Occurrence of Zucchini Yellow Mosaic Virus in Cucurbits from Connecticut, New York, Florida, and California

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ABSTRACT

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Zucchini yellow mosaic virus (ZYMV) was found infecting cucurbits in some areas of Connecticut, New York, Florida, and California during the growing seasons of 1982 and 1983. It was also recovered from mottled cucumber fruits sold in supermarkets of Geneva, NY, during the winter of 1982–1983. Two strains of this virus were recognized, ZYMV-CT and ZYMV-FL. The first strain incited symptoms resembling those described for the French and Italian isolates of the virus, whereas the second caused symptoms that could be attributed to watermelon mosaic virus 1 (WMV-1). However, both ZYMV strains appeared to have the same host range and were serologically indistinguishable. An antiserum to cytoplasmic inclusion proteins of ZYMV-CT reacted with various isolates of ZYMV but not with those of WMV-1 and WMV-2. Sources of resistance or tolerance to both American strains of ZYMV were found in accessions of *Citrullus colocynthis, Cucurbita* spp., *Cucumis sativus, Cucumis melo,* and *Lagenaria siceraria.*

In the summer of 1982, a severe viral disease occurred on a 0.5-ha squash field near Warehouse Point in northern Connecticut. About 75% of the plants of the yellow squash cultivar Multipik (*Cucurbita pepo* L.) showed a prominent yellow mosaic, necrosis, and foliar distortion. Most of the fruits on infected plants were small and green with scattered glossy yellow knobs. Electron microscopy of negatively stained leaf dips revealed virus particles closely resembling those of a potyvirus (3). However, leaf and fruit extracts of infected plants failed to react with antisera to any of the three potyviruses known to occur in squash and other cucurbits in the Northeast, namely clover yellow vein virus (CYVV) (formerly known as the severe strain of bean yellow mosaic virus), watermelon mosaic virus 1 (WMV-1), and watermelon mosaic virus 2 (WMV-2) (15,16).

During 1983, a similar virus was recovered from severely infected cucurbits grown in fields in central Florida, western New York, and central California and cucumbers from Florida sold during the winter in two Geneva, NY, supermarkets.

This paper presents evidence that this unusual cucurbit virus was zucchini yellow mosaic virus (ZYMV), a recently recognized potyvirus that occurs in Europe, the Middle East, and Northern Africa (6). A brief account of the presence of this virus in the United States has been published (12).

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MATERIALS AND METHODS

Virus isolates and diagnostic species. Leaves and fruits of infected squash (*C. pepo*) were received from Warehouse Point, CT (August 1982), Sun City, FL (January 1983), and Santa Clara County, CA (October 1983). Leaves and fruits of infected cucumber, melon, squash, and watermelon were collected in fields of an experimental farm in Monroe County, NY, during July and August 1983. From 8 January to 26 March 1983, one cucumber showing a chlorotic mottle and some distortion was purchased each week from a local supermarket. All specimens were individually triturated with 0.05 M potassium phosphate buffer, pH 7.2, and the extracts rubbed on Carborundum-dusted leaves of diagnostic species reported in Table 1. Isolates of CYVV, WMV-1, and WMV-2 were used for comparative host range studies. To ensure the purity of each virus, all isolates were passed through three successive single-lesion transfers on *Chenopodium quinoa* Willd. Each isolate was maintained separately in Seneca zucchini squash, which also served as the source of inoculum.

Electron microscopy. Expressed sap from infected leaves was stained with 2% phosphotungstate (PTA), pH 6.5, and examined with a Jeol 100B electron microscope.

Purification of cytoplasmic inclusion proteins. Cytoplasmic inclusion proteins (CIP) from leaf tissue of Seneca zucchini squash infected with Connecticut isolate CT82-35 were partially purified according to the method of Dougherty and Hiebert

Table 1. Reaction of diagnostic species to four potyviruses infecting cucurbits: clover yellow vein virus (CYVV), watermelon mosaic virus 1 (WMV-1), watermelon mosaic virus 2 (WMV-2), and two strains of zucchini yellow mosaic virus, ZYMV-CT and ZYMV-FL

Species	CYVV	WMV-1	WMV-2	ZYMV-CT	ZYMV-FL
Chenopodium quinoa	L	L	···	L	L
Cucumis melo					
B66-5 or WMR-29	–	–[c]	SM	L,SYM,D,N	L,SM,D
Iroquois	–	SM,D	SM,D	L,SYM,D,N,	L,SM,D
Cucumis metuliferus					
PI 292190	L	–[c]	SM	L,SYM,D	L,SM,D
Acc. 2459	L,SCS	SM,D	SM	L,SYM,D,N	L,SM,D
Cucumis sativus					
Marketer	L	SM,D	SM	L,SYM,D	L,SM,D
Cucurbita pepo					
Seneca Zucchini	L,SCS	SM,D	SM	L,SYM,D	L,SM,D
Multipik	L,SCS	SM,D	SM	L,SYM,N	L,SM,D
Luffa acutangula	–	SM	–	L,SYM,D	L,SM,D
Nicotiana benthamiana	SM	–	SM	–	–
Phaseolus vulgaris					
Black Turtle 1	SYM,N	–	–[c]	–	–
Black Turtle 2	SYM,N	–	SM	L	L
Pisum sativum					
Alaska or Ranger	SYM,N	–	SM	L	L
Bonneville or	–[c]	–	–[c]	–	–
Little Marvel	–[c]	–	–[c]	–	–

[a] L = local infection, SM = systemic mottle or mosaic, SYM = systemic yellow mosaic, SCS = systemic chlorotic spotting, D = leaf distortion, N = necrosis, and – = no infection.
[b] No visible symptoms with some isolates.
[c] Lack of infection due to a known genetic factor.

(1) and modified by Yeh (21). Further purification of CIP was achieved by slab-gel electrophoresis, using Laemmli's buffer system (5). CIP was eluted from gel by the procedure of Hager and Burgess (2).

Antiserum preparation and serology. To produce an antiserum, 0.6 ml of purified CIP in 1 ml of 0.05 Tris-HCl, pH 7.9, that had been emulsified with 1 ml of complete Freund's adjuvant was injected into the toe pad and a muscle of a white New Zealand rabbit. Then 1.6 and 0.2 mg of CIP with incomplete Freund's adjuvant were injected after 1 and 5 wk, respectively. The rabbit was bled 21 and 28 days after the final injection. An antiserum to intact particles of ZYMV was provided by V. Lisa (Istituto di Fitovirologia Applicata, Turin, Italy). Other antisera used during this investigation had been prepared in our laboratory or were supplied by D. E. Purcifull and F. Zettler (University of Florida). Double-diffusion tests were conducted in sodium dodecyl sulfate (SDS) agar gel (0.8% Ionagar) containing 1% NaN_3 and 0.5% SDS, as reported by Purcifull and Batchelor (17).

Screening for resistance. Domestic and foreign cultivars of cucurbit species were secured from commercial sources and from USDA Regional Plant Introduction stations. Sixteen to 20 plants of each line of *Cucurbita pepo, C. maxima* Duch., *C. moschata* Duch. & Poir., *C. mixta* Pang., wild *Cucurbita* species, *Cucumis melo* L., *C. sativus* L., *Citrullus lanatus* (Thunb.) Matsun & Nakai, *C. colocynthis* Schrad., and *Lagenaria siceraria* (Mol.) Standl. were inoculated separately with the virus culture CT82-35 and the Florida isolate FL-H-82-50. Plants that remained free of local and systemic infection were classified as immune (I). Plants in which infection remained localized or developed transitory systemic symptoms were rated as resistant (R), whereas those responding with persistent mild foliar mottle and symptomless fruits were considered tolerant (T). Virus infection in test plants was confirmed by indirect enzyme-linked immunosorbent assay (ELISA) (9,21) using the antiserum to CIP of isolate CT82-35. Results were recorded by a Microelisa Auto Reader (MR580, Dynatech Instr. Inc., Torrance, CA). All plants were grown in an insect-free greenhouse maintained at 22–28 C.

RESULTS

Virus identification. *Isolates from Connecticut.* All six virus isolates recovered from Multipik yellow squash caused local or systemic infection on 11 of the 14 diagnostic hosts (Table 1). Cucumber, melon, squash, *Cucumis metuliferus* Mey., and *Luffa acutangula* Roxb. developed local chlorotic spots and a very prominent systemic veinal yellowing followed by yellow mosaic, green veinbanding, distortion, necrosis, and plant stunting. *Chenopodium quinoa*, Black Turtle 2 bean, and Alaska and Ranger pea responded with only a localized infection, and *Nicotiana benthamiana*, Black Turtle 1 bean, and Bonneville and Little Marvel pea were not infected. Reisolates from single-lesion transfers incited identical symptoms, indicating that only one virus was involved. On susceptible hosts, these isolates caused symptoms different from those incited by CYVV, WMV-1, and WMV-2, but they resembled those reported for ZYMV by Lisa et al (8) and by Lecoq et al (7) for the nonwilt type of this virus. In double-diffusion tests, the six isolates failed to react with cucumber mosaic virus (CMV), CYVV, squash mosaic virus (SqMV), WMV-1, and WMV-2 antisera; however, they reacted with prominent precipitin lines with an antiserum to the Italian isolate of ZYMV (8). No spurs were formed between lines that completely fused with each other. No known isolate of ZYMV was available for our study. The virus from Connecticut squash was therefore designated ZYMV-CT.

Isolates from Florida. These were derived from *C. pepo* breeding lines grown on a farm near Sun City, FL. Leaves of most of the plants were severely affected by prominent chlorotic mottle, deep serrations, dark green blisters, and deformation. In many cases, the apical growth consisted of small filiform leaves, and fruits (when present) were small, discolored, and heavily knobbed. Of nine virus isolates used for identification, four reacted with a WMV-1 antiserum, three with that of CIP of ZYMV-CT, and two with both of these antisera. No reaction was visible with antisera to CMV, CYVV, SqMV, and WMV-2. The Florida virus (ZYMV-FL) had the same host range as ZYMV-CT (Table 1); however, the symptoms incited by ZYMV-FL lacked the intense yellowing caused by ZYMV-CT. In addition, the incubation period of ZYMV-FL was 3–5 days longer than that of ZYMV-CT. In many instances, squash and cucumber plants infected with ZYMV-FL recovered partially from severe symptoms and produced normally shaped but chlorotic, mottled leaves.

Isolates from supermarket cucumbers. Of 12 isolates obtained from mottled cucumber fruits, nine reacted serologically with only WMV-1 and three with CIP of ZYMV-CT; however, these last three isolates caused symptoms identical to those incited by ZYMV-FL. Although it was not possible to determine the place of origin of every fruit, most were shipped from Florida.

Isolates from western New York State. Toward the end of July 1983, most cucumber, melon, squash, and watermelon plants grown in a 4-ha experimental farm in Monroe County, NY, had developed severe foliage and fruit symptoms. Seventy specimens were collected randomly from cultivars and breeding lines of six species, and extracts were tested with antisera to CMV, CYVV, SqMV, WMV-1, WMV-2, and CIP of ZYMV-CT. Results indicated that ZYMV was the most common and widespread, followed by WMV-2 and CMV, and that only a few plants were infected by CYVV (Table 2). These isolates of ZYMV caused symptoms identical to those incited by ZYMV-FL.

Isolates from California. These isolates originated from Golden zucchini plants grown in an isolated 2.5-ha field in Santa Clara County, CA. Viral infection was severe and widespread, and most of the plants showed symptoms resembling those incited by WMV-1. The golden yellow fruits were severely green-mottled or dark green with scattered yellow knobs. Six isolates recovered from individual plants reacted strongly with the antiserum to CIP of ZYMV-CT, and one also reacted slightly with WMV-1 antiserum. No reaction was observed with other antisera to cucurbit viruses. The host range of the California isolates was the same as for ZYMV-CT and ZYMV-FL, but symptoms closely resembled those incited by the latter.

Comparative serological tests. Three each of the ZYMV isolates from Connecticut, New York, Florida, California, and supermarket (Geneva, NY) cucumbers were compared serologically using the ZYMV antiserum from Italy and the CIP antiserum of ZYMV-CT. All antigens reacted with these antisera,

Table 2. Serological identification of viruses infecting cucurbit species on a farm in western New York[a]

Species	No. of samples	No. of specimens infected with:					
		CMV	CYVV	SqMV	WMV-1	WMV-2	ZYMV
Citrullus lanatus	6*	0	0	0	0	3	4
Cucumis melo	10	3	0	0	0	2	8
Cucumis sativus	14*	2	0	0	0	3	12
Cucurbita maxima	6*	0	0	0	0	3	5
Cucurbita moschata	3	0	0	0	0	3	3
Cucurbita pepo	31*	5	2	0	0	9	25

[a] Results from immunodiffusion tests using antisera to the following viruses: cucumber mosaic (CMV), clover yellow vein (CYVV), squash mosaic (SqMV), watermelon mosaic 1 (WMV-1), watermelon mosaic 2 (WMV-2), and to cytoplasmic inclusion proteins of zucchini yellow mosaic (ZYMV); * = some of these specimens were infected with more than one virus.

forming equally prominent precipitin lines that fused with each other, indicating that they belonged to the same serogroup. However, none of these antigens reacted with antiserum to WMV-Morocco. Similarly, antigens from plants infected with WMV-1 or WMV-2 failed to react with the CIP antiserum of ZYMV-CT.

Fruit symptoms caused by ZYMV. Under natural conditions, cucumber, melon, squash, and watermelon plants infected with either ZYMV-CT or ZYMV-FL produced fruits that were often malformed (Figs. 1–3). The number of affected fruits and the severity of symptoms depended on the stage of fruit development at the time of infection. Plants infected at an early stage of growth usually failed to set any fruits, but those that were infected during the flowering stage produced severely knobbed fruits. Color break occurred on fruits of every species, but it was most noticeable on those of yellow summer squash. Severe fruit malformation was readily induced in greenhouse-grown squash plants by delaying inoculation until the flowering stage. To avoid foliar damage, petioles were inoculated instead of laminae. After an incubation period ranging from 8 to 16 days, plants developed foliar and fruit symptoms that eventually were identical to those observed under field conditions. Similar results were obtained using WMV-1, a virus that also causes severe fruit distortion.

Sources of resistance to ZYMV. Several hundred cultivars and plant introductions (PI) were screened simultaneously for resistance to ZYMV-FL and ZYMV-CT. A complete list of the accessions used for each species is available on request. Most of the germ plasm tested was very susceptible, but resistance or tolerance was found in individual accessions of seven cucurbit species (Table 3).

No resistance or tolerance was found in *C. pepo*; however, a *Cucurbita* sp. from Nigeria and a *C. ecuadorensis* from Ecuador were resistant and will be valuable germ plasm for interspecific crosses. *C. ecuadorensis* was demonstrated previously to be resistant to several viruses (14). Line TMG-1, a single-plant selection of the Chinese cucumber Taichung Mou Gua, was resistant to both strains of ZYMV. A few other Oriental cucumbers showed some tolerance to ZYMV-FL but not to ZYMV-CT. Immunity was found in some plants of *C. melo* (PI 414723), originally from India, confirming the results with French isolates of ZYMV (18). All accessions of watermelon tested were susceptible to both strains of the virus, but two accessions of *C. colocynthis* from Nigeria were tolerant. Resistance was encountered in an accession of *Lagenaria siceraria* (PI 271353), which is also resistant to other viruses (11). Both the *Cucurbita* sp. 'Nigeria Local' and *C. colocynthis* 'Nigeria Local' were recently reported by Igwegbe (4) to be immune to a potyvirus

Fig. 1. Zucchini squash (*Cucurbita pepo*) severely affected by zucchini yellow mosaic virus.

Fig. 2. Yellow summer squash (*Cucurbita pepo*) from zucchini yellow mosaic virus infected plants; mostly green with yellow knobs.

Fig. 3. (Left): A fruit of line TMG-1 resistant to zucchini yellow mosaic virus. (Right): Cucumber fruits (*Cucumis sativus*) from plants infected with the same virus.

Table 3. Sources of resistance and tolerance in cucurbit species to two strains of zucchini yellow mosaic virus, ZYMV-Conn and ZYMV-Fla

Species	ZYMV-CT	ZYMV-FL
Citrullus colocynthis		
Nigeria Local (Nigeria)	T[a]	T
Egusi (Nigeria)	T	T
Cucurbita ecuadorensis		
(Ecuador)	R	R
C. foetidissima (Texas)	T	T
Cucurbita sp.		
Nigeria Local (Nigeria)	R	R
Cucumis sativus		
TMG-1 (selection from Taichung Mou Gua) (China)	R	R
Cucumis melo		
PI 414723 (India)[b]	I/S	I/S
Lagenaria siceraria		
PI 271353 (India)	R	R

[a] I = immune, R = resistant, T = tolerant, and S = susceptible.
[b] This line is a heterogeneous population containing immune and susceptible individuals.

infecting Ahu (*Cucumeropsis edulis* L.) in that country.

DISCUSSION

ZYMV was reported in Italy and France as a new and destructive pathogen of cucurbits in 1981 (7,8). In France, this virus was initially named muskmelon yellow stunt virus, but the nomenclature was changed after comparative studies (6). With antisera provided by French and Italian workers, ZYMV was detected in plants grown in Germany, Israel, Morocco, Spain, and the United States (6,12). In May 1983, this virus also was found to infect cucurbits in Egypt (R. Provvidenti, *unpublished*). It is also possible that the potyvirus affecting cantaloups in southern California (10) and that reported by Igwegbe in Nigeria (4) are related to ZYMV. Consequently, it appears that this virus is more widespread than initially suspected. The genesis of ZYMV and the reason it was not detected earlier are open to speculation.

ZYMV is spread by aphids in styletborne manner (7,8). An attempt to demonstrate seed transmission in *C. melo* was unsuccessful (7), but further studies are needed on this avenue of dissemination. Very little is known about the overwintering hosts of ZYMV. The experimental host range of French and Italian isolates (7,8) includes mostly cucurbit species and a few species in the Chenopodiaceae, Compositae, Leguminosae, Ranunculaceae, Scrophulariaceae, and Umbelliferae. However, none of these noncucurbit species is sufficiently widespread to be considered an important source of the virus. Thus, studies are needed on the epidemiology of ZYMV, particularly in the northern regions, due to the absence of overwintering cucurbit species.

The devastating epidemic caused by ZYMV in Egypt and the severe outbreak of the same virus on farms in western New York State, Florida, and California in 1983 illustrate the economic importance of this potyvirus. Consequently, one of the major objectives of this investigation was to find sources of resistance to ZYMV. Our screening for resistance and that in Montfavet (France) has established that sources of resistance or tolerance, although rare, are available and in most cases can be readily used in breeding programs. It is encouraging that our results agree with those reported by French workers (7,18,19). They have also differentiated two pathotypes of ZYMV on the basis of the reactions of *C. melo* cultivars (19). The American isolates of this virus can be categorized into two distinct strains: 1) ZYMV-CT, which incited very prominent yellow symptoms similar to those described in Italy and France (7,8); and 2) ZYMV-FL, which causes symptoms that could be attributed to WMV-1 infection. ZYMV-FL was recovered from cucurbits grown in New York, Florida, and California and thus appears to be the most prevalent strain.

This study has confirmed the results of European researchers regarding the absence of a serological relationship between WMV-1 and ZYMV (6–8). In addition, it has demonstrated that ZYMV is able to infect *C. melo* 'B66-5' and 'WMR-29' and *C. metuliferus* (PI 292190), which are highly resistant to WMV-1 (13,20). Although WMV-1 and ZYMV are distinct entities, it is often difficult under field conditions to differentiate the symptoms caused by these two viruses.

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