A New Grapevine Disease Induced by Tobacco Ringspot Virus

R. M. Gilmer, J. K. Uyemoto, and L. J. Kelts

Professor, Assistant Professor, and Experimentalist, respectively, Department of Plant Pathology, New York State Agricultural Experiment Station, Geneva 14456.

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ABSTRACT

A new grapevine disease discovered in several New York vineyards was induced by tobacco ringspot virus (TRSV). In two vineyards containing a mixture of European and American grape cultivars, only vines of European cultivars were infected. Symptoms were similar to those of grape fan leaf disease: delayed initiation of growth; stunting; and chlorotic rings or watermarking of foliage. In midsummer, leaves were mottled and malformed, with prominent irregular serrations and broad petiolar sinuses. Diseased vines were severely injured by cold; they set few or no fruits the 2nd year of infection.

The causal virus was identified as TRSV by host range, serology, and electron microscopy. TRSV was readily isolated by mechanical transmission from foliage of infected vines and from roots or callus of infected cuttings. TRSV was sometimes irregularly distributed in cuttings from infected vines. The disease syndrome was partially reproduced in young grape seedlings that were mechanically inoculated with TRSV isolates from infected vines.

TRSV was recovered from cucumber or snapdragon seedlings grown in soil from an infected vineyard, suggesting soil transmission of the virus to grape. The nematode *Xiphenema americanum*, a vector of TRSV, was abundant in vineyard soil. Phytopathology 60:619-627.

In a recent review of grapevine virus diseases, Hewitt (12) listed five soil-borne viruses of grape (*Vitis vinifera* L.). Only three of these were reported in North America: grape fan leaf virus (GFLV) in California (11), Canada (3), and Mexico (15); grape yellow vein virus (GYVV) in California (7); and peach rosette mosaic virus in Michigan (2, 4).

Two of these viruses were found in surveys of New York vineyards in 1962 (6). Restricted occurrences of GFLV were observed twice, involving five White Riesling (Johannisberger Riesling) vines in one instance and six Concord vines on 1202 rootstocks in the second. Propagating materials of White Riesling and 1202 rootstocks both originated in California, and the circumstances suggested that both lots of vines were infected when planted. GFLV did not spread to adjacent healthy vines within a 4-year period before the infected vines were destroyed.

A few isolated and scattered infections with GYVV were found in certain American grape cultivars (*V. labrusca* L.) (6). Tomato ringspot virus, synonymous with GYVV (7), is common on other woody host plants in New York.

In 1968, prominent chlorotic rings and watermarks were observed in the foliage of a number of indexed White Riesling and Mission vines of an experimental vineyard at Geneva planted in 1963. A single White Riesling vine with these symptoms was found shortly afterward in a second vineyard at Geneva. In 1969, surveys in commercial vineyards near Hammondsport and Urbana, New York, disclosed a number of diseased vines of White Riesling and Pinot Chardonnay.

This paper presents evidence that this grapevine disease was induced by tobacco ringspot virus (TRSV), together with observations suggesting that the virus was soil-borne and spread selectively in own-rooted *V. vinifera* vines.

Previous history of affected vineyards.—1). *Vineyard A, Geneva.*—This vineyard (Fig. 1) of four rows of 102 vines each was planted in June 1963. Each row comprised three own-rooted six-vine replicates of the following cultivars: Niagara; Delaware; Concord; Catawba (each *V. labrusca*); and White Riesling (*V. vinifera*). The respective ends of each row were terminated by own-rooted six-vine plots of Mission (*V. vinifera*) and St. George (*V. rupestris* Scheele). Guard rows were added at each side of the original planting in 1964. These rows contained an assortment of own-rooted cultivars: those named previously; the French hybrids LN-33 and Baco 22-A; and Pinot Blanc, Pinot Chardonnay, and Pinot Noir (each *V. vinifera*).

Delaware, Niagara, Concord, Catawba, and White Riesling vines of this planting were derivatives of a single parent vine. Each parent vine was indexed in 1959-1962 by bud-inoculating St. George and Mission indicators, and by mechanically inoculating seedlings of *Chenopodium quinoa* Willd. The sole virus detected in the parent vines was tobacco mosaic virus (5). Vines of St. George, Mission, Pinot Noir, LN-33, and Baco 22-A were derived from healthy propagating materials supplied from the grapevine indexing program of University of California, Davis, courtesy of A. C. Goheen.

The vineyard site had probably never been planted previously with grapevines. Apple, pear, and cherry nursery trees were grown there in 1944-1954; in 1955, the site was planted with Montmorency sour cherry trees that were removed in 1962.

Vines planted in 1963 grew normally. The initial symptoms of virus infection were noted in July 1968 in scattered vines of White Riesling, Mission, and Pinot Chardonnay (Fig. 1).

2). *Vineyard B, Geneva.*—This vineyard was located about 1,000 meters from vineyard A, in an area

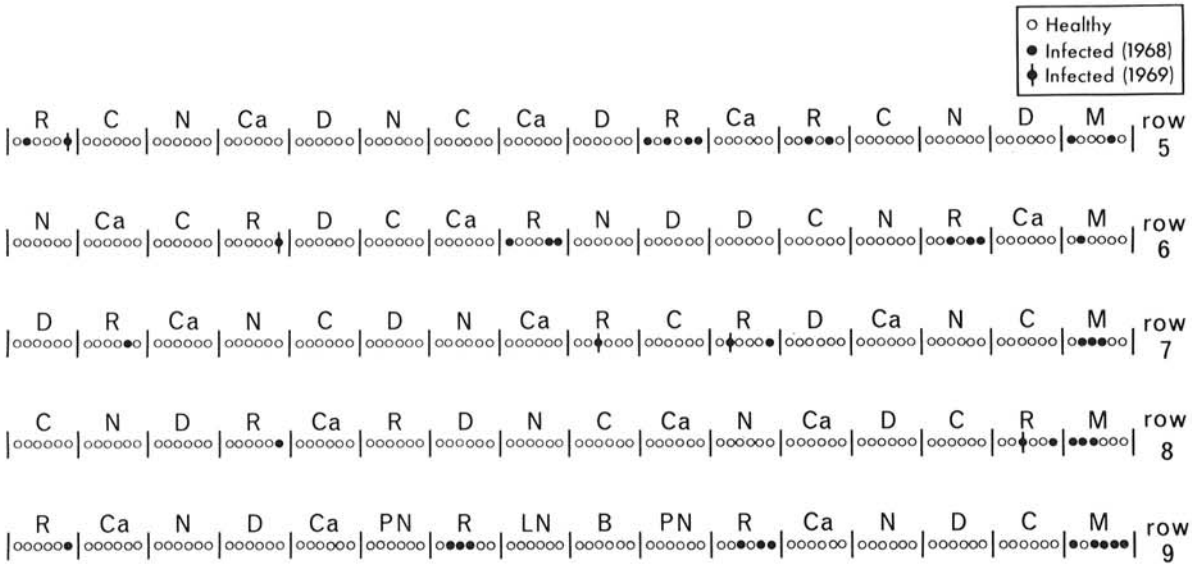

Fig. 1. Spread of tobacco ringspot virus in part of Vineyard A, Geneva, 1968-1969. Variety key: B = Baco 22-A; C = Concord; Ca = Catawba; D = Delaware; LN = LN-33; M = Mission; N = Niagara; PN = Pinot Noir; R = White Riesling.

completely isolated from other grapevines. The site had not been cultivated for at least 15 years before it was planted in 1959 with White Riesling, Traminer, Muscat Ottonel, and Pinot Chardonnay vines introduced directly from Europe under postentry quarantine. These vines were grafted on an assortment of phylloxera-resistant rootstocks: 5-BB, 5-C, 26-G, and 18815. Own-rooted vines of Delaware and Concord were interspersed in the vineyard for comparisons of cold-hardiness.

Each of the introduced vines was indexed for viruses by bud-inoculating St. George and Mission indicators, supplemented with two or more indexings by mechanically inoculating *C. quinoa*. Careful visual inspections of each vine were made in July and August for a 5-year period (1959-1963).

As soon as virus symptoms were discovered in Vineyard A, Vineyard B was inspected in July 1968. A single infected White Riesling vine was found; its debilitated condition suggested that the vine had been infected at least the previous year. No new infections were discovered in the 1969 surveys.

3). *Vineyard C, Hammondsport.*—This vineyard was a large commercial planting containing a number of *V. vinifera* and French hybrid cultivars planted in 1958-1962. In a block containing 1,200 White Riesling vines grafted on an unknown phylloxera-resistant rootstock, four widely separated infected vines were found in July 1969. No infected vines were discovered in a contiguous planting of about 500 Seibel-1,000 vines. In a block of 1,250 Pinot Noir and 1,300 Pinot Chardonnay vines about 400 meters distant, one infected Pinot Chardonnay vine was found. This vine was also grafted on an unknown phylloxera-resistant rootstock.

4). *Vineyard D, Urbana.*—This vineyard was a large commercial planting containing White Riesling, Pinot Chardonnay, and several American grape cultivars. Scions of White Riesling and Pinot Chardonnay of California origin were grafted on the phylloxera-resistant rootstocks 5-C, 26-G, and 3309. In 2400 White Riesling vines planted in 1961, three widely separated vines with symptoms were found—one on 26-G and two on 3309 rootstocks. Their symptoms suggested that the vines had been infected for at least 1 year. In the planting of 2400 Pinot Chardonnay vines located about 600 miles distant and planted in 1962, three widely separated infected vines were found. Symptoms in one of these vines suggested a fairly recent infection; the remaining two vines had probably been infected for at least a year or more.

Spread of TRSV in vineyard A.—In the initial surveys of Vineyard A (July-September 1968), 24 infected White Riesling, 15 infected Mission, and two infected Pinot Chardonnay vines were identified by foliage symptoms. Examinations of each vine of Niagara, Concord, Delaware, Catawba, and St. George for foliage symptoms were negative.

Canes from 510 vines were individually labeled and collected in November 1968. The following February, 25-40 two-bud hardwood cuttings/vine were planted in sterilized rooting medium in a propagating bench. In April, a small amount of young leaf tissue from each living cutting was indexed by mechanically inoculating *C. quinoa* or snapdragon (*Antirrhinum majus* L.) indicators. Cuttings were similarly re-indexed from succulent root or callus tissue in May.

Three White Riesling vines without symptoms in 1968 yielded TRSV in these indexings. Each of these vines showed foliage symptoms in June 1969, as did an additional four White Riesling vines that did not yield TRSV in the April or May indexings. A single Pinot Blanc vine of a guard row, without symptoms in 1968 and not indexed in April 1969, also developed symptoms in June 1969. Three other White Riesling

vines, without symptoms in 1968, negative in the April indexing, and without symptoms in June 1969, developed symptoms in late July 1969.

In the April or May indexing of cuttings, TRSV was not recovered from any of 84 Niagara, 84 Delaware, 90 Catawba, 78 Concord, 30 St. George, 12 Pinot Noir, 6 LN-33, or 6 Baco 22-A vines. None of these vines showed foliage symptoms in the 1968 and 1969 field inspections.

Symptoms of the disease.—Symptoms of the disease resembled those of grape fanleaf (11). Growth of diseased vines was initiated 7-10 days after that of healthy vines. In newly infected vines, chlorotic rings, spots, or watermarks appeared in the basal leaves about 3-4 weeks after growth began. These symptoms were very prominent in early July (Fig. 2). As growth continued, leaves were increasingly malformed, with prominent irregular serrations and broad petiolar sinuses (Fig. 2). Rings or watermarks were often absent in the malformed leaves, but a diffuse mottle was present.

In the early stages of infection, foliage symptoms were often present on only one or two canes, with the remainder of the vine appearing normal, but symptoms

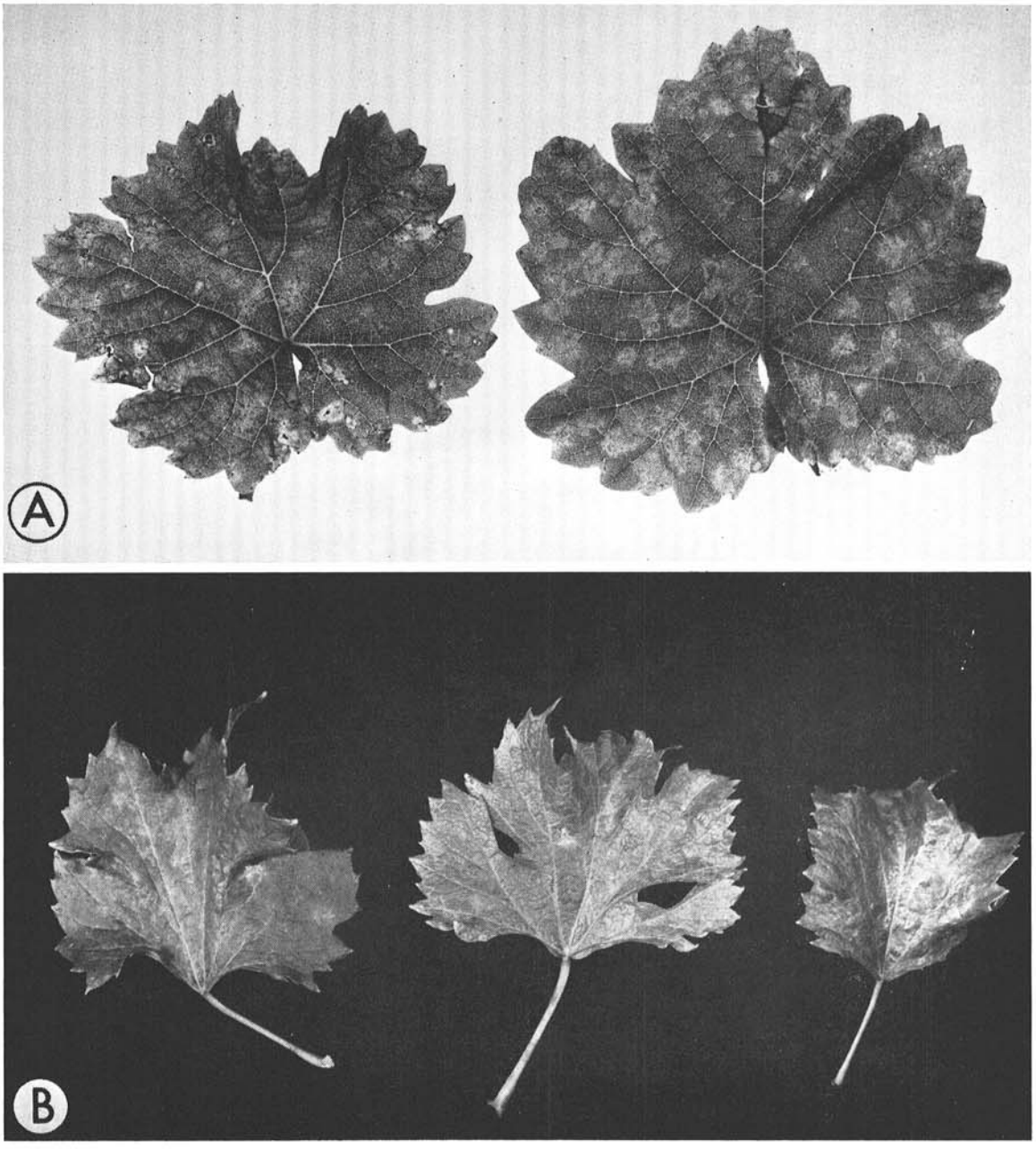

Fig. 2. Tobacco ringspot virus-induced symptoms in foliage of White Riesling grape. **A)** Chlorotic rings in basal leaves of a newly infected vine. **B)** Mottled, malformed leaves produced on later growth.

later developed on foliage throughout the vine. Shoot growth of infected canes was rosetted with small leaves and markedly reduced in diam. Vines diseased in 1968 were severely injured by cold, and many canes were completely dead in 1969. Growth from surviving buds was delayed and very rosetted; the leaves were small, mottled, and malformed (Fig. 3). These unthrifty vines set few or no fruits.

Irregular distribution of TRSV in cuttings from infected vines.—Canes from a number of healthy and TRSV-infected White Riesling vines of Vineyard A were collected in November 1968. Two-bud cuttings were prepared in February and inserted in sterilized rooting medium of coarse sand and peat moss. Cuttings were indexed from leaf tissue shortly after growth began, and were re-indexed from root or callus tissues in May.

Twenty/28, 19/30, and 21/25 cuttings from three healthy vines rooted, an over-all average of 72%. In contrast, cuttings from infected vines rooted poorly or not at all (Table 1). Of 93 surviving rooted cuttings from infected vines, only 21 yielded TRSV when indexed. The 72 healthy cuttings from these infected vines may have originated from canes not yet invaded by TRSV, even though symptoms were evident in some portions of the parent vines at least 3 months before the canes were collected. In those instances where no cuttings from an infected vine survived, it appears probable that the virus was completely systemic.

TABLE 1. Production of healthy and infected cuttings from canes of White Riesling grapevines infected with tobacco ringspot virus

Vine no.	No. cuttings rooted/ no. cuttings prepared	No. cuttings infected
4-45	15/52	1
5-28	4/28	4
5-37	0/27	
6-14	23/46	9
6-50	9/23	6
6-54	0/16	
6-73	0/25	
7-31	0/14	
8-7	24/65	1
8-73	18/48	0
Totals	93/344 (27%)	21

Indexing procedures.—TRSV was readily transmitted from infected grapevines or cuttings to herbaceous indicators with inoculum prepared by comminuting infected grape tissues in Kirkpatrick-Lindner buffer (3% nicotine; 0.05 M K_2HPO_4; 0.005 M cysteine hydrochloride, pH 9.2). TRSV was easily isolated from young grape leaves collected in the field from early June-late September, and from young leaves collected

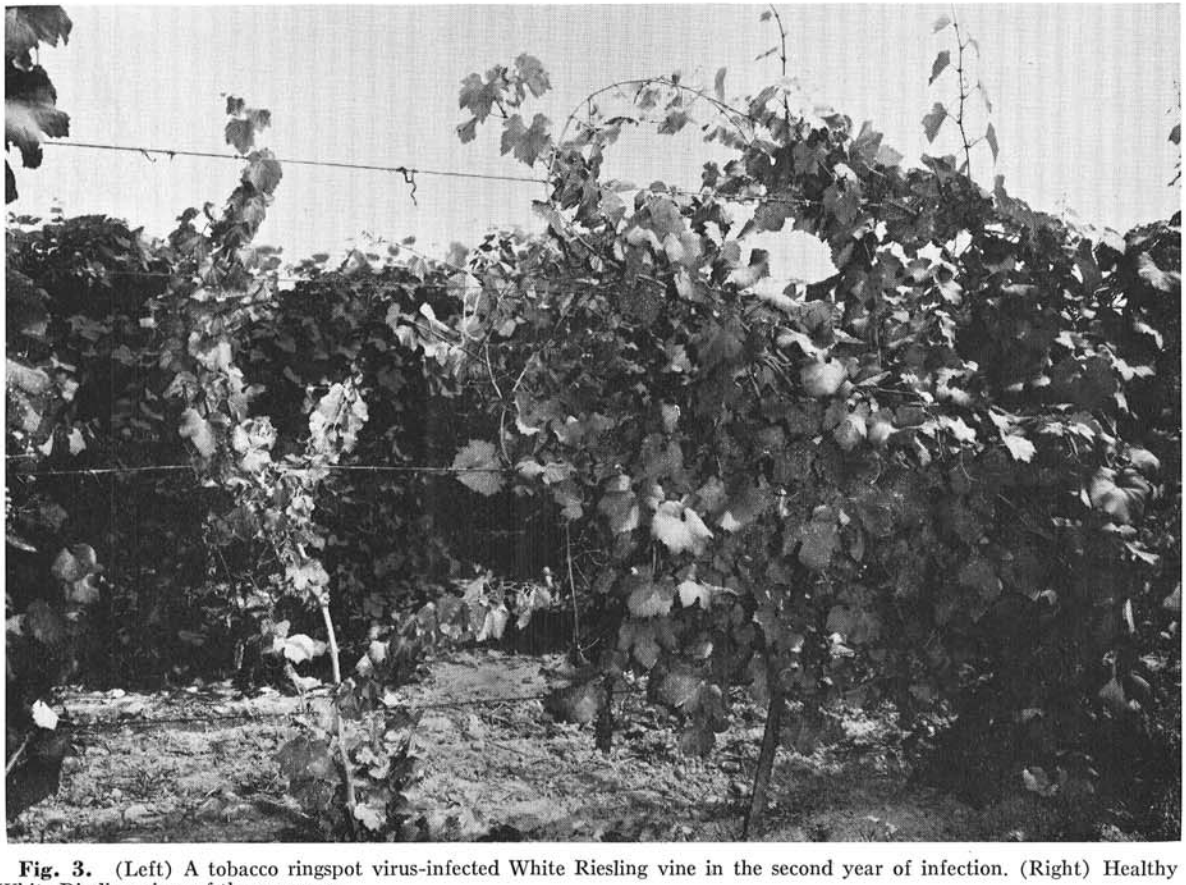

Fig. 3. (Left) A tobacco ringspot virus-infected White Riesling vine in the second year of infection. (Right) Healthy White Riesling vines of the same age.

in the greenhouse at any time. TRSV was also recovered from succulent roots or callus tissue of rooted hardwood cuttings.

In many of the early experiments, *C. quinoa* was used exclusively as an indicator. In this host, TRSV incited small necrotic local lesions within 4-6 days, later followed by necrosis and dieback of the apical shoot. These symptoms were not diagnostic for TRSV, since infection by tomato ringspot virus (TomRSV) induced similar but usually more severe symptoms in *C. quinoa*.

Snapdragon seedlings (*A. majus*) were more satisfactory indicators of TRSV (13). TRSV induced large necrotic local lesions within 3-4 days, followed by systemic necrotic spots or watermarks and severe apical dieback that sometimes killed the entire plant. These symptoms were diagnostic for TRSV; comparable inoculations with TomRSV on snapdragon induced only a faint chlorotic mottle of young leaves near the growing point. At high greenhouse temperatures of midsummer, some TomRSV isolates occasionally induced, on inoculated snapdragon leaves, faint chlorotic local lesions that slowly became mildly necrotic.

Herbaceous host range and physical properties of TRSV from grapevines.—Two TRSV isolates, Riesling 6-54 and Mission 9-1, were purified by two successive single-lesion selections in tobacco and cucumber. The symptoms induced by these isolates in various herbaceous hosts were essentially similar, and are indicated in Table 2.

In sap extracts of infected cucumber comminuted in 0.05 M phosphate buffer, the thermal inactivation point of TRSV isolates Riesling 6-54 and Riesling 9-31 was 65-68 C; extracts heated at 55 C were highly infectious. The dilution end point of each of these isolates in infective cucumber sap was 10^{-4}-10^{-5} when assayed on cucumber seedlings.

When purified by density-gradient centrifugation as later described, isolate Riesling 6-54 yielded two closely sedimenting bands. Preparations of each band were stained with 2% potassium phosphotungstate, pH 7.0, and examined with a Jeolco 100-B electron microscope. Isodiametric particles, about 24.1 ± 0.5 mμ, were present in both preparations, but numerous particles in the upper band were filled with PTA, suggesting partially empty virus particles (Fig. 4).

TABLE 2. Symptoms induced in various herbaceous plants by tobacco ringspot virus isolates from grapevines

Species	Symptoms[a]
Chenopodium quinoa Willd.	NLL, syst. RS and N
Antirrhinum majus L.	NLL, syst. RS and N
Nicotiana tabacum L. (H-423)	NLL or RS, syst. RS and WM
Callistephus chinensis Nees.	NLL, syst. N, death
Petunia hybrida Vilm.	NLL or RS, mild syst. RS
Cucumis sativus L.	CLL or NLL, syst. M or RS, stunt
Cucurbita maxima Dchsne.	CLL, syst. M or RS, stunt
Vigna sinensis (Torn.) Savi	NLL, syst. N
Phaseolus vulgaris L. 'Black Turtle Soup'	NLL, syst. M, leaf distortion
Phaseolus lunatus L. 'Fordhook'	NLL
'Henderson'	NLL, syst. N, death

[a] NLL = necrotic local lesions; CLL = chlorotic local lesions; N = necrosis; M = mottle; RS = ringspot; WM = watermark pattern.

Purification and serology.—Isolate Riesling 6-54, purified by two successive single-lesion transfers, was increased in cucumber seedlings that were harvested about 8-10 days after inoculation. Buffer (0.2 M phosphate, pH 7.6, containing 0.001 M DIECA and 0.1% mercaptoacetic acid) was added to infected cucumber tissue (1 ml/g). After comminution in a Waring Blendor, the resulting extract was strained through a double layer of cheesecloth. Butanol (10 ml/100 ml extract) was added, and the mixture was refrigerated for 9-10 hr at 2-4 C. After a low-speed centrifugation, 10% polyethylene glycol (MW 6000) and 0.3 M NaCl were added to the supernatant, and the mixture was again refrigerated at 2-4 C until a precipitate formed (8, 10). After a second low-speed centrifugation, the supernatant was discarded and the pellet was resuspended in 0.2 M phosphate (pH 7.1) by shaking for several hr. Following a third low-speed centrifugation, the supernatant was transferred to a Spinco 30 rotor and centrifuged at 28,000 rpm (68,000 *g*) for 2 hr. The resulting pellets were suspended in 0.2 M phosphate buffer (pH 7.1), and insoluble material was removed with a final low-speed centrifugation.

Density-gradient tubes (1) were prepared by layering 4, 7, 7, and 7 ml of 10, 20, 30, and 40% sucrose solutions in 0.01 M phosphate, pH 7.0. Tubes were equilibrated for 12 hr at 2-4 C before use. One to 2 ml of partially purified virus suspension was layered on each gradient column, and the tubes were centrifuged at 23,000 rpm (54,000 *g*) for 3 hr in a Spinco 25.1 swinging bucket rotor. Each gradient was scanned with an Isco Model 222 ultraviolet analyzer and a Model 180 density-gradient fractionator to locate virus bands.

Two distinct but closely sedimenting bands, corresponding to the "middle" (M) and "bottom" (B) bands described by Stace-Smith et al. (14), were located about halfway down the gradient. A sample from band B incited 200 local lesions/inoculated cowpea leaf at a dilution of 10^{-2}. Material from band M was much less infectious and at a 1:2 dilution incited only 18 lesions/inoculated cowpea leaf.

Three virus isolates from Geneva Vineyard A, four virus isolates from Hammondsport, and bands B and M of isolate Riesling 6-54 were tested with antisera of TRSV, TomRSV, cucumber mosaic virus (CMV), and GFLV supplied by K. A. Kimble and W. B. Hewitt, University of California, Davis. All antisera were 1:1 dilutions with glycerine. Except for bands B and M of isolate Riesling 6-54, which were partially purified, each of the virus isolates was in crude expressed sap of cucumber or tobacco plants infected by mechanical transmission from grapevines. The tests were conducted in agar gel double diffusion plates (Ionagar No. 2) as recommended by Grogan et al. (9), with appropriate antigen controls of TRSV, TomRSV, CMV (courtesy of R. Provvidenti), GFLV, healthy

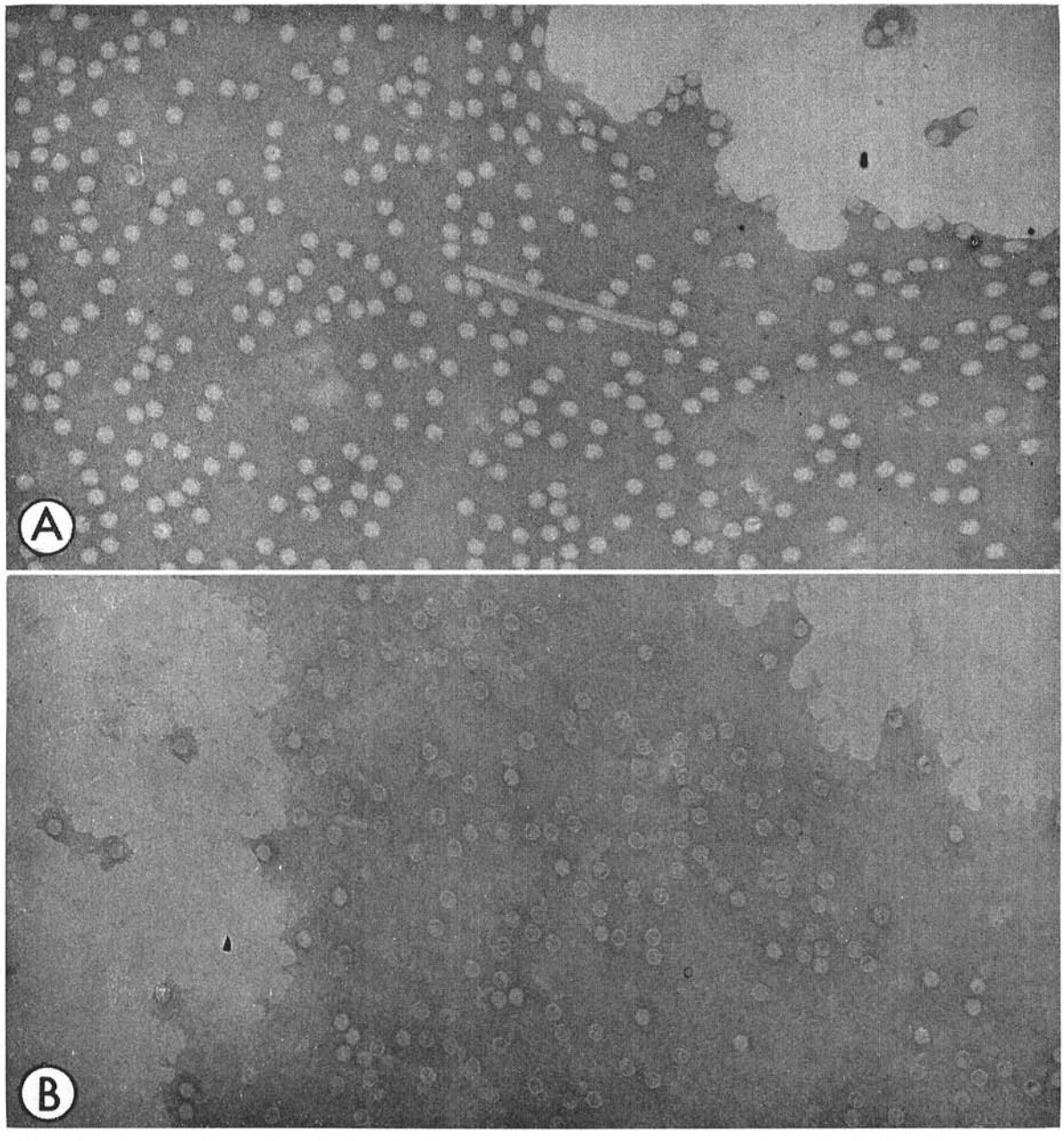

Fig. 4. Electron micrographs of sedimenting bands M and B of tobacco ringspot virus stained with 2% phosphotungstate (approx. ×100,000). **A)** Band B. A single TMV particle, added for calibration, is visible. **B)** Band M. Many particles appear devoid of RNA, as evidenced by the interior staining.

cucumber sap and healthy tobacco sap. Agar plates were incubated at 5 C and observed at 24-hr intervals for 5 days.

Bands M and B of isolate Riesling 6-54 and each of the Geneva and Hammondsport virus isolates formed precipitin lines with TRSV antiserum only. The precipitin patterns formed by two of these isolates in infected cucumber sap and TRSV antiserum are shown in Fig. 5.

Retransmission of TRSV to grape seedlings.—Initial attempts to transmit TRSV isolates from cucumber or *C. quinoa* to large, vigorously growing grape seedlings or cuttings of White Riesling were unsuccessful. Mechanical inoculations of young leaves or mature leaves, with or without preinoculation shading, or of succulent roots, did not transmit the virus.

Inoculations of very young grape seedlings in the one- or two-leaf stage were more successful. Open-pollinated seeds of Delaware (*V. labrusca*) and Pinot Noir (*V. vinifera*) were germinated and transplanted

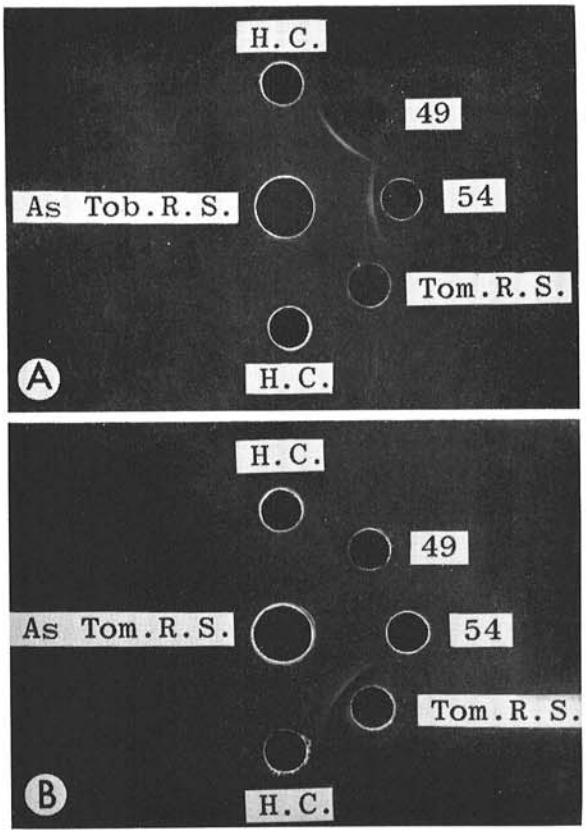

Fig. 5. Precipitin lines in agar gel diffusion plates. Key: AS Tob RS = tobacco ringspot virus antiserum; AS Tom RS = Tomato ringspot virus antiserum. Antigens in cucumber sap: HC = healthy; 49 and 54 = isolates Riesling 6-49 and 6-54, respectively; Tom RS = tomato ringspot virus.

to pasteurized soil (30 min at 85 C) in sterilized pots. In three representative experiments, 30-day-old seedlings (2-leaf stage) were heavily shaded for 72 hr before inoculation. Inoculum of two single-lesion TRSV isolates, Riesling 6-49 and Riesling 6-54, was obtained from infected cucumber tissue comminuted in 0.05 M phosphate buffer and centrifuged at low speed to remove tissue debris. Carborundum (600 mesh) was dusted on the grape foliage, and the leaves were gently rubbed with infected sap. Both lots of inoculum induced a large number of local lesions in *C. quinoa* controls in each experiment.

Inoculated grape seedlings were indexed 21-30 days after inoculation by comminuting a small amount of noninoculated leaf tissue from the growing point in Kirkpatrick-Lindner buffer and assaying the extract on young snapdragon seedlings. The results of these experiments are given in Table 3.

Infected grape seedlings grew very slowly, with short internodes and small, mottled, malformed leaves. In several instances, the growing point was killed (Fig. 6) and the seedlings ultimately died. Prominent rings or watermarks, typical early symptoms on vines infected in the field, were never observed on experimentally infected seedlings.

Recovery of TRSV from vineyard soil.—Soil samples collected in Vineyard A from the root zone of infected White Riesling 6-13 were transported to the greenhouse in October 1968 and stored in polyethylene bags. In April, aliquots were transferred to sterilized 100-mm clay pots into which healthy young cucumber, snapdragon, or Delaware grape seedlings were transplanted. The survival of cucumber seedlings was poor because of diuron herbicide residues in the soil; seedlings usually died within 2-3 weeks after transplanting. As the cucumber seedlings began to collapse, they were removed, indexed, and replaced with new cucumber seedlings. The more slowly growing snapdragon and grape seedlings showed little adverse effects from diuron.

TRSV was isolated from two cucumber seedlings, in each case within 5 weeks after transplanting into the vineyard soil. TRSV was also recovered from three snapdragon seedlings grown in the soil; one seedling developed symptoms within 6 weeks after transplanting, a second 13 weeks after transplanting, and the third 21 weeks after transplanting. No recoveries of TRSV were obtained from any of the grape seedlings grown in the soil for a 6-month period.

Other soil samples from this vineyard were examined for the presence of nematodes. Soil collected from the root zones of several infected White Riesling and Mission vines on 12 July 1968 yielded 12-220 individual *Xiphenema americanum* Cobb/100 g soil (J. A. Keplinger, *unpublished data*). Similar samples collected from the root zones of noninfected Delaware and Niagara vines yielded 16-440 *X. americanum*/100 g soil; those from the root zones of noninfected Concord vines yielded 8-80 *X. americanum*/100 g soil. These counts indicated a general, moderately heavy infestation throughout the vineyard.

DISCUSSION.—The host range, physical property, and serological data clearly identify the virus isolated from diseased grapevines as TRSV. The transmission of

TABLE 3. Transmission of two tobacco ringspot virus isolates from cucumber to grape seedlings by mechanical inoculation

Date inoculated	TRSV Riesling 6-49		TRSV Riesling 6-54	
	Pinot Noir	Delaware	Pinot Noir	Delaware
11 Dec. 1968	0/25[a]	1/20	0/26	1/16
2 Feb. 1969	1/15	0/15	0/15	0/15
9 May 1969	2/17	1/17	2/18	0/18
Totals	3/57	2/52	2/59	1/49

[a] No. infected/no. inoculated.

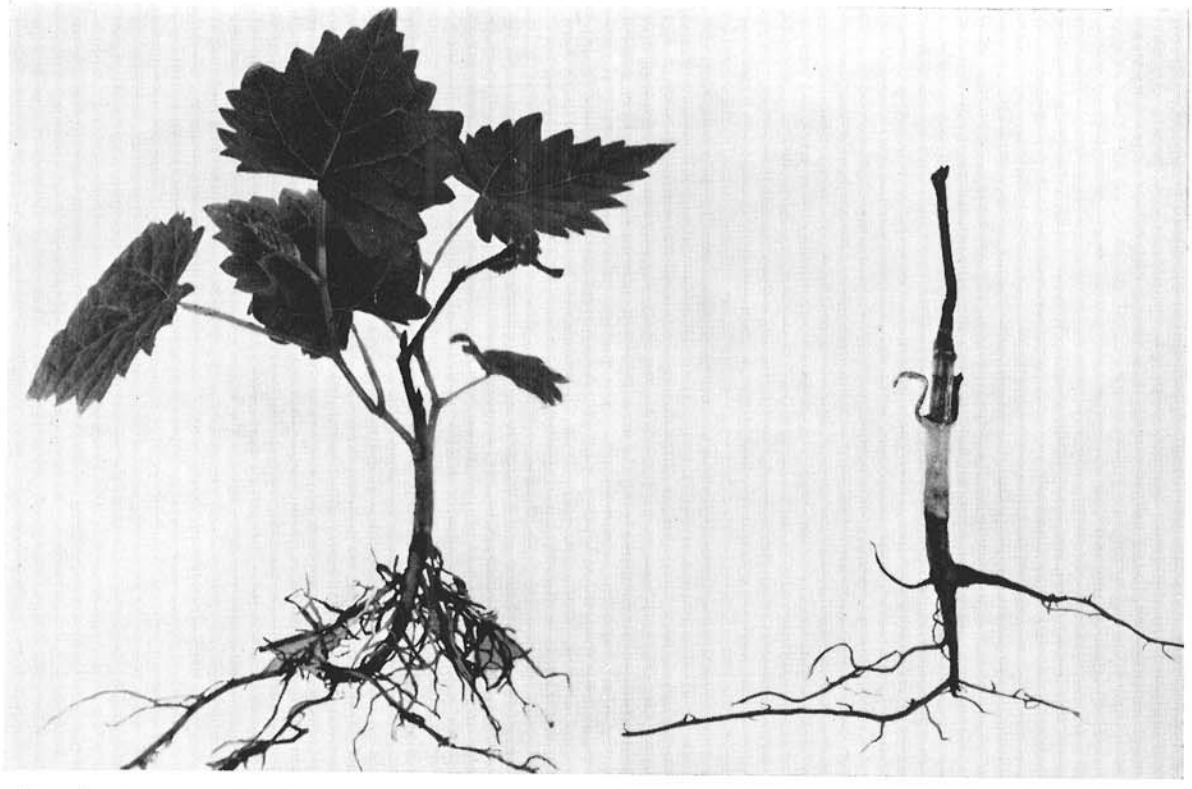

Fig. 6. Stunting and terminal dieback in Delaware grape seedlings infected with tobacco ringspot virus isolate Riesling 6-54 by mechanical inoculation.

grape isolates of TRSV to young grape seedlings by mechanical inoculation resulted in a partial duplication of the disease syndrome: stunting; shortening of internodes; diffuse mottle; leaf malformation; and general decline. The prominent chlorotic rings or watermarks present in the foliage of newly infected vines in the field were not observed in experimentally infected grape seedlings. Our observations suggest, however, that these symptoms occurred only for a relatively brief period as the virus invaded previously healthy tissues. This period may have been very short or entirely lacking in the very small grape seedlings infected by mechanical transmission. The constant recovery of TRSV from diseased vines and the duplication of most of the disease syndrome in young grape seedlings infected with these isolates suggest that TRSV is the cause of the disease.

The prospective economic importance of the disease to grape culture in New York is not clear. Infected vines rapidly became unthrifty; they produced few or no fruits in the 2nd year of infection. It is probable that the weakened vines will succumb within 2-3 years. Infection spread very rapidly in own-rooted *V. vinifera* vines of Vineyard A, where 52/228 vines (22%) became diseased within a 2-year period. In contrast, none of 336 *V. labrusca* and 30 *V. rupestris* vines was infected in the vineyard, indicating a high degree of klendusity in these species. The negligible number of infected vines grafted on phylloxera-resistant rootstocks in three other vineyards where the disease occurred and the absence of any indication of a significant rate of spread at those sites suggest klendusity in phylloxera-resistant grape rootstocks. The reasons for such klendusity are not known, but the presence of large numbers of *X. americanum* in the root zones of *V. labrusca* vines in Vineyard A and the infection of young Delaware (*V. labrusca*) seedlings by mechanical inoculation with TRSV indicate that absence of the probable vector or immunity to the virus are not the causes.

The presence of *X. americanum* (a known vector of TRSV) in large numbers in soil of Vineyard A, the infection of cucumber or snapdragon seedlings grown in this soil, and the tendency of the disease to spread to contiguous vines were factors indicating that the disease was soil-borne. The appearance of the disease in this vineyard, planted with indexed healthy vines on a site where grapevines had not grown for at least 25 years previously, suggests that TRSV was introduced from some exogenous source, possibly herbaceous weeds.

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