Incidence of Apple Union Necrosis and Decline, Tomato Ringspot Virus, and *Xiphinema* Vector Species in Hudson Valley Orchards

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ABSTRACT

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Graft-union symptoms of apple union necrosis and decline (AUND) were observed in 22 orchards involving seven scion varieties propagated on MM106 rootstock. Dandelions and individual trees in four orchards were indexed for tomato ringspot virus (TmRSV) by enzyme-linked immunosorbent assay. TmRSV was detected in the inner bark from 159 of 309 trees indexed and in 89% of trees with graft-union symptoms of AUND. Twenty-five percent of the dandelions in the four orchards were infected with TmRSV. The mean numbers of *Xiphinema* nematodes (*X. americanum* and *X. rivesi*) extracted from 100-cc soil samples were 84 and 181 for samples from beneath trees and from sodded row-middles, respectively. Nematodes transmitted TmRSV from soil samples to cucumber bait plants.

Additional key words: tobacco ringspot virus

Apple union necrosis and decline (AUND) was first reported in 1976 (18) and has been observed on many but not all varieties of apple (*Malus domestica* Borkh.) propagated on MM106 clonal rootstocks (4,11,16). Tomato ringspot virus (TmRSV) has been detected frequently in rootstocks of trees with AUND symptoms, but none of the trees inoculated with the virus have developed typical AUND symptoms (3,17). The best diagnostic symptoms of AUND are pitting, invagination, and necrosis in the woody cylinder at the graft union. Diseased trees may break off at the graft union. Tuttle and Gotlieb (21) showed weakening of the graft union is related to an increase of ray and axial parenchyma cells and a decrease of vessel and fiber cells at the unions of affected trees. Symptom development at the graft union may result from differences in rootstock and scion susceptibility to TmRSV (17,18). TmRSV has been detected in rootstocks but not in scions of diseased trees.

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TmRSV infects numerous other woody perennials including peach (*Prunus persica* Batsch) (13), cherry (*Prunus cerasus* L., *P. mahaleb* L., *P. tomentosa* Thunb.), apricot (*P. armeniaca* L.) (9), prune (*P. domestica* L.) (8), raspberry (*Rubus* spp.) (14), ash (*Fraxinus* spp.), dogwood (*Cornus* spp.), grape (*Vitis lambrusca* L., *V. vinifera* L.), and elderberry (*Sambucus canadensis* L.) (22). The virus is transmitted by *Xiphinema americanum* Cobb (20) and *X. rivesi* Dalmasso (5), infects numerous species of broad-leaved weeds (12,15), and is seed-transmitted in several plant species including dandelions (*Taraxacum officinale* Weber) (10,15). The incidence of TmRSV-infected weeds and vector nematodes in apple orchards with symptoms of AUND has not been determined in most areas.

Between 1965 and 1976, growers in eastern New York planted more than 190,000 apple trees on MM106 stocks (19). Orchards affected by AUND have been observed with increasing frequency in eastern New York since 1975. Our objectives were to document the occurrence of AUND in the Hudson Valley fruit district, to determine if TmRSV can be detected consistently in the MM106 rootstock of trees showing AUND symptoms, and to determine the incidence of *Xiphinema* vectors and TmRSV-infected dandelions in AUND-affected orchards. Because tobacco ringspot virus (TbRSV) has been reported from cherry (23) and peach (24), we also indexed AUND-affected trees for TbRSV.

MATERIALS AND METHODS

Disease incidence, nursery sources, and orchard site histories. Growers and cooperative extension agents were solicited for reports of trees breaking at the union or declining unexpectedly in apple orchards propagated on MM106. Problem orchards were investigated to determine if trees showed characteristic disease symptoms beneath the bark at the graft union. For orchards with AUND symptoms, we recorded cultivar, tree age, proportion of trees affected by AUND, nursery source for the planting stock, and previous cropping history for the orchard site. In seven orchards, rootstocks of two to 15 trees were indexed for TmRSV by enzyme-linked immunosorbent assay (ELISA).

Indexing for TmRSV and TbRSV. Four Delicious/MM106 orchards on three nonadjacent farms were selected for intensive study. Orchards A and C contained Royal Red Delicious and Sturdeespur Delicious, respectively, and both were planted in 1973 on a Bath-Nassau complex of gravelly and shaly silt loam. Orchard B contained Redchief Delicious planted in 1974 on Bath gravelly silt loam, and Orchard D contained an undetermined strain of Delicious planted on Nassau shaly silt loam in 1972.

During late June and early July 1981, 308 trees in the four orchards were indexed for TmRSV and checked for visual symptoms of AUND at the graft union. ELISA was used to index for TmRSV in the inner bark of the rootstock (2,6). Cork borers were used to remove 14-mm-diameter bark samples from opposite sides of the MM106 rootstocks just below the graft unions. Phloem and cambial tissue scraped from both the inner surface of the bark disks

and from the exposed wood on the trunk provided samples of about 0.1 g per tree. Samples were ground immediately in 4 ml of cold PBS-Tween buffer (2) with chilled mortars and pestles. The buffer and ground tissue were decanted into test tubes, kept in ice water for 1–5 hr, and processed on microtiter plates in the laboratory. Antiserum was produced from the Staff grape isolate of TmRSV (6). Plates were read visually by two persons and reactions were considered positive only when color reactions were detected by both observers.

Dandelions growing in the four intensively surveyed orchards were also indexed for TmRSV by ELISA. Composite samples consisting of a young leaf (or portion thereof) from each of three dandelions were ground in buffer and placed in microtiter plates as described for apple bark samples. Bark samples from 147 apple trees in orchards A, B, and D and 32 dandelion samples from the same orchards were also indexed for TbRSV by ELISA.

Nematode populations and TmRSV transmission to cucumber bait plants. Soil samples were collected from beneath 127 individual trees and from 40 row-middle locations in orchards A, B, C, and D on 22 July 1981. Forty-two tree locations and 11 row-middle sites in orchards A and B were resampled on 7 October 1981. Individual tree samples consisted of soil collected with a spade at three random locations within the drip line and within the herbicide strip beneath trees. Samples from the sodded row-middles contained soil from three locations not more than 2 m apart. Each soil sample was subdivided into two parts. Part of each sample was used for extracting and counting *Xiphinema* and the other part was used to grow cucumber (*Cucumis sativus* L. 'Marketmore') bait plants for detection of viruliferous nematodes. Nematodes were extracted from 100 cc of soil in the Cornell Nematode Diagnostic Laboratory by sugar-flotation centrifugation. Only *X. americanum* and *X. rivesi* in each sample were counted, but these were not separated by species.

Cucumber bait plants were established by transplanting seedlings between the cotyledon and first true leaf stages, four per pot, into 10-cm plastic pots containing about 400 ml of orchard soil. Because herbicide residues and root-rotting fungi in the soil killed or stunted many of the July-planted cucumbers, 15 cc of activated charcoal and 50 cc of vermiculite were added to each pot of October-collected soil. Plants were maintained in a greenhouse at 22–25 C for 5 and 7 wk after the July and October collections, respectively. Cucumbers were harvested by washing soil from the roots. A subsample of root tissue from surviving cucumbers in each pot was ground in buffer and tested for TmRSV by ELISA.

RESULTS

Disease incidence, nursery sources, and orchard site histories. Symptoms of AUND were found in 22 noncontiguous orchard sites and on eight apple cultivars propagated on MM106 rootstock. Some sites contained more than one cultivar. Symptoms were observed in 15 blocks of Delicious, five of Tydeman's Early, two each of Spartan and Paulared, and one each of Stayman, McIntosh, Rome Beauty, and Empire. The range of symptoms observed in the woody cylinder at the graft union of affected Delicious/MM106 trees included mild pitting, a horizontal ring of invagination, necrosis evident as a brown line, and horizontal cracking and breaking of the woody cylinder (Fig. 1). Affected Paulared and Spartan trees showed the same range of symptoms, except "brown-line" necrosis was not observed with these cultivars. Tydeman's Early, Spartan, Stayman, and McIntosh showed only the invagination and union-breaking symptoms. No union breakage or other decline symptoms were observed with TmRSV-infected Rome Beauty and Empire trees. Some infected Empire trees, however, showed slight pitting or shallow vertical grooves originating at the graft union and continuing 1–6 cm up the scion. No graft-union symptoms were observed with TmRSV-infected Rome Beauty trees, but some Rome Beauty scions were of larger diameter than their TmRSV-infected rootstocks.

The affected orchards contained about 3,700 trees. All the affected orchards were planted between 1970 and 1976. No attempt was made to accurately quantify disease losses in each orchard, but tree mortality in affected orchards ranged from 1 to 99% and most orchards contained additional declining trees incapable of bearing a full crop. Some trees with healthy terminal growth showed pitting at the graft union. ELISA confirmed the presence of TmRSV in the

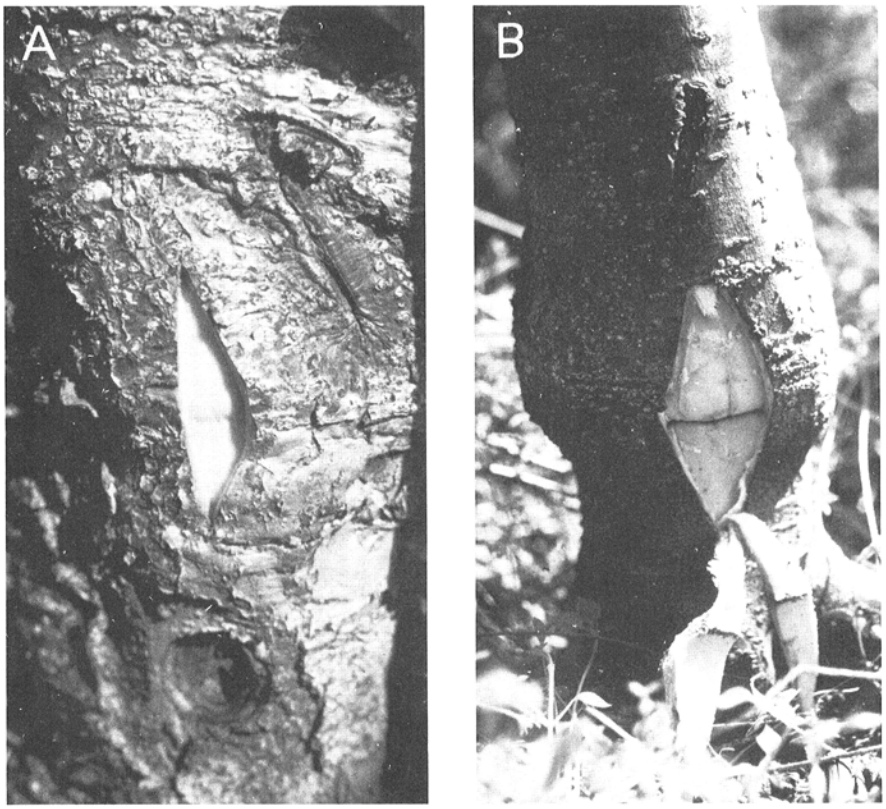

Fig. 1. Delicious/MM106 apple trees affected by apple union necrosis showing **(A)** mild pitting and **(B)** severe necrosis at the graft union where bark has been removed.

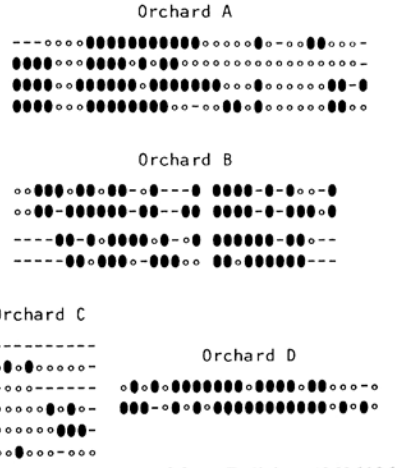

Fig. 2. Tree maps of four Delicious/MM106 orchards showing healthy trees (o), young trees not indexed (–), and trees affected by apple union necrosis and decline (●) as determined by indexing for tomato ringspot virus and inspecting trees for graft-union symptoms.

rootstocks of affected trees in all orchards where indexing was done, including three blocks of Tydeman's Early, one block of Delicious (in addition to the four intensively surveyed orchards), and one block each of Spartan, Stayman, Rome Beauty, and Empire.

Information on sources of nursery stock and orchard site histories provided no consistent clues for explaining disease incidence. Several orchard sites were previously planted with peach, prune, grape, or raspberry, which could have been TmRSV hosts, but most affected orchards were planted on old apple sites or on sites previously in field crops. The nursery sources (for trees in 16 orchards where this information was on record) represented a geographically diverse group of nurseries. In most orchards, the distribution of diseased trees was not indicative of uniformly infected nursery stock. In some orchards, disease incidence was greatest in either high potentially dry areas or in low wet areas within the orchard.

Virus-infected nursery stock could explain the high disease incidence and early symptom development in four of the surveyed orchards. These four orchards were all planted in 1970 with stock from the same nursery. In 1976, tree mortalities in these orchards were 63 and 90% for the two Delicious blocks and 13 and 21% for the two Spartan blocks. By 1981, tree mortality had not increased in the single remaining Delicious orchard, but mortality in the Spartan blocks had increased to 31 and 57% and additional trees were still declining.

Indexing for TmRSV and TbRSV. The pattern of disease incidence in the four intensively surveyed orchards is shown in Figure 2. TmRSV was detected in 159 of the 309 trees indexed by ELISA (Table 1). Graft-union symptoms, however, were found in only 93% of the 159 trees with ELISA-detectable TmRSV, and TmRSV was not detected in 11% of the trees with graft-union symptoms. TbRSV was not detected in any of the 147 indexed trees; 103 of these trees showed graft-union symptoms of AUND and TmRSV was detected in 96.

TmRSV was detected in 41 of 70 dandelion samples taken from the four Delicious/MM106 orchards (Table 1). The probability equation $P_1 = 1 - (1 - P)^{1/3}$ (where P_1 is the proportion of individual plants expected and P is the proportion of positives, 41 of 70, found in our three-plant composite samples) provides a calculated estimate of 25.5% for the proportion of individual dandelions infected with TmRSV. The proportion of infected to uninfected dandelions within the four orchards showed no relationship to the incidence of AUND-affected trees. TbRSV was detected in 11 of 32 dandelion samples, and seven of the 11 positive samples also contained TmRSV.

Nematode populations and TmRSV transmission to cucumber bait plants. The mean number of *Xiphinema* (*X. americanum* and *X. rivesi*) per 100 cc of soil was 84 for the 167 samples taken from beneath trees and 181 for the 53 samples taken from the sodded row-middles. Twenty percent of the 220 samples had fewer than 15 *Xiphinema* and 16% had more than 200. In July, *Xiphinema* populations in the sodded row-middles were usually about twice as high as beneath trees, but populations beneath trees were slightly higher than in row-middles in October (Table 2). For both sampling dates, the *Xiphinema* populations were highly variable even for samples collected from adjacent trees. Coefficients of correlation (r^2) between July and October populations for the same tree locations were 0.37 and 0.49 for orchards A and B, respectively, whereas the correlations between July and October populations for the same row-middle locations in orchards A and B were 0.85 and 0.92, respectively. Although *Xiphinema* were not separated by species in our counts, five additional samples were collected from each of the four orchards and sent to the Pennsylvania Department of Agriculture for nematode extraction and identification. In these samples, *X. americanum* was more abundant than *X. rivesi* in orchards B, C, and D and no *X. rivesi* were found in orchard A (L. B. Forer, *personal communication*).

The frequency of TmRSV transmission to cucumber bait plants was not related to presence of TmRSV in the apple tree where the soil sample was taken. Cucumber bait plants became infected with TmRSV in 14.4% (15 of 104) of July-

Table 1. Incidence of graft-union symptoms and ELISA-detectable tomato ringspot virus (Ed-TmRSV) in apple and dandelions in four Delicious/MM106 orchards

	Orchard			
	A	B	C	D
No. trees inspected and indexed	128	89	44	48
Total trees with graft-union symptoms[a]	58	68	9	31
Trees with Ed-TmRSV:[b]	57	68	6	28
With graft-union symptoms	52	66	4	26
Without graft-union symptoms[c]	5	2	2	2
Trees with graft-union symptoms but no Ed-TmRSV[c]	6	2	5	5
Total trees affected (Ed-TmRSV and/or graft-union symptoms)	63	70	11	33
Total percent trees affected	49	79	25	69
Proportion of dandelion samples with Ed-TmRSV[d]	3/16	12/12	23/35	3/7

[a] Pitting, invagination, or necrotic tissue were evident in the woody cylinder when the bark was removed.
[b] Inner bark tissue from the rootstock was used for indexing by ELISA.
[c] Numbers in this row indicate trees detected by indexing or by observation of graft unions but not by both methods.
[d] Samples were composite samples from three dandelion plants. Numerator indicates number of TmRSV-positive samples and denominator indicates total number of three-plant composite samples tested.

Table 2. *Xiphinema* spp. populations and frequency of tomato ringspot virus (TmRSV) transmission to cucumber bait plants in soil samples from four Delicious/MM106 orchards

		Soil from herbicide strip beneath trees				Soil from sodded row-middles			
			No. *Xiphinema*[a]				No. *Xiphinema*[a]		
Orchard	Sample date	No. samples	Mean of all samples	Highest count	Samples producing TmRSV-infected bait plants[b]	No. samples	Mean of all samples	Highest count	Samples producing TmRSV-infected bait plants[b]
A	22 July	39	51	190	2/31	12	108	487	1/12
	7 October[c]	20	61	192	8/30	6	47	131	2/12
B	22 July	38	98	605	3/5	8	231	612	2/8
	7 October	22	149	589	17/39	5	112	267	6/8
C	22 July	21	95	330	0/20	10	172	488	3/10
D	22 July	29	69	334	0/8	10	351	737	4/9

[a] Number of *Xiphinema* vector species (*X. americanum* or *X. rivesi*) extracted from 100 cc of soil by sugar-flotation centrifugation.
[b] Infection of cucumber bait plants (planted four per pot in 400 cc soil) was determined by indexing washed roots by ELISA.
[c] Nematode counts were not made for all of the October soil samples used for bait-plant transmission tests.

collected soil samples and in 40% (18 of 45) of October-collected samples. Comparisons of mean nematode populations for samples in which bait plants became TmRSV-infected versus samples where bait plants remained healthy showed that populations were higher for samples where TmRSV was transmitted to cucumber (Table 3).

Table 3. Incidence of tomato ringspot virus (TmRSV) infection in cucumber bait plants grown in orchard soils and *Xiphinema* spp. populations in soils producing TmRSV-infected and healthy bait plants

Sample date	Cucumber plants	Soil from herbicide strips beneath trees		Soil from sodded row-middles	
		No. samples	Mean no. *Xiphinema*[a]	No. samples	Mean no. *Xiphinema*[a]
22 July	TmRSV-infected	5	240[b]	10	365[b]
	Healthy	60	66	19	147
7 October	TmRSV-infected	13	168	5	110
	Healthy	21	93	6	49

[a] *Xiphinema* nematode counts are numbers per 100 cc soil extracted by flotation centrifugation from part of each soil sample; cucumber bait plants were grown in the other part of each sample.
[b] Differences between mean counts for samples producing TmRSV-infected cucumbers and healthy cucumber were significant ($P = 0.05$) according to the F test.

DISCUSSION

Our survey data verify that AUND is a serious problem affecting at least seven apple cultivars in commercial orchards in eastern New York, but the data provide no clear-cut evidence for determining how orchards become infected. Use of virus-infected nursery stock is a possibility not precluded by our data. We suspect, however, that contaminated stock is involved in only a few orchards where large numbers of trees develop symptoms simultaneously when trees reach bearing age. Nurseries supplying trees for the orchards we surveyed also supply trees in western New York, where AUND has been found in only a few orchards. If virus-infected nursery stock were the only source of virus and if symptom development followed the same pattern in both areas, disease incidence should be higher in western New York because 73% of the trees on MM106 stock in New York were planted in western New York (19). Reasons for the higher disease incidence in eastern compared with western New York have not been determined.

High populations of vector nematodes in orchard soils and an abundance of TmRSV-infected dandelions in the orchard ground cover suggest a more likely scenario for explaining how trees in orchards become infected. Initial contamination of orchards may come from TmRSV-infected dandelion seeds (10) blown into the orchard from orchard border areas. Plants growing from infected dandelion seeds may provide a virus source for nematodes that transmit the virus to trees. Although our survey did not prove TmRSV transmission is from weeds to trees rather than from trees to weeds, other research (D. A. Rosenberger and D. Gonsalves, *unpublished*) showed TmRSV is widespread in dandelions in old orchards (30+ yr) on seedling rootstock not susceptible to TmRSV.

This survey is the first reported attempt to systematically index rootstocks of both AUND-affected and symptomless trees throughout entire orchards. Previous reports (7,16–18) indicated TmRSV could be detected in rootstocks of AUND-affected trees, but only limited numbers of trees were indexed. In this study, indexing for TmRSV by ELISA and observation of graft-union symptoms were equally effective for diagnosing trees. Neither method detected all trees detected by the other method, but failure to detect TmRSV in trees with graft-union symptoms probably was caused by uneven distribution of the virus within the rootstock (a phenomenon reported previously by other workers [6,7]) or by virus titers below the level detectable by ELISA. Some of the discrepancies between results of indexing and visual symptoms might have been resolved by repeat sampling. In Orchard A, four trees with graft-union symptoms and a negative test by ELISA were reindexed using bark from different quandrants on the rootstock. TmRSV was detected on the second indexing in three of the four trees. We did not resample all trees where indexing and graft-union symptoms differed because repeat sampling increased physical damage to the trees.

TmRSV was detected with equal frequency in trees with only slight pitting at the union and in trees with more severe symptoms. We suspect union symptoms progress from an intermittent pitting to a continuous row of pits to a necrotic layer of tissue. Symptomless trees infected with TmRSV may be recently infected trees that have not yet developed symptoms. There is, however, no conclusive evidence that all Delicious trees on TmRSV-infected MM106 rootstock will develop symptoms of AUND or that all pitting symptoms at Delicious/MM106 graft unions are caused by TmRSV.

The 1:40 dilution of inner bark in buffer used in our ELISA indexing still allowed for clear-cut color reactions on the processed microtiter plates if samples were kept cold from the time of collection until they were processed. The first group of samples was ground in warm mortars and kept in a cold ice chest without ice water. Borderline color reactions were more common in these samples than in subsequent samples that were ground in chilled mortars and kept in ice water. Four samples kept at 4 C in a refrigerator for 24 hr indexed positive for TmRSV at the beginning but not at the end of the 24-hr period. These observations support previous reports indicating TmRSV is a relatively unstable virus (15). A low dilution factor such as the 1:20 used by Lister et al (7) would perhaps decrease chances of virus concentrations declining to undetectable levels between grinding and processing on plates. Our dilution factor, however, was determined by the amount of bark we could remove safely in commercial orchards without unduly damaging trees and by the amount of buffer needed to ensure a smooth efficient grinding and plating process.

The *Xiphinema* populations we encountered were higher than the average reported for this area in an earlier survey (1), but our highest count was only 739/100 cc of soil compared with a high count of 1,460/100 cc of soil in the earlier survey. Some of the variability in nematode populations beneath trees probably resulted from uneven distribution of weeds that survived in the herbicide-treated strip beneath trees. Variability in populations in row-middles may have been caused by variation in the ground cover species predominating at various sample sites. The increased TmRSV transmission to bait plants in October compared with June samples may have resulted from improvements we made in handling October samples and do not necessarily indicate higher infectivity in October-collected samples.

Because the large populations of *Xiphinema* vectors and infected dandelions may contribute to continued spread of AUND within affected orchards, more research is needed to determine if postplant nematicides and broadleaf herbicides can be used to reduce nematode and dandelion populations in established orchards. Long-term experiments will be required to determine if orchards with low nematode and weed populations will remain free of AUND.

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