ZUCCHINI YELLOW MOSAIC VIRUS: A PATHOGEN OF SQUASH AND OTHER CUCURBITS IN FLORIDA¹

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Abstract. In virus surveys from 1981 to 1983, 3 potyviruses were detected in naturally infected squash (Cucurbita pepo L.) plantings in Florida. The viruses were watermelon mosaic virus-1 (WMV-1), watermelon mosaic virus-2 (WMV-2), and a third virus that caused mosaic and distortion in squash and was serologically distinct from the other 2. On the basis of host range, serological relationships, and particle morphology, it was determined that the third virus was zucchini yellow mosaic virus (ZYMV). ZYMV was also identified in naturally infected cucumber (Cucumis sativus L.), cantaloupe (C. melo L.) and watermelon [Citrullus lanatus (Thunb.) Mansfield)]. WMV-1 was the most common virus in the southern counties (Dade, Palm Beach, Collier) and WMV-2 was most common in central and northern counties (Lake, Sumter, and Alachua); however, ZYMV infections increased in relative proportions as the season progressed from fall to spring in Palm Beach County and from spring to fall in the Lake-Sumter area, and were more prevalent than those of WMV-1 and WMV-2 in squash in central Florida during the fall of 1982. ZYMV was detected in counties from Palm Beach in the south to Madison in the north. The widespread occurrence and severity of fruit and foliage symptoms induced by the ZYMV-type isolates indicate that this potyvirus is an important pathogen of squash in Florida and that it represents a significant new threat to squash and other cucurbits.

Previous surveys indicated that watermelon mosaic virus-1 (WMV-1) and watermelon mosaic virus-2 (WMV-2) were the viruses most commonly affecting watermelon and wild cucurbits in Florida (1). In watermelon, WMV-1 was most common in south Florida and WMV-2 was most common in central and north Florida. The prevalence of WMV-1 in central and north Florida crops increased through the summer and fall. In the wild cucurbits, only WMV-1 was found in *Momordica charantia* L.; WMV-1 was the most common virus affecting *Melothria pendula* L. and WMV-2 was detected infrequently (1, 2, and unpublished).

WMV-1 is a significant threat to the squash industry of south Florida, especially in southeastern Florida where yields are reduced each year and there are often periods of several weeks during heavy aphid flights when crops are lost (3). We initiated a survey to confirm that WMV-1 is the main viral problem of squash in Florida.

Among the first samples collected were isolates of squash virus that differed from WMV-1 and WMV-2 with respect to host range and serological properties. These isolates caused chlorotic local lesions in *Chenopodium amaranticolor* Coste and Reyn. and systemic mosaic in *Cucumis melo* L., selection 633-3, both typical WMV-2 reactions, but they did not respond to WMV-2FL antiserum.

An antiserum was prepared for isolate 1119. Using host range, serology, electron microscopy, inclusion body assay, and aphid transmission the virus was identified (7) as zucchini yellow mosaic virus (ZYMV). The virus was indistinguishable from ZYMV from Italy (6) and muskmelon yellow stunt virus from France (5), both strains of ZYMV (4).

In this paper we report the results of a survey for viruses in squash in Florida.

Materials and Methods

Plants that vary in their response to inoculation with different viruses (1) and sodium dodecyl sulfate (SDS) immunodiffusion techniques (8, 9) have been useful in identifying cucurbit viruses. Samples of infected cucurbits were ground in potassium phosphate buffer, pH 7.0, and the crude extracts were rubbed on carborundum-dusted leaves of various test plants. WMV-1 was identified by mosaic symptoms in 'Small Sugar' pumpkin (Cucurbita pepo L.), necrotic local lesions on the cotyledons of muskmelon 633-3 (C. melo), systemic mosaic of Luffa acutangula Roxb., and failure to infect C. amaranticolor, beans (Phaseolus vulgaris L.) and peas (Pisum sativum L.). WMV-2 was identified by mosaic symptoms in 'Small Sugar' pumpkin, muskmelon 633-3, 'Alaska' pea, 'Bountiful' and 'Tendergreen' beans, chlorotic local lesions in *C. amaranticolor*, and failure to infect L. acutangula. ZYMV was identified by mosaic symptoms in 'Small Sugar' pumpkin and muskmelon 633-3, chlorotic local lesions in C. amaranticolor, and latent infections in L. acutangula and 'Alaska' pea, and in 'Black Turtle Soup 2', 'Bountiful', and 'Tendergreen' beans that were detected by back inoculation to 'Small Sugar' (7).

The SDS immunodiffusion technique was used with No. 862 antiserum to WMV-1FL, No. 868 antiserum to WMV-2FL and No. 1028 antiserum to ZYMV-FL. Crude leaf extracts prepared with a mortar and pestle and treated with SDS were used as antigens in the method of Purcifull and Batchelor (8).

Host range studies with ZYMV-FL were conducted with isolates 1119 and 81-25, both collected in Sumter county in 1981.

Results and Discussion

Symptoms of ZYMV. ZYMV causes pronounced yellowing of infected plants. In straightneck yellow squashes such as "Goldbar' and 'Multipik', infected leaves develop vein clearing followed by general chlorosis marked with distinct, variable-sized, dark green spots (Fig. 1). Severely affected leaves are filiform. The normally yellow fruit are green with yellow blisters.

In green zucchini squash, leaves show vein clearing which may develop into a general chlorosis marked with rippled dark green vein banding and some green spotting (Fig. 2). Severely affected leaves are filiform. Fruit develop large green protrusions and blisters and severe distortions.

Soon after infection, yield of marketable squash ceases, especially in yellow cultivars. Severity of symptoms from common viruses on squash in Florida was in the order WMV-2, WMV-1, and ZYMV, ranked from least to most severe.

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Fig. 1. Chlorosis and leaf spotting symptoms of ZYMV in 'Multipik' squash.



Fig. 2. Chlorosis, rippled vein banding, and green spotting symptoms of ZYMV in 'Elite' zucchini squash.

Table 1. Host reactions to mechanical inoculation by Florida isolates FC 1119 or 81-25 of zucchini yellow mosaic virus.

Host <i>Citrullus lanatus</i> (Thunb.) Mansfield Cy : 'Charleston Gray' 'Congo' 'Sugar Baby'			
Cucumis sativus L. Cv.: 'National Pickling', 'Poinsett', 'Victory', 'Chicago Pickling'	М		
Cucumis melo L. Cy.: Bit O'Honey', selection 633-3.	М		
Luffa acutangula Roxb. Melothria pendula L. Momordica charantia L. Chenopodium amaranticolor Coste & Reyn. Pisum sativum L. Cy · Alaska	SLat. M NR LL LLat.		
Phaseolus vulgaris L. Cv.: 'Bountiful', 'Tendergreen', 'Black Turtle Soup 2'.	LLat.		

 ^{z}M = systemic mosaic; NR = no reaction; LL = local lesions; SLat. = systemic, no symptoms; LLat. = local, no symptoms.

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Host range. ZYMV infects host plants outside the family Cucurbitaceae (Table 1), a characteristic shared by WMV-2, but not usually by WMV-1 (11). In the leguminous plants tested, WMV-2 causes systemic mosaic and ZYMV causes a latent infection which was detected by back inoculation to 'Small Sugar' pumpkin.

L. acutangula has been used to separate and maintain WMV-1 free from WMV-2 (10). ZYMV causes latent infection in L. acutangula, so this plant cannot be used to isolate WMV-1 from ZYMV in mixed infections. Perhaps M. charantia can be used to isolate WMV-1 from WMV-2 and ZYMV in Florida. All attempts to infect M. charantia with WMV-2 and ZYMV have failed. In France, however, ZYMV isolates infect M. charantia systemically (4).

ZYMV could be isolated from mixed infection by inoculating the plant sequence *L. acutangula* (eliminates WMV-2), *P. sativum* (eliminates WMV-1), and 'Small Sugar' pumpkin to give ZYMV alone.

Distribution. WMV-1 is common in squash in southern counties; it is not common in northern counties in the spring but increases in relative abundance in the fall (Table 2). WMV-2 is common in squash in central and north Florida, but was not recovered from infected squash in Dade, Palm Beach, and Collier counties.

ZYMV isolates were found in various locations from Collier county in the south to Madison county in the north. The virus was especially common in Lake and Sumter county plantings where it increased in relative abundance from spring to fall and in Palm Beach county where it increased from fall to spring. ZYMV was recovered in Lake county from the wild perennial cucurbit, *M. pendula*. ZYMV isolates were also identified in cultivated cucurbits other than squash. Mixed infections of WMV-1 + WMV-2,



Fig. 3. Serological relationships of a Florida isolate of zucchini yellow mosaic virus (ZYMV-FL) to other potyviruses that infect cucurbits. Well A contains undiluted antiserum to ZYMV-FL. The other wells contain sodium dodecyl sulfate (SDS)-treated extracts from *Cucurbita pepo* leaves as follows: F-infected with ZYMV-FL; Z-infected with an Italian isolate of ZYMV; M-infected with an isolate of watermelon mosaic from Morocco; H-healthy; 1-infected with watermelon mosaic virus 1; 2-infected with watermelon mosaic virus 2. The tests were conducted in agar gels containing SDS.

Table 2. Seasonal and regional distribution of cucurbit viruses in Florid	a plantings of squash,	Cucurbita pepo L	. November	1981-September	1983.
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County/Season	WMV-1z	WMV-2	ZYMV	1+2	1+ZYMV	2+ZYMV	Total of samples taken
Lake-Sumter						-	
Spring	17	15	11		2	1	30
Autumn	16	14	48	1	4	_	83
Palm Beach					_		10
Spring	5	-	10	-	3	-	18
Autumn	15	-	-	-	2	-	17
Pasco					_		10
Autumn	3	—	6	_	1	-	10
Madison							,
Spring		_	4	-			4
Alachua							
Spring		17	_	_		-	17
Autumn	9	19	-	8	2	-	38
Hamilton							•
Autumn	-	-	2	-	-	-	2
Collier					_		00
Spring	21		1	-	1		23
Autumn	11	-	-		-		11
Dade							10
Spring	19		_	-	-	-	19
Autumn	15	_	_	—	—	-	15
 Totals	115	65	82	9	15	1	287

^zWMV-1 = watermelon mosaic virus-1; WMV-2 = watermelon mosaic virus-2; ZYMV = zucchini yellow mosaic virus. Cucumber mosaic virus was detected in one sample collected in Alachua county in the spring of 1983.

Number of samples containing the virus indicated by the column heading.

WMV-1 + ZYMV, and WMV-2 + ZYMV were found in commercial cucurbits.

Although ZYMV was only recently described (6), it is widely distributed. It occurs in Italy, France, Spain, Israel, Morocco, Germany, northeastern United States (4) and southeastern United States (7). Florida ZYMV isolates were serologically unrelated to WMV-1 and a watermelon mosaic virus from Morocco, related to WMV-2, and indistinguishable from an Italian ZYMV isolate (Fig. 3). It is a new virus to Florida and much more severe than the viruses of cucurbits commonly encountered previously.

In Dade and Palm Beach counties, the number 1 and 2 summer squash producing counties, the main virus problem has been WMV-1. We felt that squash cultivars resistant to WMV-1 would provide considerable relief to South Florida growers, and a program was initiated to develop this re-sistance. Since ZYMV is now present in these areas, is more severe than WMV-1, and apparently spreads faster than WMV-1 or WMV-2, WMV-1 resistance alone could not be depended on to solve the mosaic problem in squash. Nothing is currently known about resistance to ZYMV in C. pepo. This presents a significant new challenge to squash breeders to develop cultivars with multiple virus resistance.

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