Tobacco Streak Virus Infection of Tomato and Some Natural Weed Hosts in California

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ABSTRACT

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Tobacco streak virus (TSV) was isolated from tomato (*Lycopersicon esculentum*), common yellow mustard (*Brassica campestris*), milk thistle (*Silybum marianum*), and wild radish (*Raphanus raphanistrum*) collected from three tomato fields near Sacramento, CA. All TSV isolates from these hosts were infectious to tomato cultivar Peto 81, causing necrotic symptoms on stems and leaves and ring spotting of fruit similar to symptoms observed in the field. The virus was seedborne in experimentally infected *Chenopodium quinoa* and naturally infected wild radish plants. Identification of the virus was based on symptomatology, host range, in vitro properties, morphology, and serology.

Tomato plants with necrosis on leaves, stems, and fruits were found in three tomato fields near Sacramento, CA, in the summer of 1982. Inoculation with leaf extracts from infected plants commonly used for virus study indicated that the disease was caused by a virus previously unknown from tomato in this state. Further experiments showed that the causal agent was tobacco streak virus (TSV).

This paper reports the isolation, host range, in vitro properties, seed transmission, purification, and serology of this virus. Some natural hosts of this virus also are reported.

MATERIALS AND METHODS

Isolation of the virus and host range. Field inspections in three fields of processing tomatoes near Sacramento, CA, during the summer of 1982 revealed the presence of a suspected unknown virus. Incidence of the disease was determined by recording the number of infected plants with visual symptoms in different parts of the affected fields. Roots and leaves of tomato and weeds in the field were collected for virus assay. The samples, ground in 0.03 M phosphate buffer containing 0.02 M mercaptoethanol and 0.02 M ascorbic acid and adjusted to pH 7.0 (PMA), were inoculated mechanically as reported previously (12), except that Celite was substituted for 800-mesh Carborundum. Tobacco (*Nicotiana tabacum* L. 'Havana 425'), tomato (*Lycopersicon esculentum* Mill. 'Peto 81'), *Chenopodium amaranticolor* Coste & Reyn., *C. quinoa* Willd., and *Gomphrena globosa* L. were used for routine tests. Virus isolates were maintained and multiplied in tobacco and *G. globosa*.

For host range studies, other plant species listed in Table 1 and the text were used. Recovery tests from uninoculated and inoculated leaves were made to *C. quinoa*.

In vitro properties and seed transmission. For determination of in vitro properties, infected leaves of *G. globosa* were harvested 21 days after inoculation and ground 1:1 (w/v) in PMA and the sap was centrifuged at 10,000 rpm in a Sorvall GSA rotor for 10 min. The supernatant was used for dilutions, heat treatment, and aging in vitro as reported previously (12). *C. quinoa* was used as the test plant.

To test for seed transmission, seeds from experimentally infected *C. quinoa* plants and from naturally infected wild radish were incubated at room temperature in a petri dish containing a layer of moistened filter paper. Three to 5 days later, seed coats were removed from the germinated seeds and the remaining parts (radicle, hypocotyl, and cotyledons) were ground in PMA. Twenty-five groups of five seedlings from *C. quinoa* and 35

Table 1. Experimental host range and symptoms of four tobacco streak virus isolates from tomato fields in California

Test plant	Symptoms[a]	Virus recovered from	
		Inoc. leaves	New leaves
Cucumis pepo 'Small Sugar'	ls	+[b]	+
C. pepo L. *melopepo* 'Zuccini'	ls	+	+
'Early Prolific Straightneck'	ls	+	+
'Early Summer Crookneck'	ls	+	+
C. sativus 'National Pickling'	ls	+	−/+
Chenopodium album	lc,sm	+	+
C. amaranticolor	ss,d,sn	+	+
C. quinoa	ls,sn	+	+
Gomphrena globosa	ls	+	+
Gossypium hirsutum	ls,d	+	+
Lycopersicon esculentum 'VF 145' and 'Peto 81'	lc	+	+
Nicotiana glutinosa	lc	+	+
N. tabacum 'Havana' and 'Turkish'	ls,rlp,si	+	+
N. rustica	ls	+	+
Petunia hybrida	lc,yv	+	+
Physalis ixocarpa	lc,sn	+	+
Sesbania exaltata	lc,sn	+	+
Verbesina encelioides	vs	+	+
Vigna radiata	vn	+	+
Vinca rosea	lc,sm	+	+
Zinnia elegans	ss	−	+

[a] Symptoms: d = leaf distortion, lc = local spots, ls = local and systemic spots, rlp = ring and line-patterns, si = systemic symptomless infection, sm = systemic mottle, sn = shoot necrosis, ss = systemic spots, vc = veinclearing, vn = vein necrosis, and yv = systemic yellow veins.

[b] + = Virus was recovered, − = virus was not recovered, and −/+ = inconsistent result.

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individual seedlings of wild radish were ground in 0.3 and 0.04 ml of PMA, respectively, and inoculated onto Carborundum-dusted leaves of *C. quinoa*.

Purification, serology, and electron microscopy. The virus was purified from 100 g of *G. globosa* leaves inoculated 21 days earlier, according to the procedure used by Stace-Smith and Frazier (15), with the following modifications: Extract was centrifuged at 5,000 rpm for 20 min in a GSA rotor and the supernatant was centrifuged at 27,000 rpm for 2.5 hr in a Spinco rotor 30. The pellet was resuspended in 0.01 M EDTA (disodium ethylene diamine tetraacetate) by stirring overnight at 4 C. The supernatant obtained after low-speed centrifugation was centrifuged at 45,000 rpm for 75 min in a Spinco rotor 50. The final pellet was resuspended in 0.01 EDTA, 0.02 M Tris [tris (hydroxymethyl) amino methane], or 0.02 M phosphate buffer, pH 7.0.

Partially purified virus preparations were fixed with 2% glutaraldehyde, stained with 2% potassium phosphotungstate, and examined by electron microscopy (7). For serological identification of the virus, agar gel double-diffusion tests were used. Peripheral wells were loaded with partially purified virus preparations or crude sap from healthy or infected leaves of *C. quinoa*. Central wells were loaded with antisera against the following spherical viruses: alfalfa mosaic virus (AMV), cucumber mosaic virus (CMV), tomato aspermy virus (TAV), tobacco ringspot virus (TobRSV), tomato ringspot virus (TomRSV) (9), tomato white necrosis virus (TWNV) supplied by A. P. C. Alba (1), and several different TSV strains supplied by G. I. Mink (13), R. H. Converse (2), and R. W. Fulton (8).

Attempts to recover virus and nematodes from soil. Attempts to recover the virus from soil samples were made by exposing bait plants (10). Sterilized plastic pots were filled with field soil from root zones of infected tomato plants. *C. quinoa* seedlings grown in sterilized soil were transplanted into these pots and assayed for the virus on *C. quinoa* 15–30 days later.

Extraction of nematodes from soil samples was performed by Flegg's decanting and sieving procedure (5) 8–24 hr after collection. Samples were stored at 4 C in plastic bags to avoid drying.

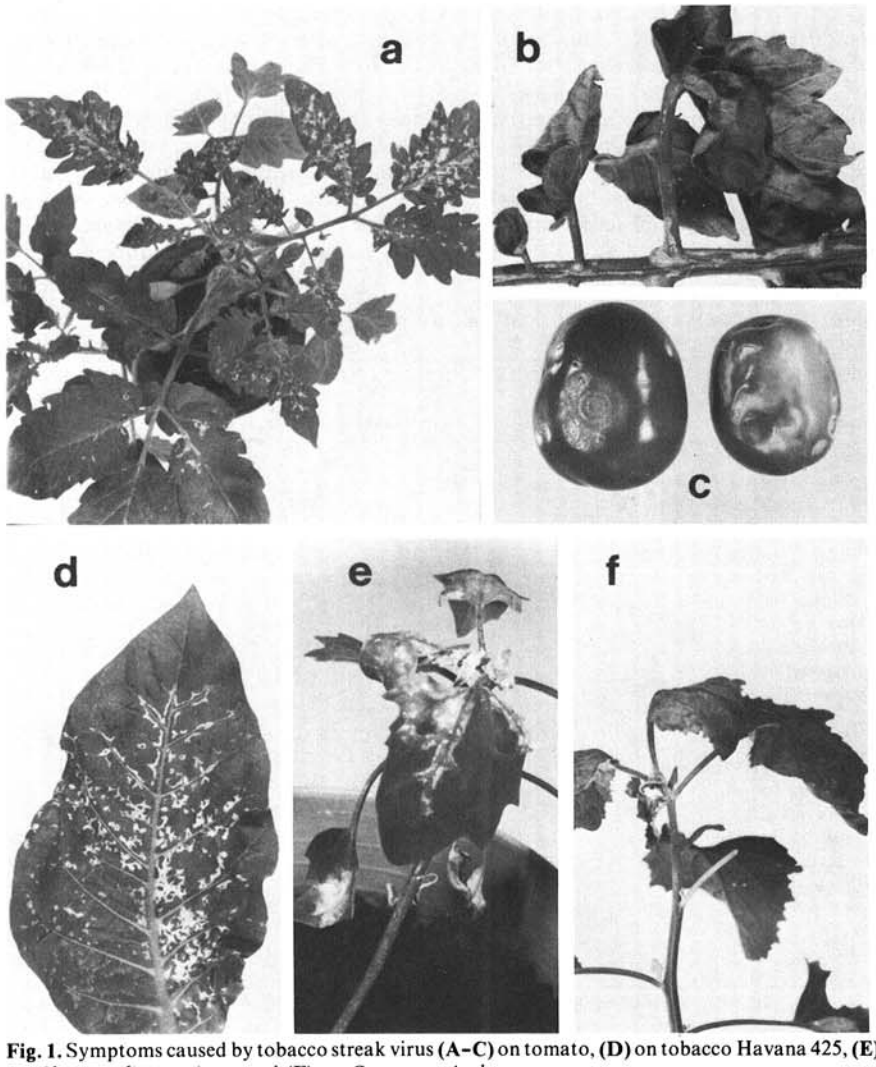

Fig. 1. Symptoms caused by tobacco streak virus **(A–C)** on tomato, **(D)** on tobacco Havana 425, **(E)** on *Chenopodium quinoa*, and **(F)** on *C. amaranticolor*.

RESULTS

Field symptoms and incidence of the disease. Characteristic symptoms of the disease in tomato cultivar Peto 81 were downward distortion of leaf blades, necrosis of veins (Fig. 1B), and ring spots on fruits (Fig. 1C). In some cases, new branches of infected plants were symptomless.

Incidence of the disease in the three tomato fields in the Delta Area near Sacramento, CA, was high. About 60% of the tomato plants near the edges of the fields showed symptoms; however, the number of diseased plants near the centers of the fields was much lower (less than 1% at 50 m from the edges).

Virus isolation from tomato and weeds. Sap inoculation from field-grown tomato samples resulted in necrotic rings and line patterns (Fig. 1D) followed by systemic symptomless infection on tobacco, local necrotic spots, and systemic tip necrosis in *C. quinoa* (Fig. 1E), and leaf mottling, distortion, and shoot necrosis in *C. amaranticolor* (Fig. 1F). Infected tobacco sap inoculated to Peto 81 tomato resulted in local and systemic necrotic leaf symptoms (Fig. 1A). New growth that developed later was symptomless. These symptoms were similar to those observed in the field.

Inoculations with extracts from leaf samples from field-grown common yellow mustard (*Brassica campestris* L.), milk thistle (*Silybum marianum* L.), and wild radish (*Raphanus sativus* L.) resulted in similar symptoms on tomato and the other hosts.

The virus was not isolated from the following weeds growing in the same area: *Abutilon theophrasti, Amaranthus albus, A. hybridus, A. retroflexus, C. album, Echinochloa crusgalli, Foeniculum vulgare, Lactuca serriola, Picris echioides, Polygonum californicum, Solanum americanum, S. sacarroides,* and *Xanthium pensylvanicum.*

Experimental host range. Thirty-five species of 11 families were infected with four isolates of the virus (from naturally infected tomato, common yellow mustard, milk thistle, and wild radish). Crucifers, including *Brassica campestris, B. chinensis* 'Pak choi,' *B. juncea, B. rapa, Raphanus raphanistrum,* and *R. sativus* developed local lesions and systemic symptomless infection. *Phaseolus lunatus, P. vulgaris* ('Bountiful,''Dark Red Kidney,''Gallatin 50,' and 'Stringless Green Pod,') *Pisum sativum, Vigna unguiculata* 'Blackeye' and 'Ramshorn,' *Convolvulus arvensis, Cucumis melo* 'Persian,' and *Cucurbita maxima* 'Sugar Meat' developed only local lesions. *Helianthus annuus, Silybum marianum*, and *B. oleracea* 'Georgia' were systemic symptomless hosts.

No symptoms developed and virus was not recovered from inoculated plants of

Amaranthus albus, A. retroflexus, Cicer arientinum 'UC-5,' *Cyamopsis tetragonoloba, Dahliae variabilis, Datura stramonium, Foeniculum vulgare, Phlox drummondii, P. vulgaris* 'Black Turtle Soup,' 'Light Red Kidney,' and 'Pinto,' *Portulaca oleracea, Tropaeolum majus, Verbena hybrida, Vicia faba, Viola cornuta*, and *Zea mays*. Types of symptoms in 19 other species and results of the recovery tests are presented in Table 1.

In vitro properties. Dilution end point of the virus was between 10^{-3} and 10^{-4}. In thermal inactivation tests, infectivity of extracts diluted to 1:10 was inactivated after 10 min at 60 C but not at 55 C. Longevity in vitro of the virus was between 52 and 54 hr at room temperature (about 25 C).

Seed transmission. The virus was seedborne by *C. quinoa* and *R. raphanistrum*, and incidence was higher in *C. quinoa* (17 positive from 25 groups of 5 seedlings) than in *R. raphanistrum* (2 positive of 35 seedlings).

Purification and serology. The ultraviolet spectrum of the purified preparations was typical of nucleoprotein with the maximum and minimum absorptions at 260 nm and 243 nm, respectively and $A_{260/280} = 1.49$. Yield was about 50 mg/kg of fresh leaf tissue. When pellets were resuspended in 0.01 M EDTA, pH 7.0, infectivity was retained longer than when resuspended in 0.02 M phosphate, 0.02 M Tris, or 0.02 M borate buffers, pH 7.0.

Purified preparations contained numerous isometric particles about 30 nm in diameter. In sucrose density gradients, the virus sedimented in two bands, and both were infectious on *C. quinoa*.

Crude sap of infected *C. quinoa* and purified preparations of four isolates of the virus (from tomato, milk thistle, yellow common mustard, and wild radish, respectively) formed a well-defined precipitin line in agar plates with antiserum to TSV-Rubus strain (2) and a faint line with the antisera to TSV-asparagus and bean strains (13); no spurs were evident. Precipitin lines were not formed when the virus was tested with the antisera to TSV-Hf strain (8), AMV, CMV, TAV, TobRSV, or TWNV.

Soil transmission attempts and nematode extraction. No virus was recovered from 20 soil samples when roots of two groups of five *C. quinoa* seedlings from each soil sample were harvested and assayed 15 and 30 days, respectively, after transplanting. On the other hand, a low population of potential nematode vectors was present in the soil. An average of one nematode of the Dorylaimid genera was isolated from each 200 g of processed soil.

DISCUSSION

Based on host range, physical properties, particle morphology, and serological reaction with antisera to the TSV-rubus, TSV-bean, and TSV-asparagus strains, the virus was identified as TSV. According to Fulton (6), many species in 31 monocotyledonous and dicotyledonous families are susceptible to TSV. In this work, TSV infected 35 species of 11 families of 49 species tested. Tomato, common yellow mustard, milk thistle, and wild radish were found naturally infected and were infected experimentally.

Tomato has been reported as an experimental host of TSV (14); however, we are not aware of a report of the natural occurrence of this virus in tomato in the United States. A necrotic strain of TSV was reported to affect tomato plantings in Brazil (3). Symptoms described for the Brazilian TSV isolate from tomatoes resemble those obtained in this study.

Necrotic symptoms in tomatoes can also be induced by tomato spotted wilt virus (TSWV), AMV, CMV, TobRSS, TomRSV (14), or TWNV (1). These viruses, however, were not detected in our tomato samples either by mechanical inoculation or serology.

Failure of our attempts to recover TSV from soil samples from root zones of infected tomato plants indicate that the virus is not soilborne. Recent reports have shown that TSV is a thrips-transmitted virus (4,11). The distribution of the diseased plants in the tomato fields indicates the possibility of an aerial vector. In addition, seed transmission of the virus through wild radish and possibly by seed of other weed hosts could provide a source of inoculum and a means for survival near or inside the tomato fields during most of the growing season and from year to year.

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