A Survey of Viruses Infecting Yellow Summer Squash in South Carolina

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ABSTRACT

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A statewide survey of yellow summer squash was conducted to determine the incidence of cucumber mosaic virus (CMV), papaya ringspot virus W (PRSV-W, formerly watermelon mosaic virus 1), watermelon mosaic virus 2 (WMV-2), squash mosaic virus (SqMV), and tobacco ringspot virus (TRSV). Squash samples were collected during the summer and fall of 1981 and 1982 in seven counties where commercial squash is grown. Virus detection methods included gel double-diffusion and enzyme-linked immunosorbent assay tests. Samples consisted of two or three leaves near the shoot apex. Some were collected from diseased plants and others were taken at random without regard for disease status. Viruses found (highest to lowest number of infected fields) were WMV-2, CMV, PRSV-W, and TRSV; SqMV was not detected. WMV-2 occurred in higher incidence than the other viruses, with 100% infection found in one field in Greenville County. Random samples were not tested for zucchini yellow mosaic virus (ZYMV), but it was identified for the first time in South Carolina from plants in three counties. ZYMV was purified and identified on the basis of host range, serology, morphology, and aphid transmission. The symptomatology of these ZYMV isolates was similar to the Connecticut biotype of this virus.

Viral infections are destructive to yellow summer squash (*Cucurbita pepo* L.), often rendering the fruit produced unmarketable (7,11,29). Proper virus identification and knowledge about which viruses are likely to be most prevalent allow growers to make better decisions on disease management. Little research on viruses of cucurbits has been conducted in South Carolina since 1963. In this study, a statewide survey of yellow summer squash was conducted to determine incidence of cucumber mosaic virus (CMV), papaya ringspot virus W (PRSV-W, formerly watermelon mosaic virus 1), watermelon mosaic virus 2 (WMV-2), squash mosaic virus (SqMV), and tobacco ringspot virus (TRSV). Zucchini yellow mosaic virus (ZYMV) was found for the first time in South Carolina during this survey.

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MATERIALS AND METHODS

Survey procedures. Surveys were conducted during the summer and fall of 1981 and 1982. In the spring of each growing season, letters were sent to agricultural extension service county agents in each of the 46 counties in South Carolina requesting information on acreage and number of fields of commercial yellow summer squash in their respective counties. All fields identified by letters from county agents were surveyed. Commercial production was reported only in Bamberg, Beaufort, Greenville, Horry, Oconee, Orangeburg, and Pickens counties.

Fields were surveyed during one period from September to October. In 1982, fields in Orangeburg County also were sampled in July. All fields were planted with the squash cv. Dixie, except for an 8.1-ha field in Bamberg County in which zucchini squash cv. Senator was grown.

Random samples were collected along intersecting field diagonals. The number of paces required to traverse the field was determined and sampling was organized so that at least 12 plants per hectare were sampled, one-half from each diagonal pass across the field. Plants were selected without regard for disease status and samples consisted of two or three squash leaves taken from the shoot apex. Part of the leaf samples was used for serological assays by the gel diffusion method of Ahmad and Scott (1) for CMV, and direct, double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) (21) for the detection of PRSV-W, WMV-2, SqMV, and TRSV.

Excess tissue, after serological assays, served to start virus cultures of selected samples by sap inoculation of squash seedlings of the cultivar Dixie. Isolates from Beaufort, Orangeburg, and Pickens counties, designated SCB, SCO, and SCP, were mailed to New Jersey for comparison with a ZYMV isolate from Connecticut (ZYMV-CT), obtained from R. Provvidenti, Geneva, NY. ZYMV-TS2 was an isolate maintained in the New Jersey laboratory (6,27).

Virus incidence in plants by county was determined from leaves collected from the 12 plants per hectare (five plants per acre) selected at random. In addition, five plants exhibiting different symptoms were selected from border or internal rows of each field to detect viruses missed by random collections. An average plant population of 7,000 squash plants per hectare was determined. Proportion (p) of plants infected in a county (representing all known squash fields in the county) was estimated by: $\hat{p} = (1/t_o)\Sigma_i\ t_i r_i/n_i$ with standard error $se(\hat{p}) = \pm(1/t_o)[\hat{p}(1-\hat{p})\Sigma_i\ t_i^2/n_i]^{1/2}$, where t_o is the total plant population in all fields sampled in a county (total hectares times 7,000), t_i is the number of plants in the ith field, and r_i is the number of infected plants found in the plants sampled (n_i) in that field.

Host range. Leaves from squash plants infected for 14 days and ground in 0.02 M

sodium phosphate buffer, pH 7, served as inoculum for three or more plants per cultivar or species.

Aphid transmission. Aphids (*Myzus persicae* Sulz.) were starved for 2 hr, given a 2-min acquisition access period on cv. Multipik squash plants infected for 12 days, and placed on Multipik test plants for a 10-min inoculation period (one aphid per test plant, 10 test plants per isolate tested).

Purification. ZYMV-SCB was purified from systemically infected squash tissue by two methods. The first was described by Sako et al (31) and modified by Davis (4), with centrifugation for 10 min at 10,000 rpm before loading onto sucrose cushions. The second method was that of Lisa et al (17), modified to include a polyethylene glycol precipitation (8%, w/v, PEG 6000) and a sucrose gradient (10–50%) centrifugation after the first high-speed centrifugation.

Electron microscopy. Purified virus (0.4 mg/ml) was fixed for 15 min in 2% glutaraldehyde (15), applied to 400-mesh, formvar-covered copper grids stabilized with carbon, and stained (1 min) with 1% uranyl acetate, pH 4.2. Grids were prepared for immunosorbent electron microscopy (30) and were stained with 2% aqueous ammonium molybdate. A diffraction grating (2,160 lines per millimeter) was used for calibration.

Serology. D. E. Purcifull (University of Florida, Gainesville) provided antiserum to WMV-2 FL and H. A. Scott (University of Arkansas, Fayetteville) provided antiserum to American Type Culture Collection (ATCC) strain of PRSV-W (ATCC PV23); antiserum to a legume strain of CMV from Spain ($CMV\text{-}B_{32}$) was produced previously (3). Ascitic fluids (33) with antibodies against ZYMV-SCB were produced in two mice (designated 44a and 44b) by described methods and a similar injection scheme (4).

Ouchterlony gel double-diffusion and indirect, antigen-coated ELISA (I-ELISA) tests were performed as previously described (5). Unfractionated ascitic fluid or purified immunoglobulin G (IgG) from crude ascitic fluid (5,12) from mice 44a and 44b were compared by I-ELISA at various dilutions and tested against fresh extracts of tissue from uninoculated plants (healthy) and tissue infected with ZYMV-SCB or WMV-2 (1:20, w/v). Each I-ELISA plate contained healthy tissue and tissue infected with homologous and heterologous virus, each in at least two wells. Results were considered positive if the ratio of absorbance (405 nm) in the test wells divided by that of the healthy controls was greater than 2.0. PRSV-W IgG was absorbed with plant proteins by the method of Lommel et al (19).

Table 1. Incidence of five viruses in cv. Dixie squash fields by county in South Carolina

County (date sampled)	Samples per field[a]	Frequency of infection (%)[b]					Average infection by county (%)[c]
		TRSV	PRSV-W	WMV-2	CMV	SqMV	
1981							
Beaufort	20	–[d]	20.0	35.0	s[e]	–	
(21 Oct.)	20	–	5.0	5.0	s	–	
	40	–	–	–	–	–	
	40	–	–	–	–	–	10.8 ± 2.8
Greenville	15	–	–	100.0	–	–	
(9 Oct.)	25	4	–	64.0	–	–	80.0 ± 6.3[f]
Oconee	5	–	–	20.0	–	–	20.0 ± 17.9
(9 Sept.)							
Orangeburg	15	–	–	13.3	–	–	
(12 Sept.)	20	–	–	s	s	–	
	10	–	–	s	–	–	
	10	–	–	20.0	–	–	
	35	–	–	s	s	–	
	35	–	–	17.1	–	–	
	20	–	–	95.0	–	–	20.0 ± 3.3
Pickens	20	–	5.0	5.0	s	–	10.0 ± 6.7
(2 Oct.)							
1982							
Bamberg[g]	100	–	5.0	34.0	–	–	
(28 Sept.)	10	–	10.0	80.0	–	–	40.0 ± 4.8[f]
Horry	10	–	–	–	–	–	
(1 Sept.)	15	–	80.0	46.0	–	–	50.0 ± 10.2[h]
Orangeburg	20	–	15.0	15.0	5.0	–	
(3 Oct.)	40	–	25.0	15.0	10.0	–	
	20	–	60.0	10.0	15.0	–	55.6 ± 5.8
Orangeburg	6	–	–	16.6	33.3	–	
(2 July)	14	–	–	7.1	28.6	–	
	15	–	–	26.6	26.6	–	46.7 ± 8.8

[a] To determine the size of a field, divide number of samples from that field by 5 (acres) or by 12 (hectares). Samples harvested sometimes exceeded 12 plants per hectare. In Bamberg Co., 10 samples were harvested from a 0.2-ha field; both fields in Horry Co. were 0.2 ha each; in Orangeburg Co. (2 July 1981), the six and 14 samples were harvested from 0.2- and 0.8-ha fields, respectively; and in Orangeburg Co. (3 October 1982), 20 samples were harvested from 0.8- or 1.2-ha fields.

[b] Percent infection determined by dividing the number of samples with a specific virus by the total number of samples harvested in that field. TRSV = tobacco ringspot virus, PRSV-W = watermelon type of papaya ringspot virus, WMV-2 = watermelon mosaic virus 2, CMV = cucumber mosaic virus, and SqMV = squash mosaic virus.

[c] Average infection calculated by assigning weights related to field size. See Materials and Methods for formulae for this and for standard errors.

[d] – = No virus detected.

[e] s = Virus detected in nonrandom samples only.

[f] One plant was doubly infected.

[g] The 100 samples from Bamburg Co. were of zucchini squash, cv. Senator.

[h] Four plants were doubly infected.

RESULTS

Survey. Four viruses were detected in samples collected from 15 fields in 1981 and 10 fields in 1982 (Table 1); SqMV was not detected. In this survey, WMV-2 was the most widespread virus (22 fields), followed by CMV (11 fields) and PRSV-W (nine fields). Tobacco ringspot virus was detected only in one plant in one field in Greenville County. WMV-2 was detected in every county sampled and its incidence generally was greater than that of other viruses (e.g., all plants sampled in one field in Greenville County were infected with WMV-2). The maximum incidence of CMV was 33.3% in Orangeburg County in a field surveyed in July 1982. Maximum incidence of PRSV-W was 80% in Horry County in a field surveyed in September 1982.

Percent infections by county were adjusted for field size to allow better comparisons of total virus incidence. A high incidence of infection was detected in Greenville County (80%) in 1981 and in Orangeburg County and other counties in 1982 (Table 1). In Orangeburg County, the total percent infection was similar in the July and October samples (47 and 56, respectively). Although only two viruses (WMV-2 and CMV) were detected in the July samples, a third virus (PRSV-W) also was detected in the October samples. Other counties, in descending order of virus incidence, were Horry, Bamberg, Oconee, Orangeburg (1981), Beaufort, and Pickens. Three counties had one or more fields in which no virus was detected in randomly collected samples. These counties with percent of fields infected and average incidence of infection in infected fields were Beaufort, 50% infected fields with 32.5 ± 7.4% incidence; Orangeburg (1981), 57% infected fields with 36.2 ± 5.4% incidence; and Horry, 50% infected fields with 100% incidence.

Double infections of WMV-2 in combination with PRSV-W were identified in Bamberg, Beaufort, and Horry counties. In Orangeburg County, the combination of WMV-2 and CMV was detected in fields during both 1981 and 1982. The combination of WMV-2 and TRSV was detected in Greenville County.

Identification of ZYMV. Three samples collected from Beaufort (1981), Orangeburg (1982), and Pickens (1981) counties showed severe mosaic and leaf malformation, but failed to react in DAS-ELISA with antisera to four viruses or in the gel diffusion test for CMV. These three isolates were sent to New Jersey for comparison with ZYMV. Symptoms produced by ZYMV-SCB, ZYMV-SCO, and ZYMV-SCP, when sap-inoculated onto squash cultivars Early Prolific Straightneck, Multipik, Senator, and Zucchini Elite, consisted of veinclearing in 5 days, followed at 10–14 days postinoculation by severe mosaic, malformation, and blistering. Symptoms and I-ELISA absorbance readings of other cucurbits inoculated with these isolates are given in Table 2. *Phaseolus vulgaris* L. 'Black Turtle 2', resistant to systemic infection by ZYMV (25), did not develop systemic infection when sap-inoculated with the South Carolina ZYMV isolates. All isolates produced local lesions on *Gomphrena globosa* L. Other host reactions were similar to those previously described for ZYMV (4).

ZYMV-SCP was most efficiently transmitted by *M. persicae* (60%), followed by ZYMV-SCO (50%), ZYMV-SCB (48%), and ZYMV-CT (7%) (average for four experiments, except three for ZYMV-CT). ZYMV-SCB was purified by both the modified methods with single bands in density gradients and yields of about 1.5 mg (using an extinction coefficient of 2.4 $[mg/ml]^{-1}cm^{-1}$ at 260 nm) per kilogram of tissue. Absorbance ratios (260/280 nm) were 1.22–1.25 (not corrected for light scattering). Flexuous rod-shaped particles were observed in purified solutions of ZYMV-SCB and were trapped and decorated by ZYMV-TS2 ascitic fluid. ZYMV-SCB virions were 726 ± 41 nm in length (average of 32 decorated particles from two grids).

The ZYMV isolates from South Carolina reacted positively with ZYMV antiserum in both Ouchterlony and I-ELISA tests in which they were serologically indistinguishable from the ZYMV-CT isolate. In Ouchterlony tests with antiserum to WMV-2 FL, the ZYMV isolates formed precipitin bands that spurred with WMV-2 FL. In I-ELISA tests, original desiccated samples of squash infected with ZYMV-SCB, ZYMV-SCP, and ZYMV-SCO reacted with antibodies to ZYMV-TS2, but not with antibodies to CMV-B_{32} or PRSV-W.

Crude ascitic fluid to ZYMV-SCB diluted up to 1:16,000 reacted with homologous virus in I-ELISA. Use of purified IgG reduced reactions to healthy tissue. Neither the ascitic fluid nor purified IgG of any draining from either mouse 44a or 44b reacted with WMV-2, indicating a high degree of virus specificity.

DISCUSSION

Deformed and abnormal green coloration occurs on squash fruit from plants infected with any one of the five viruses for which we assayed (7). Plants infected by the time fruit set begins will produce unmarketable fruit. In this survey, samples were collected within a 5- to 6-wk period from September to October, at a time when most of the fields were being harvested. Virus was detected in most fields and, during this time period, some counties had higher incidences of infection than others. However, large differences in incidences were observed among fields in the same county, probably because presence of a source of inoculum is more important to virus disease increase than the field's location within a particular county.

In this survey, TRSV was detected in one field in Greenville County. The reason for such limited distribution in South Carolina is unknown. Although Greenville County is located in the Piedmont, the nematode vector, *Xiphinema americanum* Cobb., is not restricted to the Piedmont, but occurs in coastal areas of the state (S. A. Lewis and W. R. Sitterly, *personal communications*). A TRSV isolate from Greenville County is not translocated from roots to leaves efficiently at warm temperatures, such as 32 C (32), and perhaps warm temperatures limit transmission or distribution of TRSV in South Carolina. However, in other areas, such as southern Texas, strains of TRSV readily infect cucurbits systemically (22). It is also possible that TRSV has not been introduced to other areas of the state.

ZYMV is a recently described virus (17,18) that already is widely distributed (28). It was first observed in Italy in 1973 (17), in France (13) and Lebanon (15) in 1979, and in Turkey in 1981 (6). In the United States, the first observation of ZYMV was in 1981 in Florida (28). It was then observed in 1982 in Connecticut (26), California, and Oregon (24); in 1983 in New York (26); and in 1985 in New Jersey (5) and Arkansas (37). Antiserum for ZYMV was not available for the surveys in 1981 and 1982, but cultures from that survey were maintained in our greenhouse. The Beaufort County isolate of ZYMV was collected on 21 October 1981, the Pickens County isolate on 2 October 1981, and the Orangeburg County isolate on 2 July 1982. These three South Carolina isolates were

Table 2. Symptomatology and indirect, antigen-coated ELISA (I-ELISA) detection of four zucchini yellow mosaic virus (ZYMV) isolates in local and systemic tissues of selected cucurbits 14 days after mechanical inoculation

Test plants	ZYMV isolate[a]				
	SCB	SCP	SCO	CT	Healthy control
Citrullus lanatus (Thunb.) Matsum. & Nakai 'Charleston Grey'	+/Mo[b] 0.18/0.27[c]	+/+ 0.33/0.72	−/VC 0.09/1.30	−/+ 0.08/0.87	−/− 0.07/0.08
Cucumis melo L. 'B63-3'	−/− 0.07/0.07	−/− 0.07/0.07	−/− 0.07/0.07	−/Mo,VN 0.09/0.76	−/− 0.07/0.07
Cucumis sativus L. 'Lemon'	−/VC 0.08/0.79	+/VC 0.76/1.27	+/Mo,VC 1.74/1.42	−/VN 0.08/0.59	−/− 0.08/0.08
Cucumis sativus 'Marketmore 76'	+/VC 0.28/1.16	+/VC 1.04/1.25	+/Mo,VC 1.04/1.37	+/VN 0.62/0.86	−/− 0.08/0.88
Cucumis sativus 'Straight 8'	+/Mo,VC 1.23/1.14	+/VC 1.21/1.44	+/Mo,VC 1.52/1.59	+/VC 1.68/1.20	−/− 0.08/0.09
Cucurbita pepo L. 'Goldbar'	LLc/Mo 0.76/0.28	LLc/Mo 1.10/0.68	LLc/Mo 0.92/1.27	+/Mo 1.08/0.59	−/− 0.08/0.08
Cucurbita pepo 'Zucchini Elite'	LLc/Mo,VN 0.13/1.50	LLc/Mo,VN 0.74/1.60	LLc/Mo 1.74/2.0	LLc/Mo,VN 0.79/0.34	−/− 0.08/0.08

[a] Isolate origin: SCB, SCP, and SCO from Beaufort, Pickens, and Orangeburg counties in South Carolina, respectively; CT from Connecticut.
[b] Local/systemic symptoms: LLc = local lesions, chlorotic; Mo = mosaic; VC = veinclearing; VN = vein necrosis; − = no symptoms; + = no symptoms, virus detected by I-ELISA. Symptoms are at 14 days only and became more severe over time.
[c] ELISA results; absorbance (405 nm) from inoculated tissue/absorbance from uninoculated (systemic) tissue of three plants combined, average of three wells each. Values for uninoculated (healthy) plants given for each test plant; two times healthy considered positive. See text for other details.

indistinguishable by symptomatology from the Connecticut biotype (25). ZYMV was widely distributed in South Carolina in 1981 because these isolates were obtained from three widely scattered locations. This is the first confirmation of ZYMV in South Carolina.

Although SqMV was found in the coastal region of South Carolina in 1963, it was not detected in 1981 or 1982 (including the Charleston area where the earlier survey was conducted [35]). Failure to detect SqMV may be explained by exclusive use of certified seed (Dixie) in 1981 and 1982, eradication of wild cucurbit hosts, and decreased squash production in the coastal area of South Carolina (W. R. Sitterly, *personal communication*). Seed certification programs can limit spread of SqMV (20,27) because infected seed often are the source of the virus (14,23,36). Eradication of wild cucurbits in and around squash fields can limit SqMV because it has a natural host range restricted to the Cucurbitaceae (9,16). The use of insecticides is of little consequence in the incidence of SqMV in South Carolina where squash is not routinely treated with insecticides, unlike cucurbit crops in Israel (2).

Squash is often double-cropped in South Carolina and in other areas (7). Virus infections generally are sparse early in the growing season (9,10), but as the growing season progresses more infections occur causing virus diseases to limit production in the second crop (7,8). Increases of aphid vectors and the number of infected squash plants in the first crop make growing squash in the fall more difficult (34).

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