

## Interactions Between Satellite Tobacco Mosaic Virus, Helper Tobamoviruses, and Their Hosts

Rodrigo A. Valverde, James A. Heick, and J. Allan Dodds

Department of Plant Pathology, University of California, Riverside 92521.

Present address of first author: Department of Plant Pathology and Crop Physiology, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge 70803.

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### ABSTRACT

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Satellite tobacco mosaic virus (STMV) was detected frequently in California in association with TMV-U5 in naturally infected plants of *Nicotiana glauca*. Replication of STMV was not helper virus specific because eight tobamoviruses were able to support its replication in experimental infections in hosts that included crop plants such as tobacco and tomato. Two other tobamoviruses as well as several taxonomically distinct viruses could not serve as a helper. Infection with STMV did not alter symptoms induced by the helper viruses in any of the hosts tested. The level of accumulation of STMV-specific double-stranded RNA in infected plants was affected by the helper virus and the host used and was low

in tomato, with TMV-U1 as helper virus and high in tobacco or *N. glauca* with TMV-U5 as helper virus. Yield of STMV from tobacco or *N. glauca* ranged from 1.8 to 3.0 mg per 10 g of leaf tissue. Yields of TMV-U1 and TMV-U5 were reduced 32-48% by coinfection with STMV. After serial passages through *N. tabacum*, STMV reappeared in some but not all subcultures of TMV-U5 that were initially free of detectable amounts of STMV but that had been derived from isolates that once had STMV. STMV did not appear after field isolates of TMV-U5 that were free of STMV when isolated were transferred.

Plant viral genomes that depend on a helper virus for their replication that are not defective interfering RNAs (8,16) are known as satellites. Those with the capacity to encode their own virion capsid protein are referred to as satellite viruses, whereas those that do not are called satellite RNAs and are typically encapsidated by the helper virus coat protein (1-3,13). Satellite tobacco mosaic virus (STMV) depends on strains of tobacco mosaic virus (TMV) for replication (29,30). The single-stranded (ss) RNA of STMV ( $0.3 \times 10^6 M_r$ , 1,059 bases) is encapsidated in a 17-nm-diameter isometric particle made from 60 copies of a 17.5-kDa protein (15). Hybridization experiments have indicated no significant nucleotide sequence similarity between STMV RNA and TMV-U1 or TMV-U5 RNA (29,30). Despite this, sequence data reveal a similarity between the 3'-terminal 240 bases of STMV, TMV-U1, and TMV-U5 RNAs (20). STMV coat protein is coded by STMV RNA (20) and is not serologically related to TMV coat protein. STMV can be differentiated from other satellite viruses such as satellite tobacco necrosis virus (STNV) and satellite panicum mosaic virus (SPMV) by a lack of nucleic acid sequence similarity, lack of serological relatedness, and the need for different specific helper viruses.

Interactions between helper viruses and satellite RNAs and their systemically infected hosts have been studied for the satellite RNAs of cucumber mosaic virus (CMV) (6,7,11,32,23), tobacco ringspot virus (TRSV) (26), tomato bushy stunt virus (TBSV) and other tobamoviruses (3,9), and tomato black ring virus (TBRV) (21,22). Similar studies with a satellite virus involved panicum mosaic virus (PMV) and SPMV (1) and tobacco necrosis virus (TNV) and STNV (13). TNV and STNV did not establish a systemic infection.

The purpose of this investigation was to extend initial attempts to characterize the interaction between TMV and STMV in local and systemic mixed infections (29,30) and further determine the degree of helper virus specificity for STMV, the effect of STMV infection on virion and dsRNA accumulation of the helper virus (and vice versa), and the nature of other satellite-helper virus interactions including incidence, host range, and symptomatology in natural and experimental hosts.

### MATERIALS AND METHODS

**Virus isolates.** California isolates of TMV-U5 with (TMV-U5/S<sup>+</sup>) and without (TMV-U5/S<sup>-</sup>) STMV were those described in previous studies (29-31). TMV-U2 and green tomato atypical mosaic virus (GTAMV) were obtained from A. Siegel (Wayne State University, Detroit, MI); tomato mosaic virus (ToMV) (strains purple or ToMV-P, grey wall or ToMV-G, and fruit necrosis or ToMV-F) and TNV were obtained from J. P. Fulton (University of Arkansas). Isolates of TMV-U1, TMV-cowpea (TMV-CP, also known as sunn-hemp mosaic virus), TMV-yellow (TMV-Y), odontoglossum ringspot virus (ORSV), cucumber mosaic virus (CMV), alfalfa mosaic virus (AlMV), pepper mottle virus (PeMV), potato virus X (PVX), and tobacco etch virus (TEV) were those used in previous studies (29-31).

**Field collection of *Nicotiana glauca* Grah.** Leaves from young shoots of *N. glauca* with or without clear symptoms of virus infection were collected from 269 plants growing near the coast between Los Angeles and Santa Barbara, CA, and within a 10-mile radius of Riverside, CA. They were stored frozen at -20 C before virus assay.

**Virus and RNA detection.** STMV and TMV-U5 were detected in infected plant tissue by the Ouchterlony double-diffusion test (0.9% agarose in 0.15 M NaCl, pH 7.2) using STMV- and TMV-U5-specific rabbit polyclonal antisera (titer 1:1,024 used at a dilution of 1 to 100 and titer 1:128 used undiluted, respectively). Leaf tissue (1.0 g) was extracted with 0.15 M NaCl (1.0 ml) and used to fill wells cut in agarose. Samples for dot-spot hybridization were extracted from 1.0 g of tissue ground in 1.0 ml of 0.1 M sodium phosphate buffer, pH 7.0, containing 1.5% SDS and centrifuged at 10,000 g for 5 min. The supernatant was used without dilution except in some initial experiments designed to determine assay sensitivity. Extract samples (10  $\mu$ l) were applied to nylon membranes (Zeta-Probe, Bio-Rad, Richmond, CA) with a dot-spot template apparatus and baked in a vacuum oven at 80 C for 2 hr.

STMV ssRNA was extracted from purified virions (see next section) using the sodium perchlorate method (33). STMV-specific probe was prepared as randomly primed cDNA transcribed from STMV ssRNA with avian myeloblastosis virus reverse transcriptase (Bethesda Research Laboratories, Gaithersburg, MD) in the

presence of ( $\alpha$ - $^{32}$ P)dCTP (3,000 Ci/mmol; Amersham, Arlington Heights, IL) as described by Maniatis et al (17). Prehybridization, hybridization, and washes of blotted RNA or sap extracts on nylon membranes were carried out as previously described (29).

DsRNAs of STMV, helper viruses, and other viruses were isolated from 3.0–7.0 g of leaf tissue by two cycles of chromatography on columns of CF-11 cellulose powder as previously described (29). DsRNA was resuspended in 40 mM Tris-HCl, 20 mM sodium acetate, 1 mM EDTA, pH 7.8 (electrophoresis buffer). Approximately 0.5  $\mu$ g of dsRNA was loaded into channels formed in a 6% polyacrylamide gel and electrophoresed at constant voltage (100 V) for 3 hr in a vertical slab gel apparatus (90  $\times$  80  $\times$  1.5 mm) in electrophoresis buffer. Gels were stained for 15 min in ethidium bromide at 20 ng/ml.

Field samples of *N. glauca* were analyzed for TEV by ELISA and for CMV and CARNA-5 satellite RNA by dsRNA analysis.

**Purification of STMV.** STMV was purified from *N. tabacum* L. 'Xanthi' coinfected with TMV-U5 and STMV 14–28 days after inoculation. Frozen tissue was extracted at a 2:1 buffer to tissue ratio in 0.2 M potassium phosphate buffer, pH 7.2, 2.0 ml of 2-mercaptoethanol in a blender. The extract was strained, stirred with 8% (v/v) butanol, and centrifuged at 8,000 g for 15 min. The supernatant was adjusted to 4.0% polyethylene glycol (PEG) and 0.1 M NaCl and, after the development of a precipitate (mostly TMV) and low-speed centrifugation (8,000 g for 20 min), additional PEG was added to the new supernatant to a final concentration of 8.0%. The precipitate (mostly STMV) was collected by centrifugation, resuspended in 50 ml of H<sub>2</sub>O, and centrifuged (8,000 g for 20 min). A second cycle of purification involving sequential precipitations with 4% and then 8% PEG was performed as above in the presence of 0.1 M NaCl. The final suspension of STMV was centrifuged at 90,000 g for 120 min and the pellet was resuspended in H<sub>2</sub>O. Aliquots were subjected to two cycles of rate zonal density gradient centrifugation (10–40% sucrose in H<sub>2</sub>O) at 27,000 rpm for 180 min in a Beckman SW 27 rotor. Absorbance profiles were recorded at 254 nm and fractions corresponding to the peak of absorbance, which contained STMV, were collected, diluted in H<sub>2</sub>O, and centrifuged at 90,000 g for 120 min. Pellets were resuspended in H<sub>2</sub>O and the STMV concentration was determined using an extinction coefficient ( $E_{0.1\%}^{1\text{cm}}/_{260\text{nm}}$ ) of 6.5 (J. A. Dodds, unpublished results). Aliquots at 0.5 mg/ml were mechanically inoculated to *N. tabacum* 'Xanthi' to test for contamination with TMV-U5.

**Helper virus specificity, host range, and symptom effects of STMV.** Mechanically transmissible viruses TMV-U1, TMV-U2, TMV-U5, TMV-Y, ToMV (all three strains), GTAMV, ORSV, CMV, AIMV, PeMV, PVX, and TNV were propagated in *N. tabacum* 'Xanthi.' TMV-CP was propagated in *Phaseolus vulgaris* L. 'Pinto.' To determine if these viruses could act as helper viruses for STMV, a mixture of 1 g of leaf tissue infected with the potential helper virus was ground in 1 ml of 0.2 M potassium phosphate buffer, pH 7.2, and mixed with 1 ml of purified STMV (0.5 mg/ml) in the same buffer. The mixed inoculum was mechanically inoculated to *N. tabacum* 'Xanthi' (all viruses except TMV-CP, 10 plants per treatment) and other hosts (selected viruses, three to five plants per treatment); Carborundum was used as an abrasive (Table 1). TMV-CP mixed with STMV was inoculated to *P. vulgaris* 'Pinto' and *Vigna unguiculata* (L.) Walp. 'Blackeye.' Other plants were inoculated either with the helper virus under test or with purified STMV.

Three plant species, *N. tabacum* 'Xanthi,' *N. rustica* L., and *Lycopersicon esculentum* Mill. 'Rutgers,' and three helper viruses for STMV (TMV-U1, TMV-U5, and ToMV-P) were used for a single experiment. Ten plants were mechanically inoculated with isolates of helper viruses and STMV and 10 plants were inoculated with the helper virus alone.

Eight days (inoculated leaves) and 2 wk (systemically infected leaves) after inoculation, plants were assayed by double-diffusion, dot-spot hybridization, and dsRNA analysis for both the helper virus and STMV, and symptoms were recorded.

**Reappearance of STMV after local lesion passage.** Five isolates of TMV-U5/S<sup>-</sup> (determined to be free of STMV by serology,

dsRNA analysis, and dot-spot hybridization assays) were obtained after one single local lesion transfer of TMV-U5/S<sup>+</sup> from leaves of *N. tabacum* 'Xanthi' (29). They were propagated independently in *N. tabacum* 'Xanthi' and inoculum from systemically infected tissue was transferred every 3 wk to new plants for a total of 10 transfers. Five TMV-U5 field isolates lacking detectable STMV (as determined by serology, dsRNA analysis, and dot-spot hybridization assays) also were serially transferred (10 cycles) to new plants. Two weeks after inoculation, newly developed leaves on each plant were tested for STMV by dsRNA analysis and dot-spot hybridization.

**DsRNA analysis of singly and doubly infected plants.** The accumulation of STMV-specific dsRNA in infected *N. tabacum* 'Xanthi' was evaluated in a single experiment using eight helper viruses. Ten plants were used for each mixed inoculum containing STMV and a helper virus. Five to 10 plants were also inoculated with STMV or the helper virus alone. DsRNAs from infected plants were analyzed 2 wk after inoculation. In all cases the amount of dsRNA loaded onto each gel channel corresponded to one-tenth (approximately 0.5  $\mu$ g) of the total dsRNA obtained from 7.0 g of infected tissue.

Some electrophoresed gels were soaked with 50% DMSO and 1 M glyoxal, in 25 mM sodium phosphate buffer, pH 6.5, at 50 C for 1 hr (18). The denatured RNA was electroblotted from the treated gels to nylon membranes in the presence of 10 mM Tris-HCl, 5 mM sodium acetate, 0.5 mM EDTA, pH 7.8, for 24 hr at 40 V (Bio-Rad Trans-Blot apparatus). Electroblotted nucleic acids were hybridized with a STMV-specific cDNA probe. Autoradiographs of blotted membranes were scanned at 560 nm with a gel-scanning attachment in a Beckman DU-50 spectrophotometer and the area of the absorbance peaks was determined.

**Virus yield in singly and doubly infected plants.** Two STMV helper viruses (TMV-U5 and TMV-U1) were mechanically inoculated alone and together with STMV to *N. glauca* and *N. tabacum*

TABLE 1. Ability of plants systemically infected with tobamoviruses to support infection of satellite tobacco mosaic virus (STMV)

Host <sup>a</sup>	Tobamovirus	STMV
<i>Nicotiana tabacum</i>	TMV-U1	+ <sup>b</sup>
	TMV-U2	+
	TMV-U5	+
	TMV-Y	—
	ToMV-P	+
	ToMV-F	+
	ToMV-G	+
	ORSV	+
	GTAMV	+
	<i>N. glauca</i>	TMV-U1
TMV-U5		+
TMV-Y		—
<i>N. benthamiana</i>	GTAMV	+
	TMV-U1	+
	TMV-U5	+
<i>N. rustica</i>	ORSV	+
	TMV-U1	+
<i>N. sylvestris</i>	TMV-U1	+
<i>Lycopersicon esculentum</i>	TMV-U1	+
	TMV-Y	—
	ToMV-P	+
<i>Physalis floridana</i>	TMV-U1	+
	TMV-U5	+
	TMV-U5	+
<i>Datura metaloides</i>	TMV-U1	+
	TMV-U5	+
<i>Vinca rosea</i>	TMV-U1	+
	TMV-U5	+
<i>Phaseolus vulgaris</i>	TMV-CP	—
	TMV-CP	—

<sup>a</sup> Plants were coinoculated with a tobamovirus and purified STMV. Both inocula were also inoculated alone to the hosts listed.

<sup>b</sup> Systemically infected leaves with tobamovirus symptoms were assayed for STMV by dot-spot hybridization 2 wk after inoculation. Positive (+) or negative (—) results were confirmed in at least three doubly inoculated plants. STMV was not detected in any of the singly inoculated plants (data not shown).

'Xanthi' (five plants per treatment). Three weeks after inoculation, 10 g of leaf tissue (inoculated leaves plus two to four younger leaves showing mosaic symptoms) was harvested from individual plants for virus purification. Viruses were purified from clarified extracts as described above except for the use of a single precipitation with 10% PEG (4 and 8% PEG steps were not used), followed by alternate low- (8,000 g for 15 min) and high- (90,000 g for 120 min) speed centrifugation. Final pellets were resuspended in 20 ml of distilled H<sub>2</sub>O and analyzed by rate zonal density gradient centrifugation. Virus was obtained from gradient fractions corresponding to absorbance peaks and its concentration was determined using extinction coefficients ( $E^{0.1\%, 1\text{ cm}/260\text{ nm}}$ ) of 3.0 and 6.5 for TMV and STMV, respectively. Yield of helper virus and satellite virus was calculated by averaging the amount of virus recovered from five purifications.

## RESULTS

**Detection methods.** Experimental systemic infections of STMV in *N. tabacum* and *N. glauca* were readily detected by any of the three techniques (Fig. 1). Local infections were readily detected by dsRNA analysis, a technique that avoids the problem of detecting the relatively large amounts of virions remaining on the surface of leaves inoculated with purified virus. Double-diffusion tests reliably detected STMV from systemic infections in *N. tabacum* and *N. glauca*, but not in *L. esculentum* and *N. rustica*. With a few exceptions, detection of STMV by dsRNA analysis was always correlated with positive results from dot-spot hybridization. All helper and non-helper viruses for STMV were readily detected by dsRNA analysis. In a favorable host-helper virus combination, TMV-U5/STMV in *N. tabacum*, STMV could still be detected at a sap dilution of 1/50 in the double-diffusion assay and 1/100 in the dot-spot assay, but not at greater dilutions. However, samples were used undiluted for the following experiments.

**Incidence and serological relatedness of STMV in *N. glauca*.** STMV was not detected by double-diffusion tests in any of 65 *N. glauca* plants not exhibiting foliar symptoms collected from the field. However, STMV was detected in 99 (49%) of the remaining 204 plants in which mosaic and/or chlorotic symptoms were observed. There was a near equal distribution of STMV

between plants expressing strong (49 plants) or medium to mild (50 plants) foliar symptoms. Twenty of the STMV positive plants were additionally tested for TMV-U5 in double-diffusion assays and all were positive. TEV and CMV, as well as its associated CARNA-5, were also detected in several symptomatic plants, but the distribution of these viruses among all of the samples was not determined.

Double-diffusion assay plates for STMV were designed so that each field sample was tested in a well that was adjacent to a well containing sap from a plant of *N. tabacum* infected with a standard laboratory isolate of TMV-U5 and STMV. No spur formation was observed between any positive field isolate and the standard isolate when an antiserum raised against the standard isolate was used.

**Infectivity, host range, and helper virus specificity of STMV.** When purified STMV was inoculated to *N. tabacum*, no symptoms typical of TMV-U5 infection developed and no STMV- or TMV-U5-specific antigen or nucleic acid was detected by either ELISA, dsRNA analysis, or dot-spot hybridization assay for 30 days postinoculation. STMV was not detected in any of the plants used to maintain virus isolates for this study, or in any of the plants singly inoculated with either STMV or helper virus in any of the experiments.

The following tobamoviruses acted as helper viruses for STMV in the hosts listed in Table 1: TMV-U5, TMV-U2, TMV-U1, ToMV (all three strains), ORSV, and GTAMV. Non-helper viruses included two tobamoviruses, TMV-CP and TMV-Y (included in Table 1), the other rod-shaped viruses TEV, PeMV,

TABLE 2. Detection of three tobamoviruses and satellite tobacco mosaic virus (STMV) in three plant species after mechanical inoculation<sup>a</sup>

Inoculum	<i>N. tabacum</i>		<i>N. rustica</i>		<i>L. esculentum</i>	
	Helper	STMV	Helper	STMV	Helper	STMV
TMV-U5/S <sup>+</sup>	+/+ <sup>b</sup>	+/+	+/-	+/-	-/-	-/-
TMV-U1/S <sup>+</sup>	+/+	+/+	+/+	+/+	+/+	+/+
ToMV-P/S <sup>+</sup>	+/+	+/+	+/-	+/-	+/+	+/+

<sup>a</sup> Plants were inoculated with both helper virus and (STMV). Assay was by dsRNA analysis (tobamovirus and STMV) and dot-spot hybridization (STMV). Distribution of positive and negative results for detection of STMV were the same by both assays so only one scored result is shown.

<sup>b</sup> Results for local/systemic infections; + = virus was detected in all 10 of the plants included in each treatment and - = virus was not detected. STMV was not detected in equivalent numbers of plants inoculated with tobacco mosaic virus alone (data not shown).

TABLE 3. Occurrence of satellite tobacco mosaic virus (STMV) in single local lesion isolates and field isolates of TMV-U5/S<sup>-</sup> after 10 transfers in *Nicotiana tabacum* 'Xanthi'<sup>a</sup>

Isolate	Transfer									
	1	2	3	4	5	6	7	8	9	10
SLL-1 <sup>b</sup>	-	-	+	+	+	+	+	+	+	+
SLL-2	-	-	-	-	-	-	-	-	-	-
SLL-3	-	-	-	-	-	-	+	+	+	+
SLL-4	-	+	+	+	+	+	+	+	+	+
SLL-5	-	-	-	-	-	-	-	-	-	-
NG-12 <sup>c</sup>	-	-	-	-	-	-	-	-	-	-
NG-18	-	-	-	-	-	-	-	-	-	-
NG-28	-	-	-	-	-	-	-	-	-	-
NG-29	-	-	-	-	-	-	-	-	-	-
NG-10	-	-	-	-	-	-	-	-	-	-

<sup>a</sup> Assay was by dsRNA analysis and dot-spot hybridization. Distribution of positive and negative results for detection of STMV were the same by both assays so only one scored result is shown; + = virus was detected and - = virus was not detected.

<sup>b</sup> SLL = selected single local lesion isolate from a culture of TMV-U5 that had an associated STMV infection at the time a local lesion host (*Nicotiana tabacum* 'Xanthi nc') was inoculated. The isolate recovered from a single lesion was maintained (transfer 1) in a systemic host, *N. tabacum* 'Xanthi.'

<sup>c</sup> NG = field isolates from *N. glauca*.

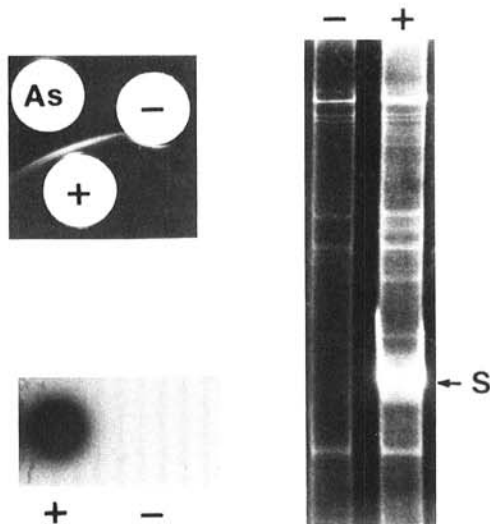


Fig. 1. Typical results of detection assays for satellite tobacco mosaic virus (STMV) from plants of *Nicotiana tabacum*. A, Ouchterlony double-diffusion test using plant sap (+ or -). As = polyclonal antiserum specific to STMV. B, Dot-spot hybridization using plant sap and cDNA specific to STMV RNA. C, 6.0% polyacrylamide gel electrophoresis of dsRNAs extracted from infected plants. In each panel - = sample from a plant infected with TMV-U5 that lacked STMV (TMV-U5/S<sup>-</sup>) and + = sample from a plant infected with TMV-U5 that had STMV (TMV-U5/S<sup>+</sup>). S = position of STMV dsRNA.

and PVX, and the spherical viruses CMV, TNV, and AIMV (not included in Table 1). *N. tabacum* was the host used for all experiments involving non-helper viruses, with the exception of TMV-CP. Two of the non-helper viruses, TEV and CMV, were detected in naturally infected *N. glauca* plants along with TMV-U5 and STMV (see previous section).

The summary of data for 11 hosts in Table 1 also indicates that no host was found that could support the systemic infection of a known helper virus but not STMV. For example, TMV-U1 was a helper virus for STMV in the following hosts tested; *N. tabacum*, *N. glauca*, *N. benthamiana* Domin., *N. rustica*, *L. esculentum*, *N. sylvestris* Speng. & Comes, *Vinca rosea* L., and *Physalis floridana* Rybd.

The experiments on host range and helper virus specificity just described were not all done simultaneously. A single experiment involving 10 plants for each treatment was done with three plant species and three helper viruses selected to give a range of host-helper virus interactions. In all cases the distribution of STMV matched that of the helper virus (Table 2). STMV was not detected in plants inoculated with TMV alone.

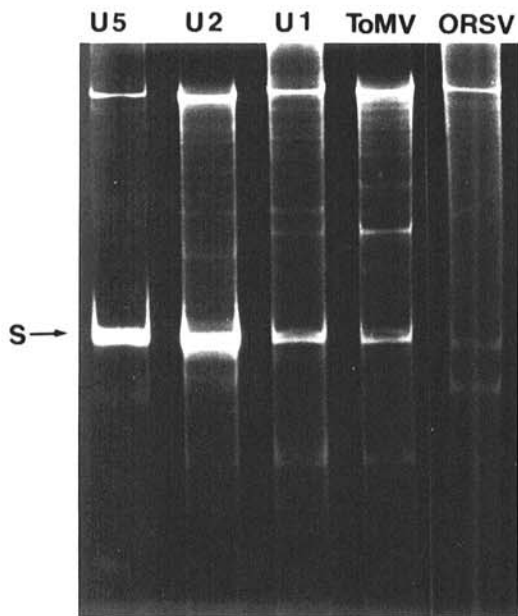
No obvious or reproducible changes in helper virus symptoms were caused by additional local or systemic infections with STMV in any host tested, either when the satellite virus was associated with its natural helper virus, TMV-U5, or with any of the other helper viruses in any of the experiments reported in this study.

**Accumulation of STMV in tomato.** Tomato is not a host of TMV-U5, the natural helper of STMV. Yields of STMV dsRNA in tomato were lower than from *N. tabacum* or *N. glauca* when a helper virus that could infect both hosts was used. For example, dsRNAs of TMV-U1 were detected consistently but little STMV dsRNA was detected in tomato. STMV could not be detected by the Ouchterlony double-diffusion test from TMV-U1/S<sup>+</sup> infected tomato plants. STMV was detected in tomato plants by dot-spot hybridization or by back inoculation to tobacco plants, which were then assayed for STMV by dsRNA analysis or the Ouchterlony double-diffusion test. Similar results were obtained when GTAMV, ToMV-P, ToMV-G, and ToMV-F were used as the STMV helper in tomato.

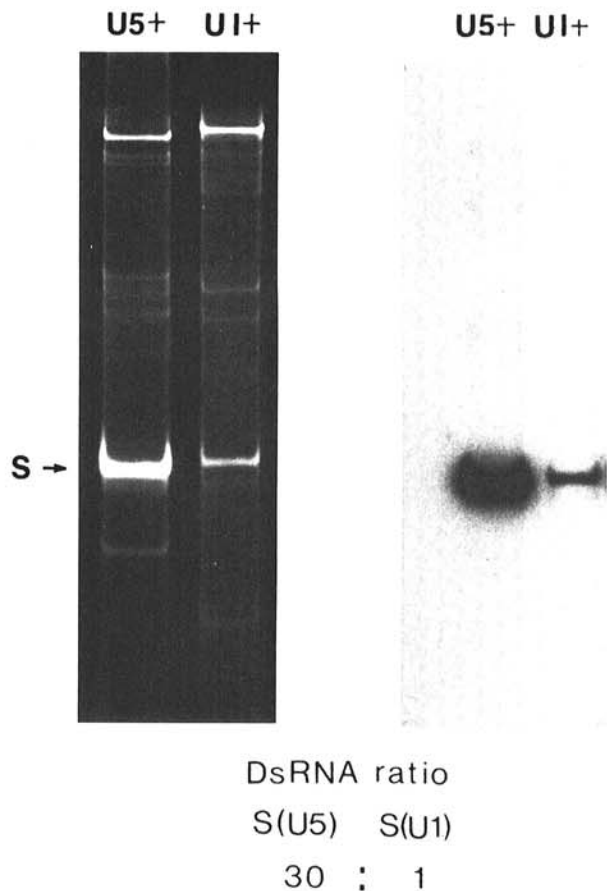
**Reappearance of STMV in subcultures.** Five isolates of TMV-U5/S<sup>-</sup> were derived from a TMV-U5/S<sup>+</sup> parental culture by passage through a single local lesion on a leaf of *N. tabacum* 'Xanthi'

and maintained in *N. tabacum* 'Xanthi.' Subsequent testing of these isolates by dot-spot hybridization indicated they were free of detectable STMV so they were selected for the experiment. STMV reappeared in three of these subcultures after serial transfer through *N. tabacum* 'Xanthi' at the second, third, and seventh passage, respectively. Parallel transfers of two other such subcultures and five different field-collected isolates of TMV-U5/S<sup>-</sup> did not result in the appearance of detectable amounts of STMV up to and including the 10th passage (Table 3). The failure of STMV to reappear in seven subcultures during the course of the experiment makes it unlikely that contamination between treatments or from outside of the experiment was responsible for the reappearance of STMV in the three subcultures where this happened.

**Effect of different helper viruses on STMV dsRNA and virion accumulation.** Relatively large quantities of STMV dsRNA in relation to helper virus dsRNA accumulated in infected plants when the helper virus was either TMV-U5 or TMV-U2, regardless of the host. In contrast, consistently lesser amounts of STMV dsRNA accumulated when TMV-U1, GTAMV, ORSV, or any of the three ToMV strains were used as helper virus (Fig. 2) in tobacco. Quantitation of northern blots indicated that 30 times (average of five determinations) more STMV dsRNA accumulated when TMV-U5 rather than TMV-U1 was the helper virus in tobacco (Fig. 3). STMV infection had no obvious effect on the amount of dsRNA of any of the helper viruses tested as compared with the results for comparable single infections. This was determined by visual comparisons of stained bands in gel channels that contained dsRNA from 0.7 g of tissue for all inoculation treatments (data not shown).



**Fig. 2.** Effectiveness of different tobamoviruses to act as helpers for satellite tobacco mosaic virus (STMV), determined by dsRNA analysis. Polyacrylamide gel electrophoresis (6.0%) of dsRNAs extracted from plants of *Nicotiana tabacum* infected with five tobamoviruses and STMV; Lanes are labeled left to right, TMV-U5, TMV-U2, TMV-U1, ToMV, and ORSV. Arrow points to STMV dsRNA (S).



**Fig. 3.** Differences in accumulation of satellite tobacco mosaic virus (STMV) dsRNAs in *Nicotiana tabacum* when supported by two different helper viruses. Left, 6.0% polyacrylamide gel electrophoresis of dsRNAs extracted from plants infected with TMV-U5/S<sup>+</sup> and TMV-U1/S<sup>+</sup>. Right, northern hybridization of blotted gel probed with labeled cDNA made from STMV ssRNA. Arrow points to STMV dsRNA (S). The ratio for STMV dsRNAs from the two treatments is an average of five comparisons.

Sucrose density gradient profiles of TMV-U5 and STMV from two different hosts are shown for one purification in Figure 4. A slowly sedimenting distinctive component (Fig. 4, S) consisting of STMV particles was readily detected by absorbance in the doubly infected plant preparations. It was a major contributor to the total absorbance recorded for the entire gradient and was absent in extracts from plants infected with TMV alone. In the experiment illustrated in Figure 4 the amount of STMV relative to that of TMV was higher than in other experiments, particularly in *N. glauca*. When the data from five purifications were averaged, the estimated yield of STMV ranged from 1.8 to 3.0 mg per 10 g of leaf tissue, an amount 5- to 10-fold less than the yield of TMV from comparable singly infected plants (Table 4). In contrast to the effect of different helper viruses on the dsRNA results for STMV, TMV-U1 and TMV-U5 supported similar amounts of STMV virions. The purification yields (average of five trials) of the two helper viruses were reduced between 32 and 48% by coinfection with STMV compared with single infections in each of two hosts (Table 4).

## DISCUSSION

Replication of STMV was supported by and dependent on infection of plants with some tobamoviruses but not with other viruses tested. The ability to act as a helper virus for STMV is not particularly strain or isolate specific, unlike several others satellite-helper virus systems (1,14). Distinct tobamoviruses that have not been reported to be associated naturally with STMV were able to support its replication in our experiments. Similar results have been reported for the satellite RNA of TBSV (3). The experimental hosts of STMV include crop plants such as tomato and tobacco, none of which has been reported to be infected with STMV in the field.

It is unclear why TMV-CP and TMV-Y are not helper viruses for STMV. The degree of relatedness between TMV-CP and other tobamoviruses is thought to be small (5), and this may be the basis for its inability to act as a helper virus for STMV. A close sequence similarity between the 3'-terminal 240 bases of TMV-U1, TMV-U5, and STMV has been observed (20), but less sequence similarity exists in the 3' noncoding region of TMV-

CP and TMV-U1 (19). The correlation between 3'-terminal sequence similarity and ability to support STMV will be examined in future studies on the nature of STMV dependence on tobamoviruses.

The dsRNAs of some satellite RNAs such as the satellite RNA of TRSV (27) and CMV (7), and satellite viruses such as STMV (29,30) can accumulate in infected tissues in relatively large quantities in relation to the helper virus dsRNA. For STMV, the degree of dsRNA accumulation is related primarily to the specific virus used as helper, but the host plant can also have an influence. The high yield of dsRNA obtained with TMV-U5 and TMV-U2, compared with the lower yields obtained with TMV-U1, GTAMV, ORSV, and ToMV in the same host seems to be associated with the degree of relatedness among these tobamoviruses. TMV-U5 and TMV-U2 are closely related as determined by similarities in host reaction (28) and nucleic acid sequence similarity (4,24). The other tobamoviruses that acted as helper viruses are related more distantly to TMV-U5 and TMV-U2 by these criteria. The relatively low yield of STMV dsRNA decreased even more when *L. esculentum* was used as host for coinfection with TMV-U1, GTAMV, and strains of ToMV. On the other hand, there was no noticeable host effect on dsRNA yields when TMV-U5 was the helper virus in the different hosts tested in this and previous studies (29,30).

It is well established that some satellite RNAs interfere with the replication of their helper virus (2,10,21) and that the host plays an important role in determining yields of both helper virus and satellite. Results obtained here with STMV generally follow these observations. The levels of interference with helper viruses were not great, if replication is measured by accumulation of virions of TMV. STMV virions accumulated to high levels when associated with the original helper virus (TMV-U5) in its natural host (*N. glauca*). This was also true for dsRNA accumulation, but since little is known about the relationship between dsRNA levels and replication, this may not be a good parameter to use to evaluate helper/satellite virus interactions in mixed infections. This conclusion is reinforced by the observation that the relatively low STMV dsRNA level detected in tobacco when TMV-U1 was the helper was not well correlated with STMV virion accumulation, which was similar to the level for TMV-U5 in tobacco or *N. glauca*.

This satellite virus did not induce change in host symptom expression, despite having a modest negative effect on the accumulation of TMV. A similar lack of effect on symptoms has been reported with the satellite RNA of TBRV (21) and the satellite virus of PMV (1). In contrast, STNV reduces the size and number of lesions that result from localized infection with TNV (12). Strains of satellite RNAs can cause different effects in their hosts as is the case with the necrotic, chlorotic, and ameliorative strains of CARNA-5 (23,32). Electrophoretically different strains of STMV that are antigenically similar have been isolated (30 and D. M. Mathews and J. A. Dodds, unpublished results), but their ability to affect symptoms of tobamoviruses has not yet been studied.

In some instances it is known that satellites were detected unexpectedly in isolates that were presumed to be satellite free

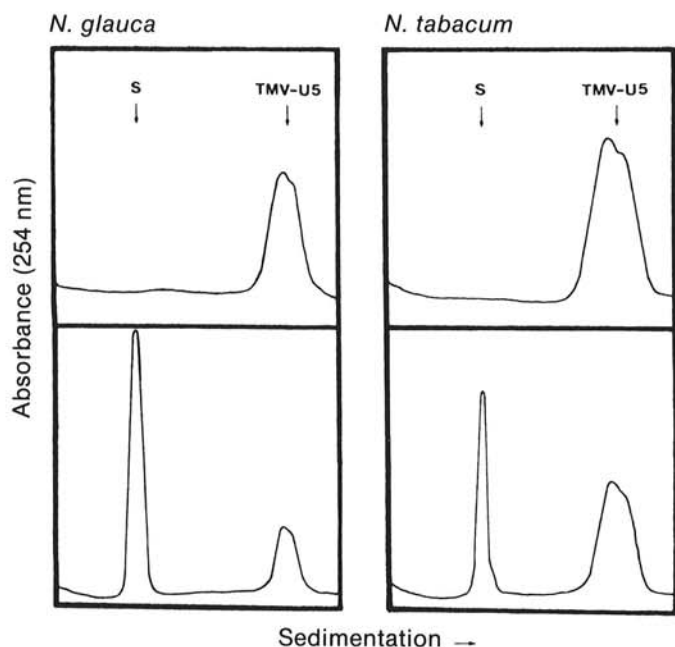


Fig. 4. Examples of the accumulation of tobacco mosaic virus (TMV) and satellite tobacco mosaic virus (STMV) virions in singly and doubly infected plants. Absorbance profiles of centrifuged sucrose density gradients used to analyze purified samples of TMV-U5 with (lower pair of profiles) and without (upper pair of profiles) STMV propagated in two different hosts (*Nicotiana tabacum* and *N. glauca*). S = STMV.

TABLE 4. Yields (mg) of TMV-U5, TMV-U1, and STMV virions from 10-g samples of infected tissue of *Nicotiana tabacum* and *N. glauca*<sup>a</sup>

Inoculation	<i>N. tabacum</i>		<i>N. glauca</i>	
	TMV	STMV	TMV	STMV
TMV-U5	20.0	0.0	15.2	0.0
TMV-U5+STMV	12.5 (37.5%) <sup>b</sup>	2.5	8.0 (48.0%)	3.0
TMV-U1	30.4	0.0	6.7	0.0
TMV-U1+STMV	19.5 (36.0%)	1.8	4.6 (32.0%)	2.5

<sup>a</sup> Values are an average of five independent purifications from different plants.

<sup>b</sup> Percentage reduction in yield of TMV in doubly infected vs. singly infected plants.

(2,10,25). Explanations for this include the presence of low levels of satellite RNA that passed undetected in the original plant, greenhouse contamination, and the activation of satellite RNA in some unknown manner from the host (2,10). Reappearance of readily detectable amounts of STMV in some but not other isolates of TMV-U5/S<sup>-</sup> that were thought to have been freed of STMV, but not in field isolates that were presumed to have never had STMV, despite the fact they were growing on the same greenhouse bench, suggests that STMV was present at undetectable levels in some of the original "satellite freed" isolates.

Only a single local lesion transfer was used in the attempt to generate STMV-free isolates of TMV-U5. This was sufficient to generate isolates free of detectable STMV for between 1 and 10 transfers in a systemic host. This indicates that freedom from STMV has to be interpreted with caution for all tobamovirus isolates. This will have to be taken into account in all future experiments, especially those using RNA transcripts of STMV from cloned cDNA.

STMV was not detected in laboratory cultures of several tobamoviruses, and in some field isolates of TMV-U5 from *N. glauca*, before experimental efforts to deliberately establish an association through the addition of STMV. By contrast, many field isolates of TMV-U5 from *N. glauca* in California have STMV associated with them. The origin of this satellite virus, as with most satellites, is not known, but the theory of origin from conventional viruses by loss of genes for replication and acquisition of sequences for foreign replicase recognition seems appropriate for STMV. The natural host *N. glauca* is a perennial solanaceous plant susceptible to infection by many different viruses (notably potyviruses, cucumoviruses, their satellite RNAs, and tobamoviruses) and genetic interaction peculiar to mixed infections may have played a role in the origin of STMV.

A question arises as to why STMV has not been detected in agronomic crops, especially since tomato and tobacco are experimental hosts and tobamoviruses of these hosts have been shown to act as helper viruses. Whereas epidemiological reasons are most likely involved, the failure to alter symptoms of helper tobamoviruses, the low titer of STMV in some hosts such as tomato, the difficulty of purifying STMV either because of the small size of single particles or of the tendency of particles to aggregate during extraction, and the ability to remain undetected through serial host passages, are all possible reasons why field detection may have been overlooked to date.

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