Seed Transmission of Tobacco Streak Virus in Strawberry

H. A. JOHNSON, JR., Director of Research, Driscoll Strawberry Associates, Inc., Watsonville, CA 95076; R. H. CONVERSE, Research Plant Pathologist, U.S. Department of Agriculture, Agricultural Research Service, Department of Botany and Plant Pathology, Oregon State University, Corvallis 97331; A. AMORAO, Plant Breeder, Driscoll Strawberry Associates, Inc.; J. I. ESPEJO, Plant Breeder, Driscoll Strawberry Associates, Inc.; and N. W. FRAZIER, Research Nematologist Emeritus, Department of Nematology, University of California, Davis 95616

ABSTRACT

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Necrotic shock disease (NSD), associated with tobacco streak virus (TSV) infection of strawberry, was detected in 73 of 392 seedlings derived from crosses among strawberry cultivars when one or both parents were infected. Transmission rates were similar among crosses when the male parent, the female parent, or both were infected, but rates varied from 0 to 35%, depending on the parental lines involved. Plants serving as female parents did not become infected with TSV when pollinated with pollen from TSV-infected cultivars. The virus was detected by enzyme-linked immunosorbent assay (ELISA) in small lots of ungerminated strawberry seed from crosses involving one or both TSV-infected parents but not from those involving healthy parents. Detection of TSV by ELISA was not influenced by surface-disinfestation of infected seed. NSD caused significant reductions in runner production and fruit yield in the patented strawberry cultivar Driscoll E18 in replicated field tests.

Necrotic shock disease (NSD) of strawberry was first observed in California in 1956 (13) and was described as a new virus disease in 1962 (12). This disease also has been found in cultivated strawberries in the Pacific Northwest (4). Tobacco streak virus (TSV) was recovered from NSD-infected strawberry plants, but sap inoculations of TSV to susceptible *Fragaria vesca* var. *semperflorens* (Duchesne) Ser. cv. Alpine seedlings failed to induce NSD symptoms. This inconsistency led N. W. Frazier (15; *unpublished*) to suggest that TSV may act with other unidentified agent(s) to cause variations in NSD. Tobacco streak virus was associated with pollen of infected Boysen blackberry and Munger black raspberry (6), and its spread in several *Rubus* cultivars is mainly flower-associated (7). NSD is known to spread in strawberries in the field, but the mechanism of transmission is unknown although an association with flowers has been noted (12). We present evidence that TSV is seedborne but does not infect pollinated plants and that NSD reduces vigor and yield in cultivated strawberry.

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MATERIALS AND METHODS

NSD was identified in parental strawberry clones and in seedlings from controlled crosses by the leaf-grafting method (2) using *F. vesca* var. *semperflorens* cv. Alpine and *F. vesca* UC-5 as indicator plants (11,12). Two scions were grafted to each indicator plant. Diagnostic symptoms appeared in infected indicators within 2 wk in summer (25–30 C) and within 4 wk in winter (20–25 C). Indicators were observed for at least 12 wk.

TSV was detected by enzyme-linked immunosorbent assay (ELISA) (7). Leaf and seed samples were triturated with a mortar and pestle in standard ELISA extraction buffer (usually at 1:10, w/v) as described previously (7). Gamma-globulin prepared from an antiserum against an Oregon TSV isolate (Munger black raspberry), TSV-R (5), was used at 1 μg/ml in the ELISA double-sandwich, alkaline-phosphatase-globulin conjugate (diluted 1:1,000, v/v, in extraction buffer), *p*-nitrophenyl phosphate system (3). Absorbances were read at 405 nm after 60 min of incubation without stopping the enzymatic reactions in Gilford ELISA plates and a Gilford PR-50 Processor-Reader (Gilford Instrument Laboratories, Inc., Oberlin, OH 44074). All samples were run in duplicate wells. Threshold-positive values used were taken as the average untransformed absorbance readings from wells of healthy sap, plus three standard deviations. These absorbance readings were assumed to be a linear function of virus concentration in the range used for calculating threshold-positive values.

Controlled cross-pollination was performed in a greenhouse, using strawberry clones whose TSV status had been determined previously by graft-indexing. All flowers were bagged from the time of emasculation and subsequent pollination until the fruit ripened. Each cross made was represented by a single berry. Fruits from these crosses were individually collected and agitated in a blender with water. The pulp was decanted and the seed was rinsed, air-dried, and stored at room temperature in glass vials before planting or serological testing.

RESULTS

Graft-indexing for TSV and TSV-ELISA results of strawberry seedlings from controlled crosses are presented in Table 1. Six crosses involving 11 parents were made in which the female parent was TSV-infected and the male parent was not. The percentage of TSV-infected seedlings varied from 0 to 35, with a weighted average of 24%. The cross E20 (infected) × C30.4s (healthy) was made three times with resulting percentages of TSV-infected seedlings of 12, 19, and 66, respectively. Two crosses involving four parents were made in which the female parent was healthy and the male parent was TSV-infected. The percentages of TSV-infected seedlings were 7 and 13, with a weighted average of 9. Both parents were infected in a single cross (E20 × self), and 11% of the resulting seedlings were TSV-infected. In four crosses in which the parents indexed free of TSV, all seedlings also indexed free of TSV. Typical symptoms of NSD developed in the grafted UC-5 indicators where leaf-graft analysis was used to detect NSD in strawberry seedlings arising from controlled crosses.

ELISA for TSV in ungerminated seed from some of the crosses (Table 1) indicated that TSV was present in seed of crosses where one or both parents were TSV-infected but not in seeds from crosses where neither parent was infected. The ELISA absorbance value ($A_{405\ nm}$) from the smallest amount of dry, infected seed tested (30 mg) was 0.14 compared with 0.04 ± 0.02 from dry, healthy seed (120 mg/ml). In a parallel study of seed

samples (12–60 mg seed per milliliter) from 20 individual fruits from a cross where the male parent was TSV-infected (G.3 × F33.5s), 18 of 20 samples tested positive for TSV by ELISA. The range of $A_{405\ nm}$ absorbance values for the 20 samples was 0.00–0.58, $\bar{x} = 0.32$.

Tobacco streak virus was detected by ELISA in batches of seed (25 mg/ml) that were soaked for 15 min in 0.1 M Na_3PO_4 + 0.05% Tween 20, or 0.5% NaOCl + 0.05% Tween 20, and then rinsed in distilled water before being triturated 1:20 (w/v) with standard ELISA extraction buffer with a mortar and pestle. Average $A_{405\ nm}$ values of the Na_3PO_4 treatment (0.30) and the NaOCl treatment (0.32) were similar to that of the untreated, infected seed (0.30), and all greatly exceeded absorbance values of healthy seed (0.02 ± 0.01) when six wells per treatment were measured.

A group of 11 plants of the greenhouse-grown, patented strawberry cultivar Heidi that had indexed free of TSV was pollinated, using three or four emasculated flowers per plant with pollen from the patented strawberry cultivar F33.5s, known to be infected with TSV. Fruit was removed when ripe, and these plants were held in the greenhouse for an additional 12 mo and were then indexed for TSV by grafting on UC-5 and by ELISA. A second group of 47 plants of the same cultivar free of TSV was self-pollinated and later indexed as controls. None of the test plants became infected.

In California field tests replicated four times, healthy and NSD-infected clones of the patented strawberry cultivar Driscoll E18 were compared for runner production and yield. Healthy plants had four times as many runners per hectare as the NSD-infected plants. Fruit yield of healthy plants averaged 47,122 kg/ha compared with 39,724 kg/ha for NSD-infected plants. Analyses of variance revealed significant differences ($P = 0.05$) between originally healthy and NSD-infected plants with respect to both runner production and yield.

Table 1. Incidence of tobacco streak virus (TSV) in progeny of strawberry crosses involving parents infected with this virus

Infection status of parents[a]		Infection status of progeny: Graft detection of TSV in seedlings[b]	Average percentage infected (by graft detection)	ELISA readings ($A_{405\ nm}$) for TSV in seed[c]
Infected female × healthy male				
E20	C30.4s	52/150		0.46
A8 (F)	D5.23 (a)	0/16		NT[d]
A8 (FA)	D5.23 (b)	0/20		NT
A8 (bb)	D5.23 (b)	1/6		NT
D9 S	Z14.106	1/16		NT
B16.4	AC15.7s	3/25		NT
Total or average		57/233	24	
Healthy female × infected male				
D5.23 (A)	A8 (fs)	2/16		NT
G9	B16.4	2/29		NT
Heidi	F33.5s	NT		0.43
Total or average		4/45	9	
Infected female × infected male				
E20	E20	12/114	11	1.08
Healthy female × healthy male				
E20	E20	0/37	0	NT
E20	C30.4s	0/48	0	NT
X67.12s	B5	NT	NT	0.05
E11	D7.7	NT	NT	0.05
Total or average		0/85	0	0.05

[a] Leaf-grafting to *Fragaria vesca* UC-5.
[b] No. seedlings showing symptoms of necrotic shock disease/total no. seedlings indexed.
[c] Absorbance values from ungerminated triturated seed. Healthy control + three standard deviations = 0.06.
[d] NT = not tested.

DISCUSSION

TSV was found to occur in strawberry progeny to an equal extent whether infected male or female parents were used. Parental lines appeared to vary in their ability to produce infected progeny. A common method of spread of TSV to strawberry seedlings appears to be through infected pollen, ovules, or both. A strain of TSV detectable by antiserum against TSV-R was apparently carried inside the seed because surface-disinfestation did not influence its incidence. Although Koch's postulates have not been satisfied for the causal agent(s) of NSD, we find that TSV is routinely associated with this disease in seedlings.

Strawberry mother plants did not become infected by TSV when pollinated from TSV-infected strawberry plants. Strawberry plants are most likely to become TSV-infected when flowering (11). Because transmission of TSV by thrips (*Frankliniella* sp.) has been reported in some annual crops (9,14), the possibility that flower-inhabiting thrips can transmit TSV to cultivated strawberry plants in the field should be investigated.

Practical implications for strawberry breeding arise from these results. Indexing of parents that had been used in the isolated breeding nursery of Driscoll Strawberry Associates, Inc., in Shasta County, CA, revealed three cases where a female or a male parent was infected with TSV. Their progeny were then indexed and 1/15, 3/25, and 2/29, respectively, were infected with TSV. In the strawberry breeding program of Driscoll Strawberry Associates, Inc., the practice of indexing every strawberry selection being held for propagation at the breeding nursery is now followed. Blossoms are removed from any selection that is infected with TSV. It may be possible to reduce the incidence of TSV infection of strawberry selections by not using parents that are infected with TSV, by deblossoming any TSV-infected selections, and by eliminating nearby wild *Fragaria, Rosa,* and *Rubus* spp. that might be possible hosts of TSV (1,8,10) in the propagation nursery.

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