

Symptomatically Distinct Strains of Tomato Ringspot Virus Isolated from Grape and Elderberry

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ABSTRACT

Leaf samples of Dutchess (*Vitis labrusca*) and Riesling (*V. vinifera*) grapevines in New York yielded three symptomatically distinct isolates of tomato ringspot virus (TmRSV). One isolate induced large chlorotic lesions on inoculated leaves of lima bean and necrotic bordered ringspot in snapdragon. A second induced large necrotic local lesions and necrotic line patterns in lima bean and snap-

dragon, respectively. A third grapevine isolate and an isolate from elderberry (*Sambucus canadensis*) were symptomatically similar, but differed from the other grape isolates. These isolates failed to induce symptoms on the inoculated leaves of snapdragon and lima bean. All isolates were identified as strains of TmRSV, and appear to represent a single serotype. *Phytopathology* 60:1838-1841.

Additional key words: Tomato ringspot virus purification; tobacco ringspot virus serology.

Preliminary identification of tobacco ringspot virus (TRSV) and tomato ringspot virus (TmRSV) is often based on differences in symptoms exhibited on the selective host plants lima bean (*Phaseolus lunatus*), snapdragon (*Antirrhinum majus*), watermelon (*Citrullus vulgaris*), and cotton (*Gossypium hirsutum*) (1, 9, 10, 11). TRSV induces local necrotic lesions on the inoculated leaves of lima bean and snapdragon, with some strains inducing systemic necrosis in lima bean and apical dieback in snapdragon (5, 11, 12, 18). TRSV strains were reported to systemically infect watermelon but not cotton (11). Systemic symptoms in cotton, however, are induced by certain TRSV strains (13). Some isolates of TmRSV induced chlorotic local lesions and mild systemic mottling or severe veinal necrosis of lima bean, snapdragon, and cotton (1, 2, 7, 11), but did not infect watermelon (9, 11).

Foliar symptoms of grapevines infected with grape yellow vein virus (GYVV), a strain of TmRSV, ranged from a faint chlorotic mottling to chrome-yellow flecking with yellow vein bands (7). Diseased vines grew vigorously with partially filled clusters. Grape yellow vein virus is serologically related, but distinct from the type culture of TmRSV (ATCC-13) and peach yellow bud virus (6, 16).

TmRSV was isolated from several grapevines and elderberries in New York. Symptoms observed in diseased Dutchess grapevines were a faint mottling in the terminal leaves, increased cane size, and slight to moderate reduction of fruit set. In contrast, the diseased Riesling vine was severely stunted, with a pronounced chlorotic mottle and shortened internodes. These symptoms resembled those described in TRSV-infected Riesling and Mission (5).

A variety of foliar symptoms occurred in TmRSV-infected elderberry. Some leaves showed chlorotic line patterns, oak leaf patterns, and ringspots, while others evidenced only a general chlorosis or a light green-dark green mottle. This paper presents the host range and serology of the TmRSV isolates from grapevines and elderberry.

MATERIALS AND METHODS.—Herbaceous host range plants used in these tests included cucumber (*Cucumis sativus* L. 'National Pickling'), tobacco (*Nicotiana tabacum* L. 'Havana 423'), snapdragon (*Antirrhinum majus* L. 'Red Giant Crimson'), lima bean (*Phaseolus lunatus* L. 'Henderson Bush'), watermelon (*Citrullus vulgaris* Schrad. 'Charleston Gray'), and *Chenopodium quinoa* Willd. Several virus cultures employed herein included TmRSV isolates Dutchess 8-9; Dutchess 22-11; Riesling 28-25-281; elderberry-Johns 1-23; a cucumber isolate, and a raspberry isolate supplied by R. S. Providenti and J. A. Keplinger (Geneva, N. Y.), respectively. The TRSV culture was described elsewhere (5). Virus inoculum was obtained from infected cucumber or tobacco leaf tissues triturated in 0.01 M potassium phosphate buffer, pH 7.0. Corundum (600 mesh) was sprinkled on the leaves as an abrasive to facilitate infection. Pre- and postinoculated plants were maintained at greenhouse temp of 25-28 C.

Virus purification.—The elderberry isolate of TmRSV was propagated in cucumber plants, harvested 10 to 14 days after inoculation, and comminuted in a Waring Blendor with 1.5 ml/g, 0.05 M potassium phosphate buffer, pH 7.0, containing 0.02 M sodium thioglycollate. The resulting extract was frozen, usually for 24 hr, and thawed overnight at room temp. After low-speed centrifugation (Sorvall GSA rotor, 8500 rpm/10 min), butanol (7 ml/100 ml) was slowly added to the supernatant, and the mixture placed in a hot water bath for 30 min at 40 C (6). The warm aqueous solution was given two alternating low- and high-speed centrifugations (Spinco 30 rotor, 28,000 rpm/2 hr). High-speed sedimented pellets were resuspended in 0.01 M potassium phosphate buffer, pH 7.0.

Additional purification was achieved with a sucrose density gradient centrifugation (5) and a zone electrophoresis separation in potassium phosphate buffer, 0.037 M, pH 7.6 (17). Each of these procedures resulted in two opalescent zones, but only the bottom band (i.e., the fastest sedimenting component and the slowest migrating component formed during centrifugation and

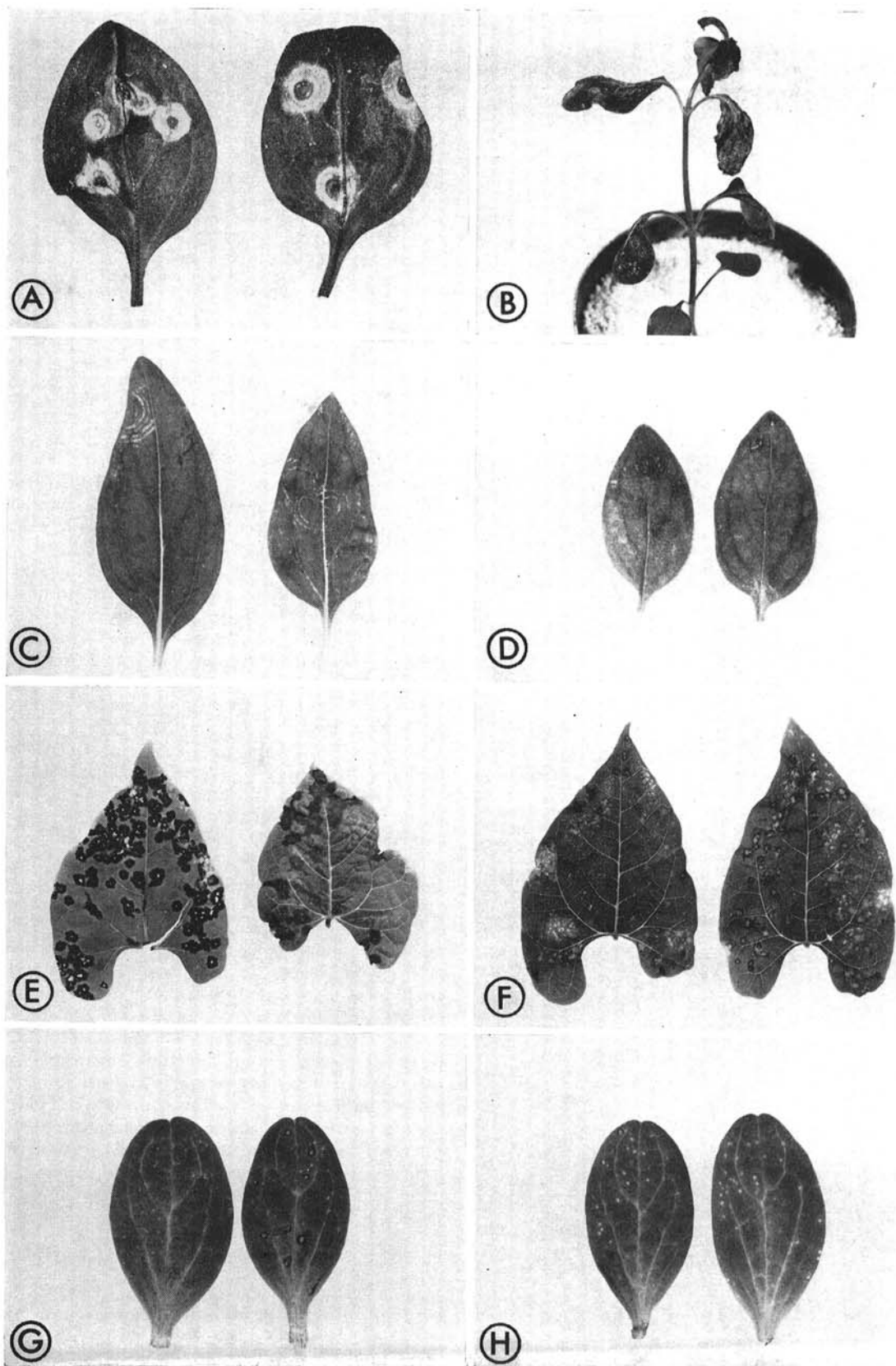


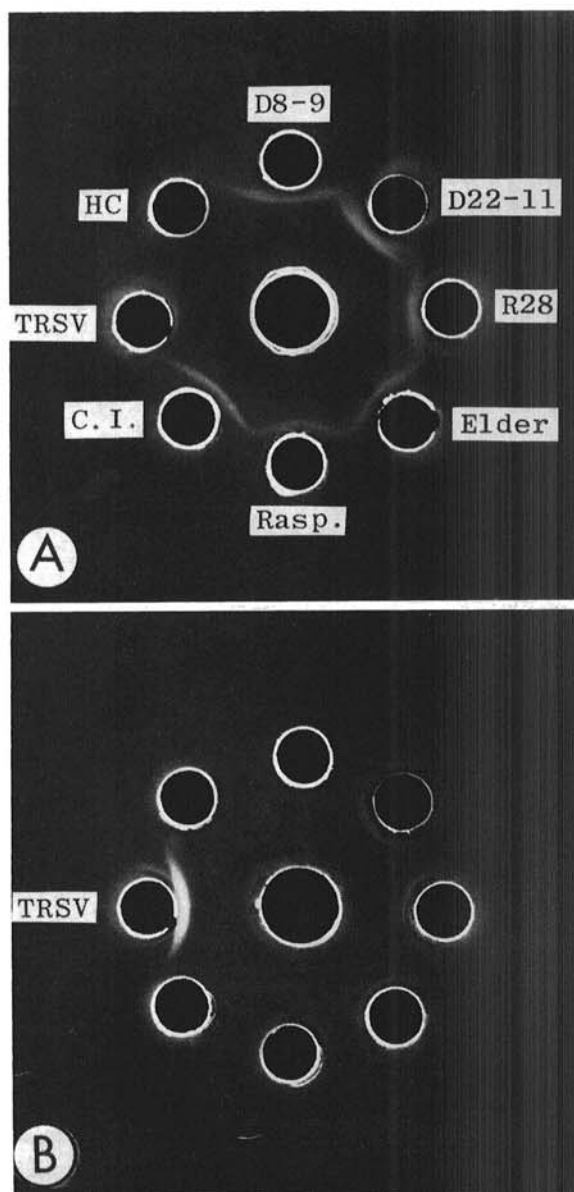
Fig. 1. Symptoms induced by strains of tobacco ringspot virus (TRSV) and tomato ringspot virus (TmRSV) isolates Dutchess 22-11 and 8-9. **A, B)** TRSV-induced local necrotic ringspots and systemic apical dieback, respectively, on snapdragon. **C)** Dutchess 22-11-induced concentric line patterns; and **D)** Dutchess 8-9-induced necrotic bordered ringspots on snapdragon. **E, F)** Local necrotic lesions induced by TRSV and Dutchess 22-11, respectively, on lima bean. **G)** TRSV- and **H)** TmRSV-induced local lesions on watermelon cotyledons.

TABLE 1. Symptoms induced in herbaceous plants by tomato ringspot virus (TmRSV) and tobacco ringspot virus (TRSV) isolates

Virus/Hosts ^a	<i>Antirrhinum majus</i> L.	<i>Chenopodium quinoa</i> Willd.	<i>Phaseolus lunatus</i> L.	<i>Citrullus vulgaris</i> Schrad.
Dutchess 8-9	RS, SCM, + ^b	CLNC, AT, AD	CLL, SCM	NLL, —
Dutchess 22-11	CLPR, +	CLNC, AT, AD	NLL, SNL	NLL, —
Riesling 28-25-281	SCM, +	CLNC, AT, AD	SCM	NLL, —
Elderberry isolate	SCM, +	CLNC, AT, AD	SCM	NLL, —
Cucumber isolate	SCM, +	CLNC, AT, AD	SCM	NLL, SCM, SNF, —
Raspberry isolate	SCM, +	CLNC, AT, AD	SCM	NLL, SCM, SNF, —
TRSV	NRS, AD	CLNC	NLL, SNL	NLL, SCM, SNF, —

^a TmRSV and TRSV isolates induced similar responses in *Cucumis sativus* L. (CLL, SCM) and *Nicotiana tabacum* L. (NLL, RS).

^b Attempts to recover virus from apical leaves; indexed onto *C. quinoa* where '+' = recovery and '-' = no recovery. AD = apical dieback; AT = apical twisting; CLL = chlorotic local lesion; CLNC = chlorotic lesion with necrotic center; CLPR = concentric line pattern ringspot; NLL = necrotic local lesion; RS = ringspot; SCM = systemic chlorotic mottle; SNF = systemic necrotic fleck; SNL = systemic necrotic lesion.



electrophoresis, respectively) was infectious. The virus zone was removed, homogenized with Freund incomplete adjuvant, and injected into rabbits. Subcutaneous and intramuscular injections were given over a 4-week period, before bleeding.

Serological tests were conducted in Ionagar gels (0.85%) employing an 8-membered well pattern and antigen-antiserum placement as described by Grogan et al. (8). Test antigen source was expressed sap from infected cucumber. Preparation of antiserum to the grape isolate of TRSV was described earlier (5). Antiserum to a strain of TmRSV was kindly supplied by K. A. Kimble (University Calif., Davis).

RESULTS.—Host range.—TRSV and TmRSV were readily differentiated in infected snapdragon seedlings. TRSV induced necrotic ringspots and apical dieback; TmRSV induced concentric line patterns or necrotic bordered ringspots and/or a systemic chlorotic mottle, depending on the isolate (Fig. 1). *Chenopodium quinoa* and lima bean were less satisfactory diagnostic indicators. Local lesions were produced on inoculated leaves of *C. quinoa* with either TmRSV or TRSV, but TmRSV rapidly became systemic, inducing twisting and dieback of the apical leaves. In lima bean, TRSV incited necrotic local lesions, but the TmRSV isolates, with the exception of Dutchess 22-11 isolate, did not (Fig. 1).

Other hosts tested, i.e., watermelon, tobacco, and cucumber, were not satisfactory differential hosts (Table 1). For example, members of both virus groups induced local lesions on watermelon cotyledons (Fig. 1), ringspots in tobacco, and a systemic mottle in cucumber. TRSV and two isolates of TmRSV (cucumber and raspberry isolates) induced an apparent systemic chlorotic mottle and necrotic flecks in watermelon. Two

Fig. 2. Serological reaction in agar double-diffusion plates. A) Tomato ringspot virus antiserum in center well; B) Tobacco ringspot virus antiserum in center well. Peripheral well contained virus isolates in expressed cucumber sap and were designated as follows: D 8-9 = Dutchess 8-9; D 22-11 = Dutchess 22-11; R 28 = Riesling 28-25-281; Elder = elderberry isolate; Rasp = raspberry isolate; CI = cucumber isolate; TRSV = tobacco ringspot virus; and HC = healthy cucumber.

separate attempts to transfer virus from these leaves to *C. quinoa* failed.

Serology.—Titer of elderberry TmRSV antiserum was determined in agar gel plates and expressed as reciprocal of dilution of homologous antigen (virus in expressed cucumber sap) and healthy cucumber sap (1024 and 8, respectively).

All TmRSV isolates, regardless of host origin, produced a single congruent precipitin line in agar double-diffusion plates using either TmRSV antisera (Fig. 2-A), suggesting an identical antigenic relationship. Until further tests are made, using additional homologous antisera, the presence of serologically distinct strains among the isolates reported here cannot be excluded. TmRSV antibodies failed to react with TRSV, and in reciprocal tests with TRSV antiserum, only the homologous antigen-antibody interaction was evident (Fig. 2-B).

DISCUSSION.—Although unrelated serologically (15), TmRSV and TRSV share many common physical and biological properties. The two viruses have a common soil-borne vector (4, 16), are morphologically similar (3, 14), and infect a wide range of host plants (11). Despite similarities in symptoms produced in several test plants, selective host plants can serve as differential hosts for tentative virus identification. Of sensitive indicator plants, snapdragon was superior, followed by *C. quinoa* and lima bean. Watermelon was useless, as isolates of either virus incited similar symptoms.

Antigenically distinct strains of TmRSV have been reported (6, 16); however, based on a precipitin pattern achieved with two TmRSV antisera, only a single serotype was demonstrated. Distinct serotypes among these isolates might be detected were additional homologous antisera available for testing. Production of antisera to the various isolates is in progress.

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