

# Occurrence of Tobacco Streak Virus in Strawberries in Israel

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## ABSTRACT

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Tobacco streak virus (TSV) was detected in cultivated strawberry plants at one location in northern Israel. Mechanical transmission of the virus from infected, symptomless field plants was obtained during the winter season only. However, serological detection with the same plants was obtained throughout the year using the enzyme-linked immunosorbent assay (ELISA) and the decoration method of immunosorbent electron microscopy (ISEM). This is the first report on the occurrence of TSV in any crop in Israel.

Strawberry plants remain symptomless after infection with most viruses. Identification of these viruses generally depends on symptoms observed on indicator plants after grafting leaves from infected plants (7). A survey using graft inoculations to identify virus diseases in commercial strawberry cultivars was started in Israel in 1980 (16). During this work, severe necrotic shock symptoms followed by apparent recovery were observed in the indicator *Fragaria vesca* var. *semperflorens* (Alpine) after grafting leaflets from cultivated strawberry plants collected from one field in northern Israel. Necrotic shock disease (NSD), described in 1962 by Frazier et al (8), was reported in California (8,17) and the Pacific Northwest (3). Stace-Smith and Frazier (17) recovered tobacco streak virus (TSV) from ground tissue of graft-inoculated *F. vesca*, but sap inoculations of TSV to susceptible *F. vesca* seedlings failed to induce symptoms of NSD. In strawberries, TSV is seedborne, and although a reduction in vigor and fruit yield was noted, infected plants remained symptomless (11). This paper reports the first occurrence of TSV in Israel.

## MATERIALS AND METHODS

**Grafting.** *F. vesca* var. *semperflorens* and *F. vesca* UC-5 indicator plants were graft-inoculated with leaflets from field-collected strawberry plants. Indicators were maintained for 6 wk in a glasshouse for diagnostic symptoms to develop.

**Mechanical inoculation.** Leaf triturates (1:10, w/v) used as inoculum were ground

either in 0.05 M phosphate buffer, pH 7, containing 2% polyvinyl pyrrolidone (PVP, mol wt 10,000) (PPVP buffer) (14), or in 0.013 M Tris-HCl, 0.067 K<sub>2</sub>HPO<sub>4</sub>, pH 8, containing 0.02 M 2-mercaptoethanol and 2% PVP (mol wt 40,000) (TPPVPM buffer) (18). Inoculum was rubbed onto Carborundum-dusted leaves of test plants, which were maintained in a glasshouse at 22 ± 2 C for 14 days and observed for symptom expression.

**Serology.** Antiserum against a TSV isolate from *Rubus occidentalis* L. var. Munger (4) was obtained from R. H. Converse, USDA, ARS, Oregon State University, Corvallis. For the agar gel double-diffusion test, peripheral wells were loaded with crude sap from healthy or infected *Cucumis sativus* cotyledons. Central wells were loaded with antiserum against TSV. Enzyme-linked immunosorbent assay (ELISA) was performed following the alkaline phosphatase, *p*-nitrophenyl phosphate method of Clark and Adams (2). Coating and conjugate gamma globulins were each used at concentrations of 1 µg/ml. Samples were run in duplicate wells.

**Immunosorbent electron microscopy (ISEM).** The decoration method of ISEM was used according to Milne and Luisoni (15). Leaf samples were ground in a mortar and pestle with 10 volumes of 0.1 M phosphate buffer, pH 7, containing 2% PVP (mol wt 40,000) (1). Grids were coated with antiserum diluted 1:1,000 for 30 min; trapping of virus particles in leaf sap lasted 60 min and the decoration step with antiserum diluted 1:25 with 0.1 M phosphate buffer, pH 7, lasted 15 min. All steps were done in a humid box at room temperature. Grids were negatively stained with 2% uranyl acetate and examined in a Jeol 100 CX electron microscope.

## RESULTS

**Incidence of the NSD in strawberries.** Strawberry plants were sampled at random from 20 fields; 10–30 plants were

collected from each field. NSD was detected in only one of the fields examined. At that one location in northern Israel, eight of 30 plants indexed positive for NSD by graft analysis. NSD symptoms were clearly observed on indicator plants grafted at any time of the year.

**Mechanical transmission of TSV from graft-inoculated indicators.** Sap inoculation from graft-inoculated *F. vesca* indicator plants with NSD symptoms to a number of herbaceous hosts resulted in necrotic local lesions followed by systemic tip necrosis in *Chenopodium quinoa*, chlorotic spots and systemic mosaic in *C. sativus*, brown ring spots in *Vigna unguiculata* cv. Blackeye, and systemic mosaic in *Nicotiana glauca*. Identification of TSV was confirmed in agar gel double-diffusion plates and by ELISA, with antiserum prepared against the TSV isolate from *Rubus* (4). A single precipitin line was formed in the double-diffusion test with sap from infected *C. sativus* cotyledons diluted 1/20. With ELISA, inoculated *Gomphrena globosa*, *Tetragonia expansa*, and *N. glutinosa* were found to be symptomless hosts of TSV.

**Detection of TSV in strawberry field plants.** Mechanical transmission of TSV from leaf extracts of field-grown strawberry plants to *C. quinoa* was tested throughout the year with both the PPVP and TPPVPM buffers. Transmission was achieved using either buffer only during the winter season. Data summarized in Table 1 show that TPPVPM buffer was superior to PPVP buffer both in the number of *C. quinoa* plants infected and the number of local lesions produced.

TSV was easily detected in infected symptomless strawberry field plants by ELISA. In a typical experiment, the  $A_{405}$  value for infected leaf tissue (ground 1:20, w/v) was 0.70 (average of 10 plants), whereas healthy plants gave  $A_{405}$  values <0.1. Even after leaf sap was diluted 200-fold,  $A_{405}$  values were still four times higher than those obtained with healthy plants.

TSV was also detected in symptomless, infected strawberry leaf tissue by the decoration method of ISEM (Fig. 1). An average of 29 decorated particles was counted in randomly selected fields of view (average of 30 fields in a grid, three grids) at ×40,000. The reliability of the method was verified in a blind test, using eight infected and two healthy plants. All eight infected plants were identified by

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**Table 1.** Effect of buffers on mechanical transmission of tobacco streak virus from field-grown plants of cultivated strawberry to *Chenopodium quinoa*

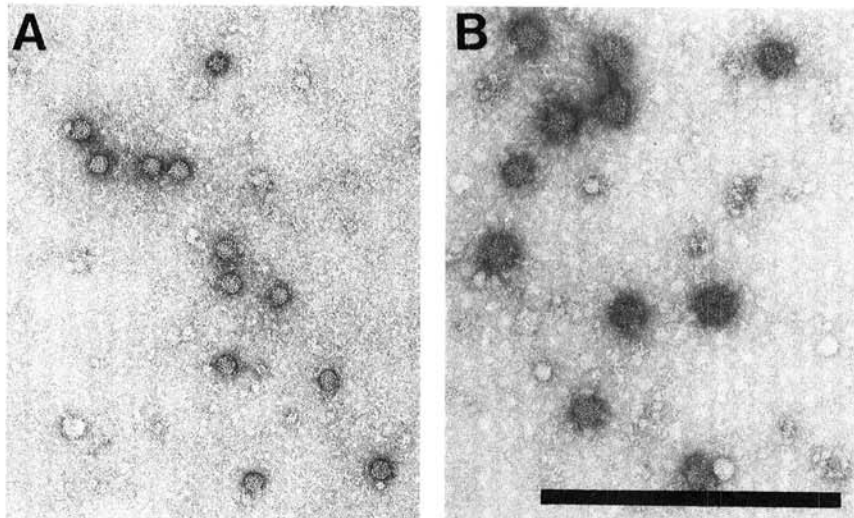
Source plant no.	TPPVPM <sup>a</sup>		PPVP <sup>b</sup>	
	Infected/inoculated <sup>c</sup>	Lesions <sup>d</sup>	Infected/inoculated <sup>c</sup>	Lesions <sup>d</sup>
1	6/6	16	2/6	<1
2	6/6	3	0/6	0
3	6/6	7	4/6	4
4	6/6	10	2/6	<1
5	2/6	3	0/6	0
6	6/6	5	4/6	2
7	6/6	16	4/6	2
8	5/6	3	2/6	<1

<sup>a</sup>0.013 M Tris-HCl containing 0.067 M K<sub>2</sub>HPO<sub>4</sub>, pH 8, 0.02 M 2-mercaptoethanol, and 2% polyvinyl pyrrolidone (PVP) (18).

<sup>b</sup>0.05 M phosphate buffer, pH 7, containing 2% PVP (14).

<sup>c</sup>Ratio of plants infected (numerator) to plants inoculated (denominator).

<sup>d</sup>Number of lesions per three leaves inoculated when plants were at the six- to eight-leaf stage. Each value represents the mean from six plants.



**Fig. 1.** Tobacco streak virus trapped by immunosorbent electron microscopy. (A) Particles trapped on grids coated with rabbit anti-TSV gamma globulins. (B) Particles trapped on coated grids and secondarily decorated with rabbit anti-TSV. The increase in diameter resulted from attachment of gamma globulin to virus particles. Bar = 0.5  $\mu$ m.

this method, whereas no virus particles adsorbed to grids in healthy controls.

## DISCUSSION

Strawberry leaf sap is notorious for its high content of phenolic compounds and viscosity, both of which interfere with detection of viruses in infected tissue. Mechanical transmission of viruses from chronically infected strawberry tissue has been reported for nematodeborne viruses (13) and for pollenborne TSV (10,17). A comparison was made of two buffers reported by Martin and Converse (14) and Thomas (18) to transmit two ilarviruses from their rosaceous hosts to herbaceous hosts. The buffer reported by Thomas (18) for transmission of prunus necrotic ringspot virus (PNRSV) from infected rose plants to leaves of *C. quinoa* was superior under our conditions to the buffer reported by Martin and Converse (14) for transmission of TSV. In any case,

mechanical transmission of TSV was achieved in Israel during the winter season only. Fulton (9) and Thomas (18) failed to transmit PNRSV from rose plants to herbaceous hosts in midsummer in the United States and England, respectively. The failure to transmit TSV mechanically in a hot climate severely limits the reliability of this method to detect TSV.

Serological detection of the virus in symptomless strawberry field plants was obtained using ELISA and the decoration method of ISEM. In the latter, grinding the tissue in a PVP-containing buffer (1) facilitated detection of numerous virus particles in symptomless infected leaf tissue.

Rapid and reliable detection of TSV in strawberry plants can be obtained throughout the year with both serological methods, thus avoiding laborious grafting to indicator plants and the long

waiting periods required by standard bioassay procedures for diagnostic symptoms to develop (5).

The occurrence of TSV in Israel is reported here for the first time. The presence of this virus, reported to be seedborne and transmitted by thrips (6,12), in symptomless strawberries represents a potential danger for introducing the disease into commercial strawberry growing areas. This danger can be minimized by use of reliable detection methods.

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