between WMV-1 and WMV-2, and these two viruses were also included in the host range experiment. The three viruses could be distinguished from each other by their effects on these hosts (Table 1).

Aphid transmission. Fourteen days after viruliferous aphids were placed on 32 healthy Small Sugar pumpkin plants, severe mosaic developed on all inoculated plants. Sap from all 32 plants tested positive for the presence of ZYMV-Ca in SDS immunodiffusion and in ELISA. Eight plants on which nonviruliferous aphids were placed showed no mosaic and gave negative reactions in ELISA.

Table 1. Enzyme-linked immunosorbent assay (ELISA) and symptom reactions of plants to zucchini yellow mosaic virus-Ca (ZYMV-Ca), watermelon mosaic virus-1 (WMV-1), and WMV-2, on selected hosts after mechanical inoculations

Virus	*Luffa acutangula*[a]	*Phaseolus vulgaris* 'Black Turtle 2'	*Chenopodium amaranticolor*	*Cucumis melo* 'Topmark'	*Cucurbita pepo* 'Early Prolific'
ZYMV-Ca	SM[b] (1.95)[c]	NR (0.03)	LL (2.50)	SM (2.55)	SM (2.60)
WMV-1	SM (2.35)	NR (0.045)	NR (0.03)	SM (2.43)	SM (1.98)
WMV-2	NR (0.04)	SM (2.22)	LL (2.56)	SM (1.94)	SM (2.60)

[a] Plants chosen were those normally used to distinguish between WMV-1 and WMV-2.
[b] SM = systemic mosaic, ELISA-positive; NR = no local or systemic reaction, ELISA-negative; and LL = local lesion, ELISA-positive.
[c] Numbers in parentheses are ELISA values representing the average of five samples for each plant species tested.

Serology. Antiserum developed against ZYMV-Ca did not react with sap from plants infected with WMV-2 and WMV-1 in immunodiffusion tests but did react with sap from plants infected with ZYMV-Ca (Fig. 1). An antiserum to an Italian strain of ZYMV reacted strongly with sap from plants infected with ZYMV-Ca, ZYMV-Mexico, and ZYMV-Oregon (Fig. 1). In reciprocal tests, WMV-2 and WMV-1 antisera did not react with sap from plants infected with ZYMV-Ca.

In ELISA, antisera developed against WMV-2 and WMV-1 did not react ($A_{405\ nm} < 0.05$) with ZYMV-Ca-infected plant sap. Similarly, antisera to ZYMV-Ca did not react with plant sap infected with WMV-1 or WMV-2 ($A_{405nm} \leq 0.06$). In comparable tests, homologous reactions (e.g., WMV-1 antiserum and sap from WMV-1-infected plants) produced values of $A_{405\ nm} \geq 1.0$.

Cross-protection. Cross-protection tests involving ZYMV-Ca, WMV-1, and WMV-2 indicated that infection with ZYMV-Ca did not protect squash plants against challenge inoculations of either WMV-1 or WMV-2. Conversely, infections of WMV-1 or WMV-2 did not protect squash plants against a challenge inoculation of ZYMV-Ca. This was true in all 10 plants tested per virus. Antigens of the challenge viruses were readily detected by ELISA in test plants.

Cultivar trials. Mosaic and stunting was observed on all cantaloupe, mixed melon, and watermelon cultivars inoculated with ZYMV-Ca. Cantaloupe cultivars Topmark, Topscore, PMR45[E], Mainstream, Perlita, Dulce, Westside, Exp. 484, Cinco, Hales Best Jumbo, Edisto 47, Smiths Perfect, Iroquois, Spartan Rock, Schoon's Hard Shell, ARTM (1804), AR5 (18006), ARHBJ (17900), Magnum 45, Harvest Pride, Valley Gold, Imperial 45[E], and S.J. 43; mixed melon cultivars 61254, G. F. Honeydew, Crenshaw, Juan Canari, Casaba, Bush Crenshaw, and U. C. Honeyloupe; and watermelon cultivars Klondike, Dixie Lee, and Charleston Gray showed severe mosaic and stunting. Only cantaloupe cultivar Perfection and watermelon cultivars Peacock and Congo showed moderate mosaic and stunting. Cantaloupe cultivars Topmark and Topscore (commonly grown in southern California with tolerance to WMV-2) showed no tolerance to ZYMV-Ca.

Seed transmission. Of the 1,400 squash seedlings tested for seed transmission of ZYMV-Ca, none gave a positive reaction for ZYMV-Ca in ELISA and none developed symptoms of ZYMV-Ca.

Field incidence. Samples of melon and watermelon from plants with mosaic from 20 fields were subjected to ELISA during the spring 1984 growing season. Of these, 75% contained WMV-2, 40% contained WMV-1, and 40% contained ZYMV-Ca (Table 2). More than one virus was detected in at least 50% of the samples tested. Incidence of ZYMV-Ca was increased dramatically from 2% in 1982 to 40% in 1984, and WMV-1 (which was not present in 1982 or 1983) had an incidence of 40% in 1984. Incidence of WMV-2 was 90, 85, and 85% in 1982, 1983, and 1984, respectively. The increase in the incidence of ZYMV-Ca was paralleled by an increase in the frequency of melon fields affected by severe mosaic diseases. In fields that contained plants infected with ZYMV-Ca, severe foliar and fruit deformation were common. Cantaloupe, watermelon, Crenshaw, and honeydew melon all showed severe leaf symptoms and fruit deformations such as large bumps and concavities, and in many cases, severe to mild cracking of fruit (Fig. 2). These symptoms are similar to those originally described for ZYMV infection in France (5).

DISCUSSION

On the bases of both particle morphology and aphid transmissibility, the virus associated with severely diseased melon and watermelon plants in the Imperial and Coachella valleys of California in 1984 was determined to be a member of the potyvirus group. This virus was not serologically related to WMV-1 or WMV-2 and differed in host reaction. Host range and serological tests

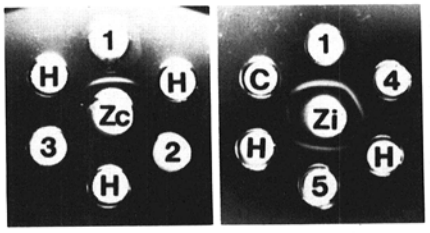

Fig. 1. SDS-immunodiffusion plate showing the relationship of zucchini yellow mosaic virus from California (ZYMV-Ca) with watermelon mosaic virus-1 (WMV-1) and WMV-2 and the relationship of ZYMV-Italy with ZYMV-Ca, ZYMV-Mexico, and ZYMV-Oregon. Zc = antisera to ZYMV-Ca, Zi = antiserum to ZYMV-Italy, 1 = squash sap infected with ZYMV-Ca, 2 = squash sap infected with WMV-2, 3 = squash sap infected with WMV-1, 4 = squash sap infected with ZYMV-Mexico, 5 = squash sap infected with ZYMV-Oregon, H = healthy squash sap, and C = buffer control.

Table 2. Incidence of watermelon mosaic virus (WMV-1), WMV-2, and zucchini yellow mosaic virus-Ca (ZYMV-Ca) identified by the enzyme-linked immunosorbent assay in samples of mosaic diseased plants collected in the Coachella and Imperial valleys of southern California (1982–1984)

Virus	Incidence (%)		
	1982[a]	1983[b]	1984[c]
WMV-2	90	85	85
WMV-1	0	0	40
ZYMV-Ca	2	10	40

[a] About 100 samples tested.
[b] About 100 samples tested.
[c] About 200 samples tested.

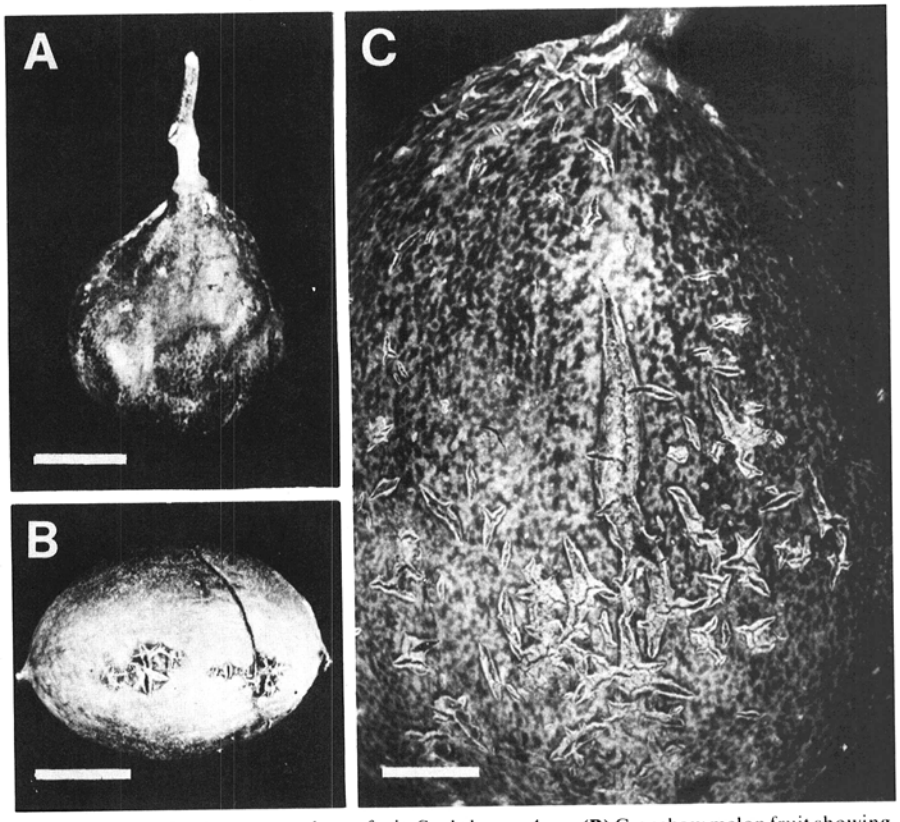

Fig. 2. (A and C) Immature cantaloupe fruit. Scale bars = 4 cm. **(B)** Crenshaw melon fruit showing deformation and star-shaped cracking associated with a field infection with zucchini yellow mosaic virus from California. Scale bar = 1 cm.

proved it to be an isolate of ZYMV. In a recent study, WMV-2 was reported to be the only potyvirus associated with melon mosaic diseases found in 10 fields in the Imperial Valley in the spring (3). Incidence of WMV-2 has continued to be high in subsequent years. The increasing occurrence of ZYMV-Ca, however, is of concern because of the severe losses associated with this virus. ZYMV has been recently reported as a new problem in cucurbit production in New York, Connecticut, Florida, and in one isolated area in central California (1,10,11). The virus was probably present in southern California as early as 1981 (S. T. Nameth, *unpublished*) and was probably a localized problem in cantaloupe production in 1982 and 1983 (8). It was not until the spring of 1984 that the virus caused severe losses in melon production and the incidence of ZYMV-Ca rose to 40% compared with 2% in 1982 and 10% in 1983 (Table 2).

This dramatic increase in virus incidence may be a result of the mild winter of 1983–1984, which favored record numbers of aphids in both the Imperial and Coachella valleys. The high vector population, along with earlier planting by some growers may have been key factors in the severe yield reduction in spring melons. Melon yields in the Imperial Valley in the spring of 1984 were reduced 40–50% compared with past years (F. Laemmlen, *personal communication*). Although the unusually high yield loss may be the consequence of the high incidence of ZYMV-Ca, the increased presence of WMV-1 and any interaction between the two viruses and WMV-2 cannot be ruled out as contributing factors. Mixed infection studies involving WMV-1, WMV-2, and ZYMV-Ca should be performed to clarify this point. More work is also needed on the possible involvement of seed transmission in the rapid spread of ZYMV. A variety of hosts other than squash and cantaloupe should be tested. Lack of seed transmission in muskmelon was previously reported (5), and it appears the virus is not seedborne in squash. There is some indication that the California isolate may have properties slightly different from those of isolates previously reported in the United States (10,11). The California isolate of ZYMV does not incite local infection in inoculated leaves of *P. vulgaris* 'Black Turtle 2' as do the Florida and Connecticut isolates (10,11), and the virus cannot be detected by serology in inoculated leaves. More detailed serological and biochemical testing is needed to determine the relatedness of the California isolate to other isolates.

ZYMV-Ca, which is now a major threat to melon production in southern California, is also present elsewhere on the West Coast, including Mexico and Oregon. A survey of the distribution of this virus throughout the rest of California is needed.

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