Transmission of Satellite of Tobacco Ringspot Virus by Xiphinema americanum

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ABSTRACT

Satellite (S-TRSV) of tobacco ringspot virus (TRSV) was transmitted with activator TRSV by *Xiphinema americanum* from cucumber to cucumber. Plants became systemically infected with both TRSV and S-TRSV after nematode transmission, developing a chlorotic mottle in young leaves. Symptoms were indistinguishable between cucumber plants infected with TRSV alone and plants with TRSV plus S-TRSV.

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A satellite-like virus, associated with tobacco ringspot virus (TRSV) by Schneider (7), has since been characterized as a satellite requiring activation by TRSV for its replication, and has been designated S-TRSV (10). Particles of S-TRSV are not different from TRSV in size and shape or in exclusion of stain when negatively stained and compared by electron microscopy (8). S-TRSV is serologically indistinguishable from activator TRSV (7, 8, 9), and the activator codes for protein-coat structure of S-TRSV (9).

Xiphinema americanum Cobb is an efficient vector of TRSV (3). It transmits several serological strains of the virus (5, 6), including two strains together (6). Therefore, tests were made to determine the ability of this nematode to transmit S-TRSV with activator TRSV from cucumber (*Cucumis sativus* L. 'Model') infected with both to healthy cucumber.

A watermelon isolate of TRSV from Arkansas (PV-125, ATCC), which is transmitted by *X. americanum* (3), was used as the activator of S-TRSV. The isolate designated ST-TRSV, which could be distinguished alone or with S-TRSV by the size of lesions produced on cowpea [*Vigna sinensis* (Torner) Savi] (7, 8), produced very poor titer in the roots of cucumber and was not readily transmitted from this host by nematodes. Purified preparations from cucumber infected with PV-125 TRSV plus S-TRSV gave an ultraviolet absorption profile on a centrifuged density-gradient column which indicated replication of S-TRSV (8). Lesion size on cowpea did not distinguish presence of S-TRSV, because PV-125 TRSV alone caused many different lesion sizes.

Tests for transmission of S-TRSV by *X. americanum* were conducted in Arkansas. Virus-inoculum stocks were prepared by grinding eight lesions produced on cowpea by PV-125 TRSV alone per ml of 0.02 M phosphate buffer pH 7.2, and five tiny lesions produced on cowpea by S-TRSV plus ST-TRSV per ml buffer. One-week-old cucumbers growing in fine sand were shaded ca. 16 hr, then cotyledons dusted with Carborundum were inoculated with (i) PV-125 TRSV, (ii) S-TRSV, (iii) equal volumes of PV-125 TRSV and S-TRSV stocks, or (iv) not inoculated. Plants inoculated with PV-125 TRSV or PV-125 TRSV plus S-TRSV developed systemic chlorotic mottle symptoms within 7 days and were used as virus acquisition hosts for *X. americanum*. Subsequent mention of TRSV will refer to PV-125 TRSV.

Procedures for TRSV and satellite acquisition and transmission by *X. americanum* were as previously described (3). Following 10-days' acquisition access, groups of 10 active nematodes per plant were given 30-days' transmission access to cucumber bait plants growing in fine sand at 28 to 30 C. In one test, there were 15 bait plants per acquisition source i, ii, and iv above, and 32 bait plants for source iii. In a second test, there were 32 bait plants for each acquisition source.

Systemic chlorotic mottle symptoms developed in some bait plants 2 to 3 weeks after addition of nematodes that had acquisition access to plants infected either with TRSV or TRSV plus S-TRSV. The symptoms were not distinguishable.

Roots and top leaves of all bait plants were indexed separately by mechanical inoculation of 'Early Ramshorn' cowpea and Model cucumber 30 days after nematodes were added. Development of necrotic lesions in cowpea and a systemic chlorotic mottle in cucumber indicated presence of TRSV, but presence of satellite could not be distinguished by symptoms. Virus was recovered from all bait plants to which nematodes had

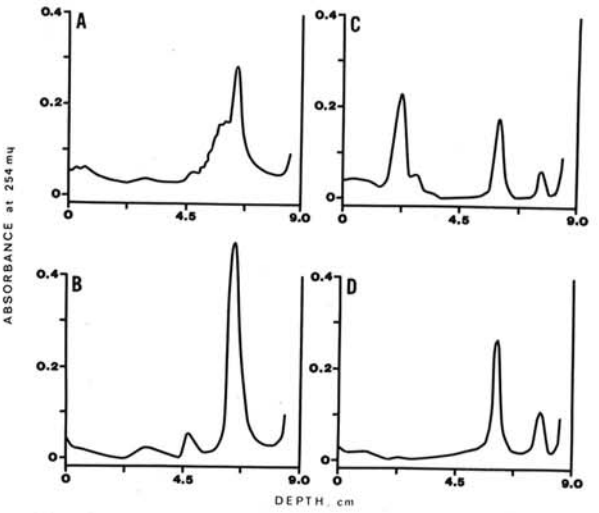

Fig. 1. Ultraviolet absorbance profiles of centrifuged density-gradient columns containing **A)** tobacco ringspot virus (TRSV) plus satellite of TRSV transmitted to cucumber by *Xiphinema americanum;* **B)** TRSV transmitted to cucumber by *X. american;* **C)** nucleic acids from A; **D)** nucleic acids from B. Centrifugation with Spinco SW41 rotor at 40,000 rpm for 90 min **(A & B)**, and at 30,000 rpm for 16 hr **(C & D)**.

transmission access following acquisition access to TRSV-infected plants, or plants infected with TRSV plus S-TRSV. No virus was transmitted by nematodes that had access to plants inoculated with S-TRSV alone or to healthy plants.

One week after initial symptom development, the cucumber indicator plants were harvested, grouped to provide more than 50 g of tissue per group, and frozen. In the first test, one group of plants had received virus from tops and one group virus from roots of TRSV plants, and two groups had received virus from tops and two groups virus from roots of TRSV plus S-TRSV bait plants. In the second test there were three groups of each. The frozen material was shipped over dry ice to the Plant Virology Laboratory, Beltsville, Md., to be analyzed for presence of the satellite.

Virus was purified from each group of plants as previously described (10). Nucleic acids were extracted from part of each purified virus preparation (2). All purified virus preparations and their corresponding nucleic acids were analyzed by sucrose density-gradient centrifugations as before (1), except that centrifugation was with a Spinco SW 41 rotor at 40,000 rpm for 90 min for virus, and at 30,000 rpm for 16 hr for nucleic acids. Ultraviolet absorption profiles after centrifugation of virus revealed components sedimenting more slowly than a homogeneous component (bottom component of TRSV). In both tests, these components indicated the presence of S-TRSV as well as TRSV in all groups of indicator plants inoculated with extracts of roots or tops of TRSV plus S-TRSV bait plants. Profiles of preparations from groups of plants inoculated with extracts from TRSV-only bait plants were characteristic of TRSV only. An example of each profile is shown (Fig. 1-A and 1-B, respectively).

A predominant fraction sedimenting slowly to a position characteristic of the nucleic acid of S-TRSV (8), and two peaks characteristic of TRSV nucleic acid (1), were present in nucleic-acid preparations from purified extracts of all groups of TRSV plus S-TRSV indicator plants from both tests (Fig. 1-C). In each group, more than 50% of the nucleic acid was satellite nucleic acid. Slightly faster fractions than the nucleic acid of S-TRSV were typically present in small amounts. These may have been specific aggregates, such as dimers and trimers, of the S-TRSV nucleic acid. Two peaks characteristic of TRSV nucleic acid were present in purified extracts of TRSV-only index plants (Fig. 1-D). A small amount of absorbance occurred near the position of satellite nucleic-acid peak in some preparations in this group, which may indicate occasional satellite contamination.

These results show that *X. americanum* acquires and transmits S-TRSV and activator TRSV together during feeding. Particles of S-TRSV are probably retained in the same manner as TRSV in the lumen of the alimentary tract of *X. americanum* (4), so there would be a mixture of TRSV and S-TRSV particles retained by nematodes feeding on a plant with mixed infection. Since the protein coat of S-TRSV is coded by TRSV nucleic acid (9), transmission of S-TRSV suggests that protein-coat structure could be important in specific acquisition of virus by *X. americanum*.

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