Tobacco Streak Virus in Black Raspberry

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ABSTRACT

Munger black raspberry stock in Oregon is generally infected with a sap-transmissible virus serologically related to tobacco streak virus (TSV). No known disease in black raspberry is associated with this infection. The lack of cross-protection between the white clover strain of TSV and the black raspberry isolate (TSV-R), and the lack of serological reaction between TSV-R and antiserum against bean red node strain (TSV-RN), indicate that TSV-R is a distinct strain of TSV. TSV-R infected many herbaceous test plants, including cucumber from which it had a dilution end point of 1:32, a thermal end point of 56-60 C, and a longevity in vitro (5 C) of 17 days. Purification by chloroform-butanol extraction from high molarity buffered sap followed by differential and rate-zonal density-gradient centrifugations yielded a partially purified preparation with a corrected sedimentation coefficient for the heavy component ($s_{20,w}$) of 83 S. The virus particle was isometric.

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A survey of the virus diseases of black raspberry (*Rubus occidentalis* L.) in Oregon revealed that sap-transmissible viruses were present in all samples examined (2). A virus causing systemic necrosis in *Chenopodium quinoa* Willd. was isolated from many stocks of Munger, the principal black raspberry cultivar grown in Oregon. Identification and characterization of this virus, the *Rubus* strain of tobacco streak virus (TSV-R), is the subject of this paper.

MATERIALS AND METHODS, RESULTS.—*Field occurrence of sap-transmissible viruses in black raspberry.*—Dormant canes were collected from 25 Munger, 1 Black Hawk, and 1 Cumberland black raspberry fields in Oregon and Washington in 1969-1971. Cane growth was forced in the greenhouse, and in all cases the resulting foliage was symptomless. Young leaves with 2% nicotine added (1:1, w/v) (1) were ground by mortar and pestle, and the resulting sap, mixed with Celite, was used to inoculate young *C. quinoa* plants. Virus isolates were recovered in all 27 cases, and these produced systemic necrosis and/or mottling on *C. quinoa*. The isolates were maintained in *C. quinoa*, either in living plants or in leaves held over $CaCl_2$ in desiccators at 5 C (12). An isolate from a Munger field at Colton, Ore., was recovered twice from dilution end point tests, and the resulting culture, C 162 SL-2, hereafter called TSV-R, was used for antiserum production and for further characterization as described later. All 27 black raspberry isolates taken from Oregon and Washington reacted positively with the antiserum against TSV-R.

Host range.—TSV-R was successfully transferred to cotyledons of young *Cucumis sativus* L. 'National Pickling' in which it produced systemic mottling and dwarfing (Fig. 1-C). Sap from young infected cucumber plants mixed 1:1 with 0.067 M phosphate buffer + 0.02 M 2-mercaptoethanol, pH 6.5 (PME 6.5), was used to inoculate test species by leaf rubbing. A few of the cucurbits tested, particularly cotyledons of Butternut squash (*Cucurbita pepo* L. 'Butternut'), developed local lesions when inoculated with TSV-R, but results were erratic. *C. quinoa* (Fig. 1-A) and cucumber were the most sensitive indicators tested, and virus concentrations were bioassayed on these hosts by dilution end point. Tobacco (*Nicotiana tabacum* L. 'Havana 425') was infected only with difficulty after repeated trials. In tobacco, TSV-R produced ring and line patterns as shock symptoms (Fig. 1-B) followed by a symptomless chronic stage. TSV-R was easily subtransferred from tobacco to tobacco. Havana 425 chronically infected with TSV-R was successfully challenge-inoculated with TSV-WC (6) from white clover and vice-versa. *Tithonia speciosia* Hook 'Torch' developed yellow spots when infected. *Torenia fournieri* Lindl. and *Vigna unguiculata* (L.) Walp. 'Blackeye' were systemically infected by sap inoculation with TSV-R but remained symptomless.

Physical properties.—Cucumber was used as source and indicator for TSV-R. Virus from sap of aboveground parts of young cucumber plants harvested 6 days after inoculation, mixed 1:1 with PME 6.5, had a dilution end point of infectivity of 1:32, but this declined to 1:2 13 days after inoculation. The thermal end point was 56-60 C. In PME 6.5, TSV-R was infective after 17 but not after 30 days of storage at 5 C. When stored at 5 C in cucumber or *C. quinoa* leaves over $CaCl_2$, the virus was infective for 12 months. A group of isolates from Munger were serologically indistinguishable from the standard TSV-R isolate. Infectivity, as determined by dilution end point bioassay on cucumber, was determined for these isolates after they were incubated for 1 hr in buffered sap at various pH

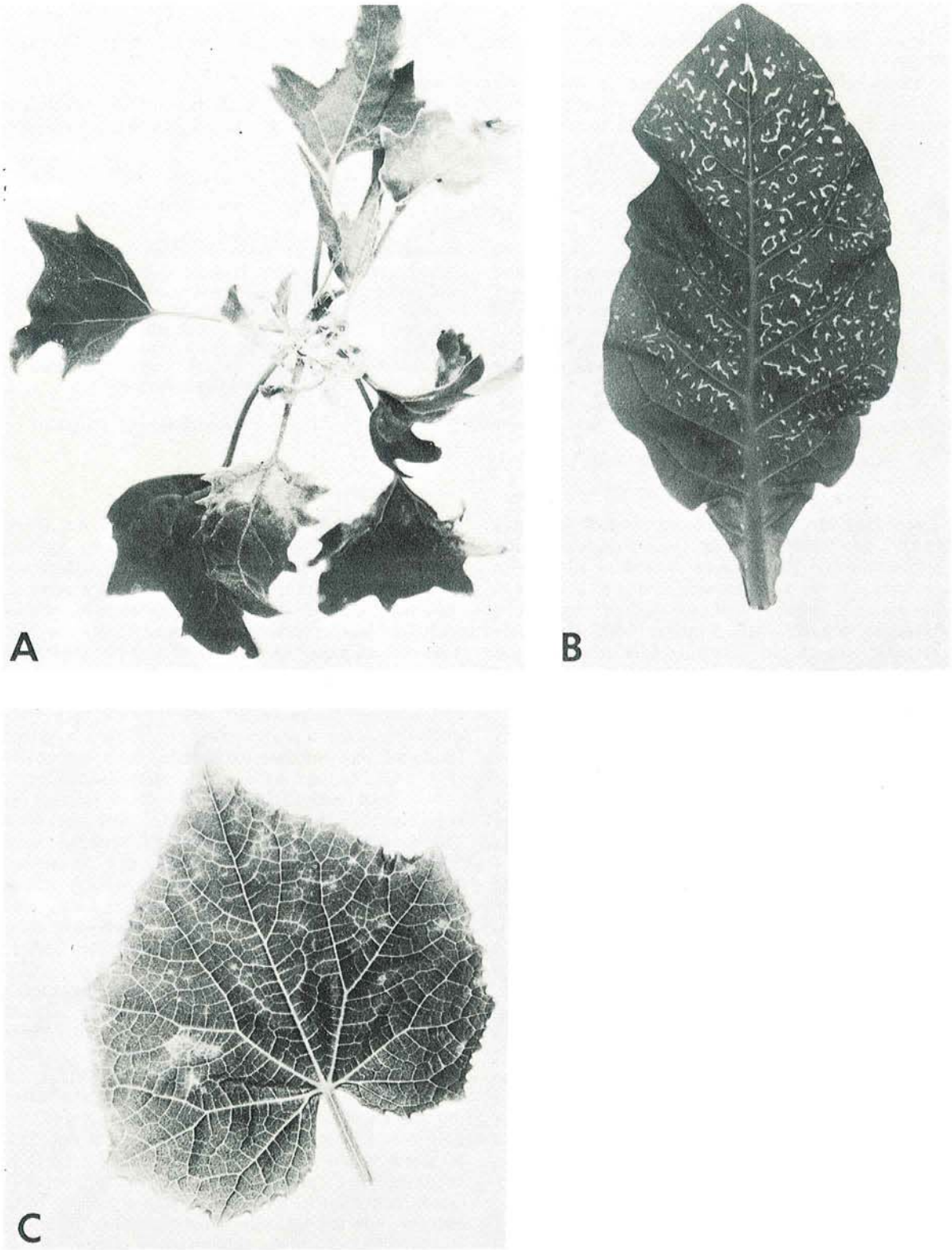

Fig. 1. Tobacco streak virus, black raspberry strain. **A)** Systemic necrosis in *Chenopodium quinoa;* **B)** Ring and line patterns in inoculated leaf of *Nicotiana tabacum* 'Havana 425'; **C)** Systemic mottling and vein clearing in *Cucumis sativus* 'National Pickling'.

levels. Infectivity was maximum at pH 6.5, and just detectable at pH 4.6 and 8.2. Six extracting buffers were tested with the standard TSV-R isolate at 0.02 and 0.2 M concentrations at pH 6.5 plus 2-mercaptoethanol. Citrate and phosphate were the best low molarity buffers, and disodium ethylenediaminetetraacetate (EDTA) and Tris [tris (hydroxymethyl) amino methane] were the best high molarity buffers as determined by infectivity tests. The antioxidants, 0.02 M 2-mercaptoethanol, 0.01 M sodium thioglycollate, and 0.1 M cysteine:0.1 M sodium diethyldithiocarbamate (DIECA) (1:1), prolonged the time TSV-R could be held at 5 C but did not increase the initial infectivity titer. Sodium ascorbate 0.01 M + 0.002 M sodium sulfite and 0.06 M dithiothreitol depressed infectivity compared with the other antioxidants tested. The virus was infective after extraction with chloroform (10% v/v), with 1-butanol (up to 8%), and with 40% diethyl ether followed by carbon tetrachloride extraction. The virus was not infective after extraction with 50% acetone + 50% ethanol or 250% chloroform-butanol.

The following procedure was used for the partial purification of TSV-R. One hundred g of young National Pickling cucumber plants harvested 6 days after infection were blended with 100 ml of 0.2 M sodium EDTA plus 0.01 M sodium thioglycollate, pH 6.5. All operations were conducted at 5 C. The sap was squeezed through cheesecloth, and 10% chloroform and 6% 1-butanol (v/v) were added. The mixture was rapidly stirred with a motor-driven paddle for 5 min, then centrifuged at 4,000 *g* for 10 min. The aqueous supernatant phase was withdrawn by syringe to avoid disturbance of the pellet and was dialyzed overnight against PME 6.5. The preparation then was subjected to two cycles of low- and high-speed centrifugation. The final high-speed pellet, taken up in 2 ml of PME 6.5, typically had an infectivity dilution end point of 1:10 and an optical density A_{260} of 2-3. Rate-zonal density-gradient centrifugation of this preparation on 5-30% sucrose gradients (in a Spinco SW27 rotor at 95,000 *g* at R_{av} for 2 hr, 27,000 rpm) failed to produce visible bands in the centrifuge tubes. Elution of such tubes (ISCO density gradient fractionator) revealed the presence of several peaks (A_{254}) (Fig. 2). Bioassay of separate 2-ml fractions indicated that maximum infectivity occurred in the middle (a) and bottom (b) peaks (Fig. 2) [terminology is that of Fulton & Potter (9)]. However, maximum serological activity against TSV-R antiserum occurred in, and somewhat above, the top (T), a, and b peaks (Fig. 2).

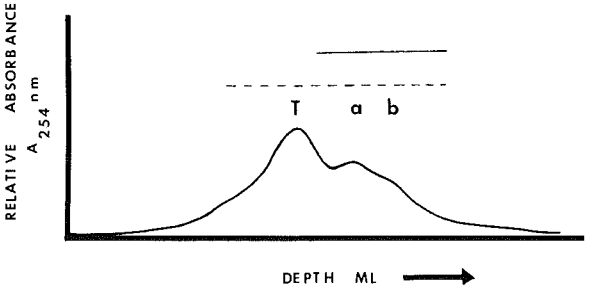

Fig. 2. Tobacco streak virus, black raspberry strain, ultraviolet absorbancy profile of rate-zonal density-gradient ultracentrifugation (2 hr at 27,000 rpm in a 10-25% sucrose gradient in a Spinco SW27 tube). Areas of maximum infectivity and maximum serological activity of 2-ml fractions are indicated above the absorbancy profile by solid and dotted lines, respectively. Top, middle, and bottom components are designated T, a, and b, respectively. The arrow shows the direction of sedimentation.

Analytical ultracentrifugation (Beckman Model E with ultraviolet optics and an ultraviolet scanning system) indicated that the $s_{20,w}$ value for bottom (b) component (six determinations) was 83.0 ± 3.3 S.

Attempts to use sodium phosphotungstate, pH 7, to prepare the partially purified virus isolate for electron micrographs were unsuccessful. However, the virus particles were successfully shadowed with platinum and palladium, and were isometric.

Antiserum against TSV-R was prepared over a 3-month period in a rabbit by two intramuscular injections of partially purified TSV-R mixed 1:1 with Freund's incomplete adjuvant followed by four intravenous injections of infective preparations from rate-zonal density-gradient centrifugations. After cross-absorption against concentrated healthy cucumber sap, the resulting serum (diluted 1:5 with respect to the crude serum) gave no reaction in agar gel against healthy cucumber sap and had a dilution end point of 1:32 against the homologous virus in buffered cucumber sap.

TSV-R isolate in cucumber sap (1:1 with PME 6.5) failed to react in agar gel tests with antisera against 13 isometric viruses, including those reported to occur in *Rubus*. In reciprocal serological gel tests, black raspberry latent virus (3) and TSV-R gave only occasional weak heterologous reactions of doubtful significance.

We tested the serological relationships of TSV-R and its antiserum in serial dilution tests in agar gel with three other TSV isolates and their antisera: TSV-WC from white clover and its corresponding antiserum, TSV-M (6); a strawberry necrotic shock isolate, TSV-SNS (14); and the bean red node isolate from R. W. Goth, TSV-RN (13). All isolates were tested from tobacco in buffered sap. TSV-R gave strong reactions with its homologous antiserum and with TSV-M antiserum, a somewhat weaker reaction with TSV-SNS antiserum, and no reaction with TSV-RN antiserum. TSV-M and TSV-RN had relatively higher end points with their homologous antisera than with TSV-R antiserum. Parallel tests with healthy tobacco sap were either negative or were accounted for in making the above statements. Normal serum reactions were negative.

We feel that tobacco streak virus in black raspberry is sufficiently distinct from previously described isolates of TSV to be designated the *Rubus* strain of tobacco streak virus (TSV-R). A culture of TSV-R, isolate C 162 SL-2, has been deposited with

the American Type Culture Collection as ATCC PV-154 as the type culture of this strain.

DISCUSSION.—The EDTA, chloroform-butanol method of preparing partially purified TSV-R yields low levels of virus which were adequate for partial characterization of the virus. However, one would expect that purification methods that gave good yields of other strains of TSV (6, 8, 10, 11, 13, 14) would be suitable for purification of TSV-R.

The corrected sedimentation coefficient ($s_{20,w}$) of the heaviest component of TSV has now been reported as 113 S for TSV-RN (11), 96 S for TSV (6), 89 S for TSV-SNS (14), and (in this paper) as 83 ± 3 S for TSV-R. This is a sufficiently wide spread of values to justify redetermination of S values for more isolates representing these different strains to see if differences of this magnitude in $s_{20,w}$ values are common among the strains of this virus.

Tobacco streak virus has a very wide host range, and in the Rosaceae has already been reported to infect strawberry and rose (5, 7, 8, 14). The recent report that tobacco streak virus is closely related, if not identical, to the virus associated with strawberry necrotic shock disease in strawberry (14) indicates that Boysen cultivar of trailing blackberry is also a host, since it has been shown to be infected with strawberry necrotic shock disease in California (4). In preliminary tests we found that several virus isolates from Boysen, including some from California, and some isolated from commercial red raspberry gave a serological reaction of identity with the TSV-R antiserum.

Rose is also naturally infected by TSV (7), but this was an isolated occurrence. Munger black raspberry stocks in Oregon and the few tested from Washington seem to be almost universally infected with TSV-R.

The isolates and strains of tobacco streak virus that were described before 1971 were all reported to be serologically indistinguishable from the standard strains of TSV described by Fulton (8). In 1971, Gooding (10) reported a strain of TSV from tobacco in North Carolina, TSV-NC, that was serologically related but not identical to Fulton's type strain. Serological comparisons between TSV-NC and TSV-R have not yet been made. If TSV-R and TSV-NC are shown to be distinct, TSV-R will be the third strain of TSV that has some distinctive serological properties.

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