Uneven Distribution of Tobacco Streak Virus in Santiam Blackberry Before and After Heat Therapy

Richard H. Converse

Plant Pathologist, Agricultural Research Service, U.S. Department of Agriculture, and Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331.

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ABSTRACT

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Santiam is an Oregon blackberry cultivar that is naturally infected with the *Rubus* strain of tobacco streak virus (TSV-R). Indexing of softwood propagants showed that TSV-R was unevenly distributed in the shoot system when Santiam plants were grown at normal greenhouse temperatures. When grown at a constant 37 C over a 14-mo period, Santiam plants also produced both healthy and virus-infected propagants. The percentage of infected propagants varied with the source of the mother plant, increased in cyclic fashion with time, but was not influenced by the nodal position on the shoot from which the propagant was taken. Santiam-75, a clone obtained free from known viruses, produced significantly (32%) more shoots in field tests than stock infected with TSV-R.

Additional key words: Rubus strain of tobacco streak virus, *Rubus ursinus,* thermotherapy, virus distribution in host plant.

Rubus ursinus Cham. & Schlecht. 'Santiam', also known at one time as 'Ideal Wild', is a blackberry that is grown on a limited acreage in Oregon for the specialty jam trade. It probably originated as a chance cross between the native Pacific Coast trailing blackberry *R. ursinus* (=*R. macropetalus* Dougl.) and *R. loganobaccus* Bailey, 'Logan' ('Loganberry') (3). In British Columbia, native *R. ursinus* is 32% infected with tobacco streak virus (11). My preliminary unpublished investigations showed that major commercial sources of Santiam plants are symptomlessly infected with the *Rubus* strain of tobacco streak virus (TSV-R). The present study was undertaken (i) to determine the uniformity of TSV-R in various segments of the normal Santiam shoot system, (ii) to determine the effect of thermotherapy on the persistence of TSV-R in these segments, and (iii) to obtain Santiam plants free from TSV-R and compare their vigor with infected plants. An abstract dealing with a part of this work has appeared (2).

MATERIALS AND METHODS

Five Santiam plants from commercial fields in western Oregon were well established in 30-cm clay pots, put in a growth chamber at 37 ± 1.7 C with 16-hr days (11,000 lux), and watered with 0.1-strength Hoagland's solution. At 1-mo intervals all new growth was cut from these plants, and from similar plants grown in a greenhouse at normal temperatures. New growth was divided into shoot tip (cut just below the first opening leaf) and four successive 3-node segments below the tip. These cuttings ranged in length from 2-60 mm and averaged 16 mm.

All cuttings were placed in a bed of 1:1 peat:perlite under intermittent mist at 25 C. The surviving rooted cuttings (propagants) were potted, grown in a screened greenhouse, and kept through winter dormancy in an unheated screenhouse before being forced and indexed for virus content.

During winter and spring, when conditions are most favorable for indexing, young Santiam shoot growth was mixed with an equal volume of 2% nicotine and ground with Celite with mortar and pestle. Sap from each propagant was rubbed on recently expanded cotyledons of four *Cucumis sativus* L., 'National Pickling' cucumber plants. The cucumbers were then observed for 10 days for expression of symptoms (yellow lesions on cotyledons, followed by severe clearing of veins and dwarfing of true leaves).

As a check on the sensitivity of the test procedure, one-third of the propagants that initially indexed negative were chosen at random to be reindexed one or more times during a period of 16 mo. Of these, 20% indexed positive in the second test. Of propagants that tested negative in two successive indexings, 3% were positive in the third indexing.

For serological identification of TSV-R from Santiam propagants, sample virus isolates were maintained in

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cucumber, and sap from recently infected cotyledons, diluted 1:1 with 0.02 M phosphate buffer, pH 8.0, was tested in agar-gel plates against an antiserum for an Oregon isolate of TSV-R from *R. occidentalis* L. (1). TSV-R was serologically identified from propagants taken from several different Santiam source plants after 0, 30, 87, 365, 390, and 421 days at 37 C. No serological differences between isolates were noted.

TABLE 1. Effect of nodal position on survival of the *Rubus* strain of tobacco streak virus in propagants obtained monthly for a 3-mo period from infected Santiam blackberry plants grown at normal greenhouse temperatures, and at 37 C in a growth chamber

Nodal position of propagants	No. virus-infected propagants/total propagants from	
	Normal greenhouse temperature	37 C growth chamber[a]
Tip	2/4	5/23
1–3	1/2	16/85
4–6	3/9	17/69
7–9	3/4	15/49
10+	1/3	10/37
Totals	10/22 (= 45%)	63/263 (= 24%)

[a]There were no significant differences between percentages of infected propagants obtained from the various nodal positions.

RESULTS

Propagants were taken at different nodal positions over a 14-mo period from TSV-R-infected Santiam plants grown at normal greenhouse temperatures and were indexed for TSV-R. Table 1 shows that 45% of these propagants were infected and that infection occurred at all nodal positions sampled.

The percentage of propagants infected with TSV-R was compared for five infected Santiam plants held at 37 C over a 3-mo period. A total of 355 propagants were indexed. The mean percentage of infection was significantly higher in plant 5 (73%) than in propagants from any of plants 1-4, which did not differ significantly from each other (mean 23, range 16-29%).

Propagants from Santiam plants 1-4 above were obtained after 30, 60, and 87 days of heat treatment, classified according to their original shoot position, and indexed for presence of TSV-R (Table 1). The resulting percentage infection data were transformed into arcsin $\sqrt{\%}$ values and were subjected to an analysis of variance which indicated that there was no significant difference in the percentage of infected propagants (mean = 24%) among the various nodal positions examined.

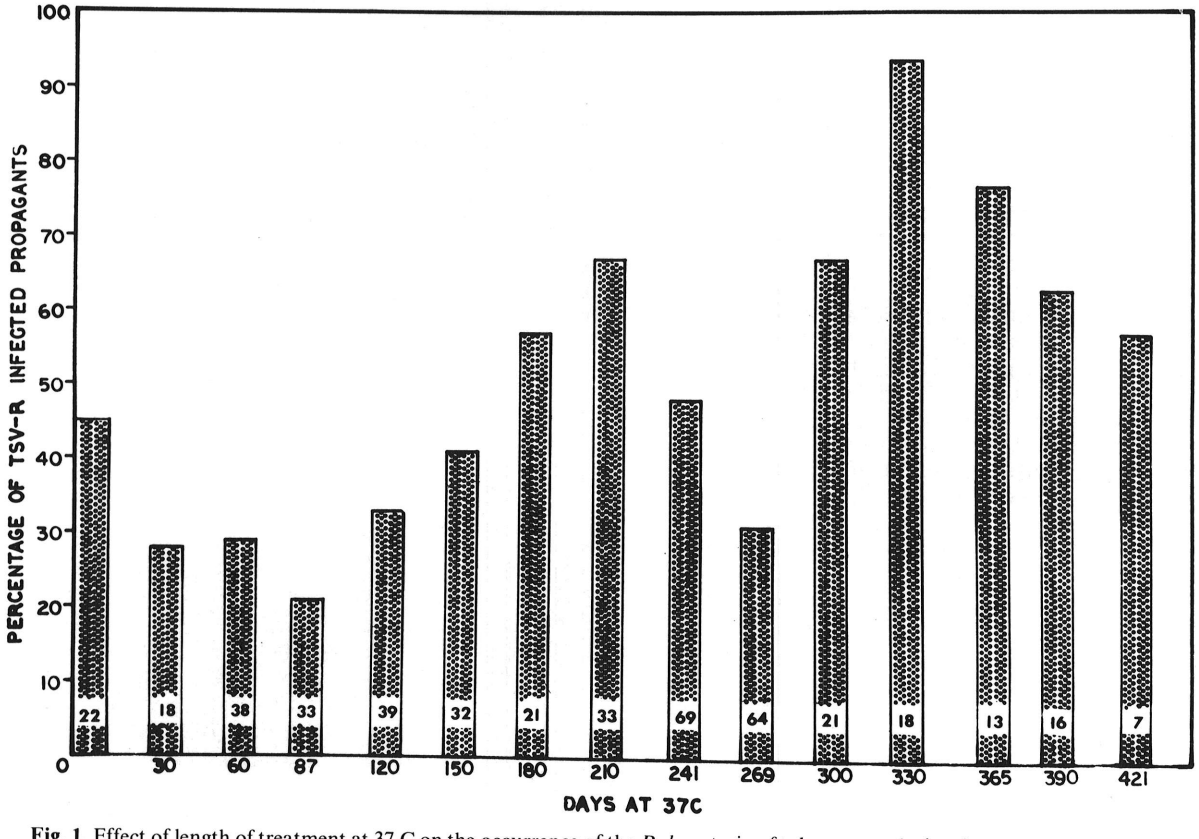

Fig. 1. Effect of length of treatment at 37 C on the occurrence of the *Rubus* strain of tobacco streak virus in propagants obtained at various times from a naturally infected Santiam blackberry plant. The number of propagants tested is given in parentheses.

Santiam plant 2 was held at 37 C for 421 days, and the 422 propagants obtained at 14 monthly intervals were indexed for TSV-R (Fig. 1). Overall, 45% of the propagants from plant 2 were TSV-R infected. The infection percentage ranged from 21 to 94 and appeared to vary cyclically. The linear regression of the percentage of TSV-R-infected propagants (arcsin $\sqrt{\%}$ transformation) against time was

$$Y = 28.5 + .077\ X$$

Variance attributable to linear regression accounted for 51% of the total variance and was highly significant.

Heat-treated plants, some of which had indexed positive and some negative for TSV-R, were planted in a new commercial Santiam planting near Silverton, OR in April 1973. In February 1974 and May 1975, all the plants that originally indexed negative, were reindexed. All indexed negative in 1974, and 23/24 still indexed negative in 1975. The number of primocanes per plant was counted in 1975 in 23 plants infected with TSV-R and 23 plants which indexed negative. The virus-negative plants produced 32% more primocanes than the virus-positive plants, a statistically significant difference.

A clone designated Santiam-75 was developed from one propagant that was obtained after 60 days at 37 C. This clone tested virus-negative repeatedly over a 3-yr period. Santiam-75 was given to State Departments of Agriculture on the Pacific Coast for distribution to commercial nurseries beginning in 1975.

DISCUSSION

In interpreting these data I have assumed that the presence of TSV-R had no effect on the rooting ability of cuttings or on survival of the resulting plants. I also have ignored any errors that may have been introduced by the repeated indexing of only one-third of the propagants found to be free of TSV-R in the first indexing.

A basic assumption involved in indexing a *Rubus* plant for the presence of a symptomless virus is that the virus is present uniformly throughout the vegetative parts of the shoot system, with the possible exception of meristematic areas. This assumption is made untenable by the present data, which suggest that, (i) TSV-R may not occur in many propagants from a diseased plant whether they are heat treated or not, and (ii) the percentage of propagants infected by TSV-R varies considerably from clone to clone within this cultivar. Furthermore, a virus-infected *Rubus* plant may escape detection if, inadvertently, only healthy tissues are sampled or if the sensitivity of the bioassay procedure for indexing is overestimated.

These observations suggest the possibility of obtaining virus-free propagants of other *Rubus* cultivars that follow the distribution pattern of TSV-R in Santiam. One need only propagate a quantity of short, softwood cuttings and some should be virus free.

Maintenance of the identity of the propagants that came from the same shoot was not an objective of this study. Therefore, it is not known whether some shoots of a given plant infected with TSV-R were completely free from virus and others were completely infected, or whether certain nodes were virus free and others on the same shoot were infected.

In the Rosaceae, irregular distribution of viruses throughout the shoot system has been reported for apple mosaic virus in chronically infected apple trees (7), chlorotic leaf spot virus in apple (5, 9), and prune dwarf virus in cherry (6). This is the first report of irregular distribution of a virus in the shoot system of a chronically infected *Rubus* cultivar.

In strawberry, TSV is associated with a disease known as necrotic shock (12). Because strawberry necrotic shock was difficult to eradicate by heat therapy (4), Nyland and Goheen (10) suggested that the causative virus might exist in its host as a free nucleic acid. The serological detection of TSV-R in cucumbers inoculated from Santiam propagants obtained throughout 14 mo of thermotherapy suggests that this virus either maintained itself during heat therapy as complete virions or rapidly recovered its ability to form them after subtransfer to cucumber at room temperature.

The equation for the linear regression of percentage of TSV-R infection on days at 37 C has a small positive slope. However, the data in Fig. 1 indicate that a somewhat more complicated, cyclical relationship may exist between these variables. Regardless of this cyclic variation, the small but statistically significant positive slope ($b = + .077$) of the linear regression equation predicts a slight increase in the percentage of infected propagants as heat treatment of mother plants is prolonged.

Although this is an unusual relationship in plant virus thermotherapy, increases in detectible virus after prolonged thermotherapy have been noted in several plant-virus combinations. The literature on this subject was reviewed by Mellor and Stace-Smith (8), who suggested two alternative explanations: (i) formation of heat-resistant virus mutants, and (ii) selection for heat-resistant strains already present in the virus population in the plants. In either case, they suggested, heat resistance in potato virus S did not seem to be a stable character. Heat resistance in TSV-R in Santiam blackberry does not appear to be a stable character either, but no data are available to suggest which of Mellor and Stace-Smith's explanations best fits the Santiam-TSV-R host-virus combination.

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