Detection of Cucurbit Viruses in New Jersey

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ABSTRACT

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During a 3-yr study of virus diseases of cucurbits in New Jersey, we found that different viruses were associated with severe disease symptoms, depending on the year. In 1983, cucumber mosaic virus (CMV) caused the most severe disease in squash (*Cucurbita pepo*) although watermelon mosaic virus 2 (WMV-2) was the most prevalent. In 1984, the watermelon mosaic strain of papaya ringspot virus (PRSV-W) caused a destructive disease of squash. Zucchini yellow mosaic virus (ZYMV) was detected for the first time in New Jersey in 1985 and caused severe losses in squash and other cucurbit crops. In field samples infected with various mixtures of ZYMV, WMV-2, and PRSV-W, ZYMV usually predominated after rub-inoculation of susceptible test plants and detection by enzyme-linked immunosorbent assay (ELISA). ELISA of field samples was thus more reliable as an indication of the viruses present in such samples than ELISA of experimental test plants rub-inoculated with sap from field samples. ZYMV is highly aggressive and appears to have a competitive advantage over PRSV-W and WMV-2 in mixed infections. Most ZYMV isolates occurring in New Jersey were similar to the Connecticut strain (ZYMV-CT); however, one isolate of ZYMV from zucchini, designated ZYMV-NJ_{sn}, unique from previously reported isolates in its ability to induce severe stunting and necrosis in squash, represents another biotype of ZYMV.

Viruses causing diseases in cucurbit crops in the eastern United States (13,14, 18,20) include watermelon mosaic virus 2 (WMV-2 [17]), watermelon strain of papaya ringspot virus (PRSV-W [16]), cucumber mosaic virus (CMV [7]), squash mosaic virus (SqMV [2]), and zucchini yellow mosaic virus (ZYMV [10]). During a survey of squash (*Cucurbita pepo* L.) conducted in Cumberland County, New Jersey, in 1969, Webb (20) found PRSV-W (formerly WMV-1), WMV-2, and CMV. SqMV and the severe strain of bean yellow mosaic virus were reported in New Jersey in 1974 (14) and 1977 (18), respectively.

Viruses continue to cause severe diseases in cucurbit crops in New Jersey, particularly in those planted midsummer to late summer. To provide a continuous supply of fruits for the fresh market, many growers plant successively at 2-wk intervals throughout the growing season. These fields are often near one another. This situation favors buildup and spread of viruses by aphid vectors. Limited control of aphid-transmitted cucurbit viruses can be accomplished with reflective mulches (12), particularly aluminum foil, but this is not a popular method because of the cost and the inconvenience of applying and removing the mulch.

This study was undertaken to assess the occurrence of viruses in cucurbits in New Jersey, more specifically: 1) the presence of any new viruses, such as ZYMV; 2) the major viruses associated with disease losses; and 3) the incidence of these viruses alone and in mixed infections. This information is needed to design improved control strategies.

MATERIALS AND METHODS

Sample collection. Foliar samples, including a young leaf and a fully expanded mature leaf, were collected in New Jersey from cucurbit plants in both research breeding plots and commercial fields from August through October during 1983–1985. In 1983, 127 samples were collected from 10 farms in Atlantic, Burlington, Cumberland, and Middlesex counties. In 1984, 24 samples were collected from one farm in Gloucester County, and in 1985, 94 samples were

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collected from nine farms in Cumberland County. An attempt was made to collect samples from plants representing a wide range of symptoms, from mild to severe, within each field. In 1985, fruits were also collected from zucchini plants. Leaf and fruit samples were taken to the laboratory for testing by enzyme-linked immunosorbent assay (ELISA) and storage by desiccation in case of future need. Selected samples were also mechanically inoculated onto Carborundum-dusted test cultivars by rub-inoculation of sap prepared in 0.02 M sodium phosphate buffer, pH 7.0. Viruses used as controls in ELISA were maintained in the greenhouse by periodic subculturing in squash.

Virus identification. Viruses were identified by ELISA as described later, and in some cases, by Ouchterlony agar gel double diffusion and electron microscopy as described previously (5). The samples collected in 1983 and 1985 were assayed by the double-sandwich indirect method of ELISA (DSI-ELISA [1,4,8]), whereas those collected in 1984 were tested by an indirect method of ELISA (I-ELISA [5]). Selected isolates were further evaluated by symptomatology after mechanical inoculation to some or all of the following hosts: Multipik and Early Prolific Straightneck yellow squash; Elite, Senator, and Cocozelle zucchini squash (*Cucurbita pepo*); National Pickling and Poinsett cucumber (*Cucumis sativus* L.); *Chenopodium quinoa* Willd.; Campbells 147 tomato (*Lycopersicon esculentum* Mill.); ground cherry (*Physalis floridana* Ryd.); jimsonweed (*Datura stramonium* L.); Yolo Wonder pepper (*Capsicum annuum* L.), Just Right turnip (*Brassica rapa* L.); globe amaranth (*Gomphrena globosa* L.); Samsun, Samsun NN, and Xanthi tobacco (*Nicotiana tabacum* L.); *N. glutinosa* L.; PI 292190, and accession 2459 of *Cucumis metuliferus* Mey.; and Black Turtle 2 (BT-2) bean (*Phaseolus vulgaris* L.). The last three mentioned hosts were provided by R. Provvidenti (Cornell University, Geneva, NY).

Virus isolates. An isolate of ZYMV from Egypt (ZYMV-E), the American Type Culture Collection (ATCC) strain (PV 23) of papaya ringspot virus (PRSV-W-ATCC), and the ATCC strain (PV 27) of WMV-2 (WMV-2-ATCC) were from H. A. Scott (University of Arkansas, Fayetteville). Florida isolates of WMV-2 (WMV-2-Fl) and SqMV (SqMV-Fl) were from D. E. Purcifull (University of Florida, Gainesville). CMV isolated from squash in New York (CMV-NY) was from R. Provvidenti. All isolates were previously tested by DSI-ELISA. Viruses were maintained by periodic subculturing in squash. ZYMV-TS2 (5) and CMV-NJ83-42 were previously isolated from squash in Turkey and New Jersey, respectively.

Antibodies. WMV-2-ATCC, PRSV-W-ATCC, and ZYMV-E antisera were from H. A. Scott. Antiserum to SqMV was from V. Lisa (Istituto di Fitovirologia Applicata CNR, Torino, Italy). D. Gonsalves (Cornell University, Geneva, NY) provided antisera to cylindrical inclusion protein of PRSV from Hawaii (PRSV-HA). D. E. Purcifull provided antisera to PRSV-W-Fl and to SqMV-Fl. Antibodies to ZYMV were prepared from Turkish and South Carolina squash isolates (ZYMV-TS2 and ZYMV-SCB, respectively), and antibodies to CMV were prepared from *Vinca minor* and *P. vulgaris* isolates (CMV-Vi and CMV-B, respectively) as described previously (5,6).

Serology. Ouchterlony agar gel double diffusion tests (15) were performed as described previously (5). For ELISA, immunogammaglobulins (IgG) were purified from rabbit antisera or murine ascites fluid by affinity chromatography on a 3-cm column of protein A-sepharose CL-4B (Pharmacia Fine Chemicals, Piscataway, NJ) prepared by suspending 0.5 g in 0.02 M sodium phosphate buffer, pH 7.3. When the absorbance of the eluate at 280 nm was 0.01 or less, the buffer was changed to 0.1 M glycine, pH 3.0, to elute the IgG. Fractions were neutralized with 40 μl of 2 M Tris buffer, pH 8.5, and IgG was precipitated with 0.9 volume of saturated ammonium sulfate. $F(ab')_2$ fragments for DSI-ELISA were isolated from pepsin-digested IgG using a column of protein A-sepharose CL-4B (8).

ELISA was performed in polystyrene plates (NUNC-Immunoplate II, Vangard International, Neptune, NJ), using the buffers described by Clark and Adams (3). For DSI-ELISA (1,4,8), plates were coated with 1 μg/ml $F(ab')_2$ in carbonate coating buffer (pH 9.6) for 4 hr at 37 C. Leaf tissue was triturated in 10 volumes (w/v) of sample buffer (PBS-Tween buffer, pH 7.4, with 2% polyvinylpyrrolidone-40,000 and 0.2% ovalbumin) and incubated overnight at 4 C. Virus-specific IgG was added at 1 μg/ml in sample buffer for 3 hr at 37 C, followed by protein A-alkaline phosphatase conjugate (Code 10-1022, Zymed Laboratories, Burlingame, CA) at 1:1,000 (v/v) dilution in sample buffer for 3 hr at 37 C. In 1983, the antisera used included PRSV-W-Fl, WMV-2-ATCC, CMV-B, SqMV-It, and ZYMV-E, and antigens were PRSV-W-ATCC, WMV-2-ATCC, CMV-NY, SqMV-Fl, and ZYMV-E. In 1985, the antisera used included PRSV-W-ATCC, WMV-2-ATCC, CMV-Vi, and ZYMV-SCB, and antigens were PRSV-W-ATCC, WMV-2-Fl, CMV-NJ83-42, and ZYMV-E. Each plate contained antibodies specific for one virus, and controls consisted of sap from uninoculated plants and from plants inoculated with each of the viruses.

I-ELISA was performed as previously described (5). Samples were triturated in coating buffer at 1:100 dilution (w/v) and incubated 4 hr at 37 C, followed by overnight incubation at 4 C with a 1-μg/ml concentration of PRSV-W-ATCC, WMV-2-ATCC, SqMV-Fl, and CMV-B IgGs or a 1:16,000 dilution of ZYMV-TS2 murine ascites fluid. Each plate contained sap from uninoculated plants and from plants inoculated with PRSV-W-ATCC, WMV-2-ATCC, SqMV-Fl, CMV-NJ83-42, and ZYMV-TS2.

Reactions were measured at 405 nm absorbance in a Titertek Multiskan MC apparatus (Flow Laboratories, McLean, VA) 20 min to 2 hr after addition of substrate. Samples and controls were tested in triplicate, and reactions were considered positive if the mean absorbance minus two standard deviations of the sample exceeded the mean absorbance plus two standard deviations of the uninoculated control.

Purification. CMV was purified by the method of Lot et al (11) with modifications as described previously (6). PRSV-W was purified by the method of Sako et al (19) with modifications as described previously (5).

RESULTS

In 1983, 67% of samples collected were infected with virus(es). Of the 127 samples collected from watermelon (*Citrullus lanatus* (Thunb.) Matsum & Nakai), cantaloupe (*Cucumis melo* L.), cucumber, winter squash (*Cucurbita maxima* Duch.), and yellow, zucchini, and pumpkin squash, 60 were infected singly with WMV-2, six with PRSV-W, five with CMV, and one with SqMV but none with ZYMV (Table 1). In addition, nine plants were infected with a mixture of WMV-2 and CMV, two with CMV and SqMV, one with WMV-2 and PRSV-W, and one with CMV, WMV-2, and PRSV-W. Symptomatology varied from mild mosaic or veinbanding to severe mosaic, malformation, and stunting. Mild symptoms were associated most often with single infections of WMV-2.

In 1983, the most severe disease of cucurbits, characterized by extreme stunting, mosaic, and malformations, occurred in a commercial field of Multipik squash in Cumberland County. Seven plants with these symptoms, collected in one location, contained both CMV and WMV-2, whereas plants with milder symptoms in the same field contained only WMV-2. Extracts from two of the plants with mild symptoms, determined by DSI-ELISA to be infected with WMV-2 alone (NJ83-54 and NJ83-56), and extracts from three plants with severe symptoms, determined by DSI-ELISA to be infected with CMV and WMV-2 (NJ83-41, NJ83-42, and NJ83-43), were inoculated onto squash and BT-2 bean. Squash plants infected by both CMV and WMV-2 developed chlorotic local lesions followed by systemic mosaic, veinbanding, malformation, and stunting, similar to the symptoms observed in the field, whereas the squash

plants infected by WMV-2 produced only mild mosaic. WMV-2 alone, and in combination with CMV, induced epinasty and mild mosaic in BT-2 bean.

To separate WMV-2 from mixed infections, two of these samples (NJ83-42 and NJ83-43) were subcultured twice through BT-2 bean, a systemic host for WMV-2 but not CMV. These two isolates and one from a field sample infected only by WMV-2 (NJ83-54) caused veinbanding and mild mosaic in BT-2 bean and mild mosaic in squash but did not cause symptoms in cucumber, tomato, jimsonweed, tobacco, or ground cherry.

CMV was separated from mixed infection by subculturing in Samsun NN tobacco. Back-inoculation with New Jersey CMV isolates NJ83-41, NJ83-42, and NJ83-43 in squash caused the same type of severe symptoms observed in the original plants infected in the field. In addition, CMV caused chlorotic local lesions in cucumber, jimsonweed, and *C. quinoa* and mosaic, malformations, and stunting in cucumber, tomato, and ground cherry. CMV isolate NJ83-42 was purified from tobacco and squash, and the host reactions were identical to those mentioned before. Electron microscopy revealed icosahedral particles characteristic of CMV. Flexuous rods were not present in the purified preparation.

During 1984, a very severe disease outbreak occurred in commercial fields in Gloucester County. One field of Multipik yellow squash and an adjacent field of Elite zucchini squash (about 1 ha each) were 100% affected for a total crop loss. Symptoms resembled those reported for ZYMV and included severe mosaic, stunting, and malformations of the foliage, such as blistering and shoestringing. Symptoms on the fruits of Multipik included malformations and color change from yellow to green, resulting in unmarketable fruits.

Two Elite squash samples were first tested by Ouchterlony gel double diffusion and found infected with PRSV-W using PRSV-HA cylindrical inclusion protein antiserum. All samples, 20 Elite and four Multipik squash, were infected with PRSV-W but not with ZYMV, WMV-2, CMV, or SqMV, as determined by I-ELISA. Extracts from four Elite and two Multipik samples were rub-inoculated onto squash and cucumber plants, which reacted with severe systemic mosaic and occasionally necrosis. No symptoms were observed in *C. quinoa* or BT-2 bean. One of the isolates from Multipik squash (NJ84-72) was purified and then maintained in squash by periodic subculturing. This isolate was used to inoculate 12 of 25 healthy Elite and 12 of 25 healthy Multipik seedlings in the field in late June 1985. Symptoms on the inoculated plants were identical to those originally observed in 1984, and aphids present in the field spread the virus rapidly to all 26 uninoculated plants. The presence of PRSV-W in all plants, but not WMV-2 or CMV, was confirmed by I-ELISA.

In 1985, a severe disease occurred in squash crops during late summer. As in 1984, symptoms included severe mosaic and malformations of both foliage and fruit, resulting in crop losses. In 1985, virus was detected in 97% of the symptomatic samples collected, and ZYMV was found in 87% of them. Of the 94 samples collected in 1985, 26 were infected with WMV-2 and ZYMV, 23 with ZYMV alone, 20 with PRSV-W, WMV-2, and ZYMV, 11 with PRSV-W and ZYMV, five with PRSV-W and WMV-2, four with WMV-2 alone, and two with CMV, WMV-2, and ZYMV (Table 2).

To confirm infection by ZYMV and the role of this virus, particularly in mixed infections, extracts from 23 samples were inoculated onto Multipik or Elite squash, Poinsett cucumber, BT-2 bean, and *C. quinoa* plants in the greenhouse. After rub-inoculation with sap, ZYMV was detected by DSI-ELISA from 83% of the samples that had originally contained this virus (Table 3).

Table 1. Number of cucurbit samples collected in 1983 that were infected with viruses, as determined by double-sandwich indirect enzyme-linked immunosorbent assay

Crop species	No. infected/ no. tested	Single virus infections					Mixed virus infections			
		PRSV-W[a]	WMV-2	CMV	SqMV	ZYMV	CMV, WMV-2	PRSV-W, WMV-2	CMV, SqMV	CMV, PRSV-W, WMV-2
Citrullis lanatus	2/4	0	1	1	0	0	0	0	0	0
Cucumis melo	3/6	0	3	0	0	0	0	0	0	0
C. sativus	2/4	0	2	0	0	0	0	0	0	0
Cucurbita maxima	5/10	0	3	2	0	0	0	0	0	0
C. pepo (pumpkin)	5/6	1	3	0	0	0	0	1	0	0
C. pepo (yellow)[b]	55/77	3	38	1	1	0	9	0	2	1
C. pepo (zucchini)	13/20	2	10	1	0	0	0	0	0	0
Total	85/127	6	60	5	1	0	9	1	2	1

[a] PRSV-W = watermelon strain of papaya ringspot virus, WMV-2 = watermelon mosaic virus 2, CMV = cucumber mosaic virus, SqMV = squash mosaic virus, and ZYMV = zucchini yellow mosaic virus.
[b] Yellow = straightneck hybrid yellow summer squash and zucchini = hybrid green zucchini squash.

Table 2. Number of cucurbit samples collected in 1985 that were infected with viruses, as determined by double-sandwich indirect enzyme-linked immunosorbent assay

Crop species	No. infected/ no. tested	Single-virus infections				Mixed-virus infections				
		PRSV-W[a]	WMV-2	CMV	ZYMV	PRSV-W, WMV-2,	PRSV-W, ZYMV	WMV-2, ZYMV	CMV, WMV-2, ZYMV	PRSV-W, WMV-2, ZYMV
Cucumis sativus	11/14	0	0	0	9	0	0	2	0	0
Cucurbita maxima	1/1	0	0	0	0	0	0	1	0	0
C. pepo (pumpkin)	4/4	0	0	0	0	2	0	1	0	1
C. pepo (yellow)[b]	14/14	0	1	0	3	0	0	5	2	3
C. pepo (zucchini)	23/23	0	1	0	4	1	1	9	0	7
C. pepo (zucchini fruit)	38/38	0	2	0	7	2	10	8	0	9
Total	91/94	0	4	0	23	5	11	26	2	20

[a] PRSV-W = watermelon strain of papaya ringspot virus, WMV-2 = watermelon mosaic virus 2, CMV = cucumber mosaic virus, SqMV = squash mosaic virus, and ZYMV = zucchini yellow mosaic virus.
[b] Yellow = straightneck hybrid yellow summer squash and zucchini = hybrid green zucchini squash.

WMV-2 and PRSV-W were only detected in 16 and 45%, respectively, of squash plants inoculated with sap from samples that had contained these viruses. Viruses in mixed infections were not transmitted very frequently or were at levels undetectable by ELISA. For example, WMV-2 was not detected by ELISA in test plants inoculated with sap from nine samples that had tested positively for both WMV-2 and ZYMV by ELISA (Table 3).

In most instances, the squash and cucumber plants infected with ZYMV alone or in combination with other viruses developed typical ZYMV symptoms consisting of veinclearing, mosaic, and malformations. One unique isolate was obtained (NJ85-42). Table 4 presents results of host reactions to zucchini isolate NJ85-42 and an isolate from cucumber that typifies isolates commonly encountered in this study. The zucchini isolate (NJ85-42), designated ZYMV-NJ_{sn}, caused very severe stunting and necrosis of the foliage and stem at the growing point followed by death in squash plants inoculated at the cotyledonary stage. The cucumber isolate used in Table 4 (NJ85-41), designated ZYMV-NJ, did not induce severe necrotic symptoms. ZYMV-NJ and ZYMV-NJ_{sn} did not induce symptoms on *C. quinoa*, BT-2 bean, turnip, pepper, jimsonweed, globe amaranth, tomato, ground cherry, or the tobacco cultivars. These hosts were not tested for latent infections.

DISCUSSION

Use of the ELISA technique greatly facilitated the identification of viruses found in this study of New Jersey cucurbitaceous crops and provided more accurate and consistent results than did symptomatology and host range. Symptomatology of plants infected in the field was of limited diagnostic value for the following reasons: 1) Symptoms caused by PRSV-W in squash during 1984 were indistinguishable from those caused by ZYMV in 1985, and 2) test plants infected with a mixture of viruses sometimes reacted identically to a single virus infection (i.e., CMV and WMV-2 together produced symptoms identical to CMV alone). In many cases, viruses in mixed infections could not be detected after rub-inoculation to susceptible hosts. For example, ZYMV was recovered more frequently than PRSV-W or WMV-2 from plants rub-inoculated with field samples originally shown by ELISA to be infected with one or both of these viruses in addition to ZYMV. ZYMV is a highly aggressive and virulent pathogen and may have a competitive advantage over PRSV-W and WMV-2. Further studies of competition among these viruses involving equivalent concentration of infectious virus will help clarify this point.

It is sometimes difficult to infect greenhouse-grown test plants with mechanically transmissible viruses from field-grown plants. However, many surveys have been based on detection of virus on test plants rub-inoculated with the field samples. Our studies suggest that, at least for some virus combinations in cucurbitaceous crops, this approach may not provide accurate results. Surveys based on the use of differential hosts might be more reliable than those using hosts susceptible to all viruses in a mixture. This would avoid the loss of one or more viruses in mixed infections. However, a sensitive serological assay of the original samples would provide more accurate and reliable results in a shorter time and with limited space than a bioassay involving differential hosts.

ZYMV isolates have been grouped by Provvidenti et al (13) into two biotypes, characterized by ZYMV-Fl and ZYMV-CT, respectively. Under our conditions, ZYMV-CT and ZYMV-Fl can be distinguished by differences in symptomatology and length of incubation period in squash. ZYMV-CT induces distinct veinclearing 5 days after inoculation, whereas the first symptoms of ZYMV-Fl infection appear within 8–10 days and consist of mild mosaic. One month after inoculation, the symptoms of both viruses consist of severe mosaic and malformation, but plants infected with ZYMV-CT generally show vein necrosis and appear more stunted than ZYMV-Fl-infected plants. Most of the ZYMV isolates obtained in New Jersey are similar to ZYMV-CT in these characteristics. However, the zucchini isolate, ZYMV-NJ_{sn}, is much more severe than other isolates and can be distinguished by tip necrosis and severe stunting in squash. We suggest that ZYMV-NJ_{sn} is a third biotype of ZYMV.

It was surprising that ZYMV isolates from New Jersey and other isolates (i.e., ZYMV-CT, ZYMV-Fl, and ZYMV-TS2) inoculated at the same time failed to produce local symptoms in *C. quinoa* or in BT-2 bean. Because we have observed local symptoms in these plants inoculated with ZYMV-CT, ZYMV-Fl, and ZYMV-TS2 at other times, we attribute the lack of symptoms in this study to variation in symptom expression probably caused by environmental conditions.

The major viruses associated with cucurbit diseases varied each year over the 3-yr period of this study. Thus, the ecology of these viruses appears to be finely balanced. ZYMV was more frequently isolated than PRSV-W or WMV-2 from single and mixed infections in 1985 (Table 2). This may be the result of greater numbers of ZYMV-infected plants in the field serving as virus sources or it may relate to differences among viruses in efficiency of aphid transmission. Lecoq and Pitrat (9) have shown that PRSV-W, WMV-2, and ZYMV are efficiently transmitted by aphids from plants infected with one virus. However, using purified viruses and their helper components (HC), they demonstrated that ZYMV was aphid-transmitted more efficiently than WMV-2 in the presence

Table 3. Detection of papaya ringspot virus (PRSV-W), watermelon mosaic virus 2 (WMV-2), and zucchini yellow mosaic virus (ZYMV) by enzyme-linked immunosorbent assay in original cucurbit samples and in Multipik squash plants 14 days after rub-inoculation with these samples

Viruses detected in:			
Original samples[a]		Multipik squash after rub-inoculation	
No.[b]	Virus(es)	No.	Virus(es)
3	ZYMV	3	ZYMV
9	WMV-2, ZYMV	8	ZYMV
		1	None
1	PRSV-W, ZYMV	1	ZYMV
10	PRSV-W, WMV-2, ZYMV	1	PRSV-W
		2	WMV-2
		3	ZYMV
		3	PRSV-W, ZYMV
		1	PRSV-W, WMV-2, ZYMV

[a] Samples were collected in 1985 from several cucurbit crops in New Jersey and are included in Table 2.

[b] No. = number of samples in which the corresponding virus(es) shown to the right was detected by double-sandwich indirect enzyme-linked immunosorbent assay.

Table 4. Symptomatology of zucchini yellow mosaic virus (ZYMV) isolated in 1985 from cucumber (ZYMV-NJ) and a particularly severe isolate from zucchini squash (ZYMV-NJ_{sn}) in New Jersey

	Virus isolates[a]	
Species	ZYMV-NJ	ZYMV-NJ_{sn}
Chenopodium quinoa	−/−[b]	−/−
Cucumis metuliferus		
PI 292190	−/Mo,VC	−/Mo,VN
Acc. 2459	−/Mo,VC	−/Mo,VC, VB,Stu, SNec
Cucumis sativus		
Poinsett	−/VC	−/VC,Mo
Cucurbita pepo		
Elite zucchini	−/Mo,VC	−/Mo,VC, Stu,SNec
Multipik	−/Mo,VC	−/Mo,VC, Stu,SNec
Phaseolus vulgaris		
Black Turtle 2	−/−	−/−

[a] ZYMV-NJ represents a strain found commonly in New Jersey, whereas ZYMV-NJ_{sn} was isolated from only one sample.

[b] Local/systemic symptoms: − = none, not tested by back-inoculation; Mo = mosaic; VB = veinbanding; VC = veinclearing; VN = vein necrosis; Stu = stunt; and SNec = severe top necrosis leading to death.

of WMV-2-HC, whereas in all other paired combinations, the HC supported more efficient aphid transmission of the homologous virus than the heterologous virus. On the basis of our results that ZYMV is more frequently detected than PRSV-W or WMV-2 in single and mixed infections and is more often transmitted than PRSV-W or WMV-2 by rub-inoculation from mixed infections, we support the suggestion by Lecoq and Pitrat (9) that ZYMV may have an important epidemiological advantage that may explain the rapid spread of this virus. Because of aggressiveness, virulence, and aphid transmission properties, ZYMV should be considered a very serious threat to cucurbit production.

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LITERATURE CITED

1. Adams, A. N., and Barbara, D. J. 1982. The use of $F(ab')_2$-based ELISA to detect serological relationships among carlaviruses. Ann. Appl. Biol. 101:495-500.
2. Campbell, R. N. 1971. Squash mosaic virus. No. 43. Descriptions of Plant Viruses. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, Surrey, England. 4 pp.
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. Davis, R. F. 1984. Comparisons of three enzyme-linked immunosorbent assay (ELISA) methods for the detection of watermelon mosaic virus (WMV), strains 1 & 2. (Abstr.) Phytopathology 74:756.
5. Davis, R. F. 1986. Partial characterization of zucchini yellow mosaic virus isolated from squash in Turkey. Plant Dis. 70:735-738.
6. Davis, R. F., and Hampton, R. O. 1986. Cucumber mosaic virus isolates seedborne in *Phaseolus vulgaris*: Serology, host-pathogen relationships, and seed transmission. Phytopathology 76:999-1004.
7. Francki, R. I. B., Mossop, D. W., and Hatta, T. 1979. Cucumber mosaic virus. No. 213 (No. 1 revised). Descriptions of Plant Viruses. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, Surrey, England. 6 pp.
8. Hsu, H. T., and Aebig, J. 1982. Enzyme-linked immunosorbent assay (ELISA) of plant viruses using protein A-enzyme conjugate and $F(ab')_2$ of immunoglobulin G (IgG). (Abstr.) Phytopathology 72:708.
9. Lecoq, H., and Pitrat, M. 1985. Specificity of the helper-component-mediated aphid transmission of three potyviruses infecting muskmelon. Phytopathology 75:890-893.
10. Lisa, V. L., and Lecoq, H. 1984. Zucchini yellow mosaic virus. No. 282. Descriptions of Plant Viruses. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, Surrey, England. 4 pp.
11. Lot, H., Marrou, J., Quiot, J. B., and Esvan, C. 1972. Contribution à l'étude du virus de la mosaîque de concombre (CMV). II. Méthod de purification rapide du virus. Ann. Phytopathol. 4:25-38.
12. Nameth, S. T., Dodds, J. A., Paulus, A. O., and Laemmlen, F. F. 1986. Cucurbit viruses of California: An ever-changing problem. Plant Dis. 70:8-11.
13. Provvidenti, R., Gonsalves, D., and Humaydan, H. S. 1984. Occurrence of zucchini yellow mosaic virus in cucurbits from Connecticut, New York, Florida, and California. Plant Dis. 68:443-446.
14. 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.
15. Purcifull, D. E., and Batchelor, D. L. 1977. Immunodiffusion tests with sodium dodecyl sulfate (SDS) treated plant viruses and plant viral inclusion. Univ. Fla. Agric. Exp. Stn. Tech. Bull. 788. 39 pp.
16. Purcifull, D. E., Edwardson, J., Hiebert, E., and Gonsalves, D. 1984. Papaya ringspot virus. No. 292 (No. 84 revised). Descriptions of Plant Viruses. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, Surrey, England. 8 pp.
17. Purcifull, D. E., Hiebert, E., and Edwardson, J. 1984. Watermelon mosaic virus 2. No. 293 (No. 63 revised). Descriptions of Plant Viruses. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, Surrey, England. 7 pp.
18. Rizvi, S. A. H., Varney, E. H., and Shifriss, O. 1977. New Jersey isolate of a cucurbit-infecting strain of bean yellow mosaic virus. (Abstr.) Proc. Am. Phytopathol. Soc. 4:196.
19. Sako, N., Matsuo, K., and Nonaka, F. 1980. Purification of watermelon mosaic virus. Ann. Phytopathol. Soc. Jpn. 46:639-646.
20. Webb, R. E. 1971. Watermelon mosaic viruses 1 and 2 in squash on the Atlantic seaboard. Plant Dis. Rep. 55:132-135.