Tobacco Ringspot Virus from Squash Grown in South Carolina and Transmission of the Virus Through Seed of Smooth Pigweed

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ABSTRACT

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Tobacco ringspot virus (TRSV) was detected in commercial fields of yellow summer squash (*Cucurbita pepo*) in only one of seven South Carolina counties. TRSV was isolated from root tissue of smooth pigweed (*Amaranthus hybridus*) growing adjacent to diseased squash plants in the field and was shown to be seed-transmitted in this host. *Xiphinema americanum* was associated with diseased plants in the field, and *Cucumis sativus* 'Model' bait plants grown in field soil became infected with TRSV. The host range and serological properties of the isolate from squash were similar to those of other TRSV isolates; the isolate belongs to the NC-38 serogroup. Estimated relative molecular masses of the RNAs and protein of the squash isolate of TRSV were consistent with values reported for other TRSV isolates. This is the first report of TRSV from squash in South Carolina.

Tobacco ringspot virus (TRSV) is widespread in North America (10). The virus causes diseases, often typified by ringspot symptoms, in perennial crops and in annual crops such as tobacco and soybean (18,19). In cucurbits, it often induces chlorotic stippling during early stages of infection followed by severe mosaic and leaf malformation (12,16). Fruit set and size are reduced, and a warty appearance makes fruit unmarketable (16).

In surveys and other studies of viruses infecting yellow summer squash (*Cucurbita pepo* L. 'Dixie'), some plants were infected with a virus that resembled TRSV. An isolate (TRSV-G) from a diseased plant was identified and partially characterized by host range, host reaction, serology, and chemical properties.

MATERIALS AND METHODS

During summer and fall of 1981–1983, samples of yellow summer squash and weeds were collected from Bamberg, Beaufort, Greenville, Horry, Oconee, Orangeburg, and Pickens counties in South Carolina. The seven counties surveyed were scattered over the state to include the major squash production areas in the piedmont and coastal plain. Samples for bioassay or serological tests consisted of two or three leaves taken from near the shoot apex of squash plants or from leaves and roots of weeds. Samples were evaluated by direct, double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) (11) for the presence of papaya ringspot virus-type W (= watermelon mosaic virus 1), watermelon mosaic virus 2, cucumber mosaic virus, squash mosaic virus, and TRSV with coating and conjugate antibody concentrations optimum for each serum with its homologous virus. ELISA results were judged by visual comparison with appropriat positive and negative controls; judgment was not confounded by intermediate reactions.

The TRSV-G isolate was initiated from an infected squash sample collected from Greenville County and maintained in the greenhouse. TRSV-F was obtained from R. W. Fulton (University of Wisconsin, Madison). TRSV was propagated in *Nicotiana tabacum* L. 'Burley-21' or 'X-73' and purified by homogenization of 1.5 g of tissue in 1 ml of 0.05 M sodium phosphate buffer, pH 7, plus 2 mM Na_4EDTA and 1% 2-mercaptoethanol and 1 ml of butanol: chloroform (1:1), precipitation from the supernatant with polyethylene glycol (10% PEG 8000) and 1% NaCl, differential centrifugation, and collection of virus bands after centrifugation on linear, 10–40% sucrose gradients. Antisera were produced in rabbits against TRSV-F and TRSV-G. Agar double-diffusion serology was in 0.8% agarose in 0.03 M sodium phosphate, pH 7.0, plus 0.1% sodium azide. Extraction and electrophoresis of viral RNA in nondenaturing conditions was by the method of Bruening et al (4), and the method of Weber and Osborn (20) was used for capsid protein electrophoresis. Relative molecular masses were estimated by measuring the distance viral RNA or protein moved in gels relative to a dye front when scanned at 260 or 280 nm, respectively, and comparison with R_f values of standards.

The following weed species were collected from the field and tested for TRSV by ELISA: *Amaranthus hybridus* L. (smooth pigweed), *Polygonum pensylvanicum* L., and *Panicum dichotomiflorum* Michaux. Several smooth pigweed plants transplanted from the field and allowed to produce seed in the greenhouse served as sources of seed for seedlings tested for seed transmission by ELISA.

To assess the effects of temperature on translocation of TRSV from roots to shoots, roots of smooth pigweed seedlings were sap-inoculated with one of the following isolates: TRSV-G, TRSV-F, TRSV-NC-38 (obtained from G. V. Gooding, Jr., North Carolina State University, Raleigh), or TRSV-ATCC-98 (obtained from the American Type Culture Collection, Rockville, MD). Inoculated seedlings were repotted and held in the greenhouse for 48 hr, then grown at 14, 22, or 32 C in growth chambers with a 12-hr photoperiod and an average light intensity of 213 $\mu W \cdot cm^{-2}$.

Field soil collected near squash infected with TRSV was used in greenhouse bait trials. TRSV was the only virus detected by ELISA in squash samples from this field. Cucumber (*Cucumis sativus* L. 'Model') was seeded (five seeds per pot) in the soil, which contained 50 *Xiphinema americanum* Cobb. per 100 cm^3. Nematode extraction was with a semiautomatic elutriator and centrifugal flotation (1). Mounted specimens were identified by S. A. Lewis (Clemson University). After germination, cucumber plants were grown for 3 wk before testing for TRSV by ELISA and bioassay.

RESULTS

TRSV was detected by ELISA in squash samples from Greenville County but not in samples from the other six counties. An isolate from Greenville County, TRSV-G, was further characterized. TRSV-G, TRSV-ATCC-98, TRSV-F, and TRSV-NC-38 all formed

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confluent bands in gel diffusion serology with TRSV-G and TRSV-F antisera.

The host range of TRSV-G was typical of other TRSV isolates (Table 1); for instance, ringspots followed by recovery from symptoms in Burley-21 tobacco, red necrotic local lesions leading to systemic necrosis and death of cowpea (*Vigna unguiculata* (L.) Walp. subsp. *unguiculata*), and necrotic local lesions usually with systemic symptoms on *Chenopodium* species. Smooth pigweed showed no symptoms when infected with either TRSV-G or TRSV-F. TRSV-NC-38 induced a systemic mosaic in this host, whereas TRSV-ATCC-98 caused systemic necrosis of leaves and stem tip followed by death about 12 days after inoculation.

The RNAs of TRSV-G were estimated to have a relative molecular mass of 2.3×10^6 ($\pm 0.1 \times 10^6$) Da for RNA-1 and 1.4×10^6 ($\pm 0.1 \times 10^6$) Da for RNA-2. The capsid protein of TRSV-G was estimated to have a relative molecular mass of 54,000 (± 484) Da.

Smooth pigweed was the only weed collected in which TRSV was detected by ELISA. Virus was detected in roots but not leaves when the plants were collected. When infected pigweed plants were transplanted and grown in the greenhouse for seed collection, TRSV was detected in both root and leaf tissue after about 2 mo. TRSV was detected in 21 of 99 seedlings grown from the seed of these infected plants. TRSV was detected in 22 of 80 smooth pigweed plants grown from this same seed lot, which had been stored 16 mo at 4 C. None of these infected seedlings or their mother plants produced visible symptoms. No positive ELISA reaction occurred when control seedlings derived from ELISA-negative smooth pigweed mother plants were assayed. Sap inoculations from ELISA-positive seedlings to *Nicotiana clevelandii* Gray, Burley-21 tobacco, *Phaseolus vulgaris* L. 'Topcrop', and Small Sugar pumpkin resulted in symptoms typical of TRSV-G and confirmed that infectious virus occurred in these seedlings.

When smooth pigweed plants were root-inoculated with TRSV isolates and held at 14 C, TRSV-G and TRSV-ATCC-98 were detected in root tissue, but no infection by TRSV-F or TRSV-NC-38 was detected (Table 2). At 22 C, TRSV-F, TRSV-G, and TRSV-ATCC-98 were detected in both root and leaf tissue, whereas TRSV-NC-38 was detected only in root tissue. At 32 C, TRSV-F was the only isolate detected in both root and leaf tissue; all other isolates were detected only in root tissue. None of the smooth pigweed plants infected by root-inoculation produced visible symptoms. In a repetition of the test, TRSV-G and TRSV-F were detected in roots and leaves at 22 C, but at 32 C, TRSV-F was detected in roots and leaves, whereas TRSV-G was not detected in roots or leaves.

Bait plants of Model cucumber grown in field soil became infected with TRSV. In soil used soon after collection, 12 of 17 bait plants became infected, whereas in soil stored at 4 C for 4 wk, two of four bait plants became infected. Tissue from ELISA-positive bait plants was used to inoculate additional cucumber plants, which subsequently were infected with TRSV.

DISCUSSION

The TRSV isolate from Greenville County was similar to other characterized TRSV isolates. The 54,000-Da major protein of TRSV-G has been reported for other TRSV isolates (8). The relative molecular masses of RNAs 1 and 2 also were similar to reported values (14). No serological differences were found among the four isolates, so TRSV-G probably belongs to the NC-38 serogroup (6).

Smooth pigweed was shown to be an alternative host for TRSV. TRSV-G was seed-transmitted in this host, and bait plants grown in soil collected near infected squash and pigweed became infected, supposedly because of viruliferous nematodes in the soil. Thus two mechanisms could contribute to persistence of TRSV in squash fields between seasons; the presence of the virus in smooth pigweed seed or internally in *X. americanum*. *X. americanum* populations stored in soil at 10 C for 49 wk are viruliferous (2,3), all stages survive winters (5,15), and four stages of the nematode transmit TRSV (9).

Temperature appears to be a major factor in the translocation of TRSV isolates from the roots to the leaves of smooth pigweed. Of the four isolates tested, only TRSV-F was translocated to leaves of smooth pigweed when plants were held at 32 C. This may explain why TRSV was detected only in roots of pigweed collected on 16 August 1982. Perhaps, the virus could be detected in leaves of smooth pigweed collected later in the fall. This would be possible because the mean frost date for Greenville County is 15 October. Lower temperatures during September and early October might allow translocation of TRSV-G from roots to foliage for easier virus detection, but seed infection might not

Table 1. Host reactions after sap inoculation of leaves with tobacco ringspot virus strains TRSV-G, TRSV-ATCC-98, TRSV-F, and TRSV-NC-38

Test species[a]	Isolate			
	TRSV-G	TRSV-ATCC-98	TRSV-F	TRSV-NC-38
Amaranthus hybridus	−/LI[b]	−/TN,W,L	−/LI	−/M,MA
Chenopodium amaranticolor Coste & Reyn.	NS/CS,VC	NS/VC,CS	NS/CS,MA,L	CS/−[c]
C. quinoa Willd.	NC/CS,VC,MA	NS/−[c]	C,NS,/CS,MA	CS/−[c]
Citrulus vulgaris Schrad. 'Sugar Baby'	−/M	−/−[c]	−/CS,MA	−/−[c]
Cucumis sativus 'Model'	−/CS	−/CS,RS,M	−/CS,RS,M	−/CS
Cucurbita pepo 'Dixie' squash	−/CS,MA	−/CS	−/CS	−/CS(R)
Cucurbita pepo 'Small Sugar' pumpkin	−/CS,MA,M	−/CS	−/CS	−/CS(R)
Dolichos lablab L.	−/LI	−/M	−/LI	−/M
Glycine max (L.) Merr.				
'Bragg'	−/M	−/M	−/M	−/M
'Davis'	−/M	−/M,SN	−/M	−/CS,M
Lupinus albus L.	NS,RS,/M,MA	NS,RS/−	NS,RS/SN,TN,L	NS/−[c]
Nicotiana clevelandii	−/C,M	N,RS,LI/TN	C,M/−	RS/C
N. tabacum 'Burley-21'	CS,LP,/RS	CS,VC/LI	−/CS,LP	LP,CS/−
N. sylvestris Speg. & Comes	M/M	N/VC,N	N,M,LP/M	RS/VC
Phaseolus vulgaris 'Top Crop'	−/M,MA	N,D/N,MA,RL	D/M	−/M
Trifolium pratense L.	−/−[c]	−/−[c]	−/−[c]	−/−[c]
Vigna unguiculata subsp. *unguiculata*				
'California No. 5 Black Eye'	D,RL,/TN,L	RL,D,/W,L	RL,D/TN,L	RL/W,L
'Early Ramshorn'	−/M,MA,L	N,D/N,MA,RL	D/M,L	−/M

[a] Plants dusted with corundum and wiped with phosphate buffer and mercaptoethanol served as inoculated controls.
[b] Local/systemic symptoms: C = chlorosis, CS = chlorotic spots, D = defoliation, L = lethal, LI = latent infection, LP = line pattern, M = mosaic, MA = malformed leaves, N = necrosis, NS = necrotic spots, (R) = recovery, RL = red lesions; RS = ringspots, SN = systemic necrotic spots, TN = terminal necrosis, VC = veinclearing, W = wilting, and −, no visible slymptoms.
[c] Plants assayed by ELISA and found negative for TRSV.

Table 2. Effects of temperatures on infection and translocation of virus in smooth pigweed after root inoculation with tobacco ringspot virus isolates TRSV-F, TRSV-G, TRSV-ATCC-98, and TRSV-NC-38

	32 C		22 C		14 C	
Isolate	**Roots**	**Leaves**	**Roots**	**Leaves**	**Roots**	**Leaves**
TRSV-F	+[a]	+	+	+	−	−
TRSV-G	+	−	+	+	+	−
TRSV-ATCC-98	+	−	+	+	+	−
TRSV-NC-38	+	−	+	−	−	−
Uninfected[b]	−	−	−	−	−	−

[a] All samples were assayed by ELISA for TRSV and scored as: + = positive for virus and − = negative.

[b] Control plants of smooth pigweed roots abraded with phosphate buffer and mercaptoethanol.

occur if seed development were already at an advanced stage.

TRSV-G and TRSV-F induced no symptoms in smooth pigweed, whereas the other two isolates were injurious to the growth of this host. Perhaps, the less virulent TRSV-G has a selective advantage that enables it to be seed-transmitted in smooth pigweed.

Although TRSV has been reported in surveys of cucurbit crops in Texas (13) and in squash in North Carolina (7), an earlier survey of cucurbit viruses in the Charleston area of South Carolina failed to detect TRSV (17). This is the first report of TRSV from commercial squash fields in South Carolina.

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