

Isolation of Tomato Mosaic Virus from Red Spruce

VOLKER JACOBI, Graduate Research Assistant, and JOHN D. CASTELLO, Associate Professor, Faculty of Environmental and Forest Biology, State University of New York, College of Environmental Science and Forestry, Syracuse, NY 13210-2788; and MARTINA FLACHMANN, Graduate Research Assistant, Institut für Botanik-210, Universität Hohenheim, Garbenstrasse 30, D-7000 Stuttgart 70, Germany

ABSTRACT

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A tobamovirus was transmitted to *Chenopodium quinoa* from a purified, composite 40-g needle sample collected from red spruce on Whiteface Mountain in the Adirondack Mountains in New York. The virus was identified as tomato mosaic virus (ToMV) based on host range and symptomatology and supported by immunoelectron microscopy (IEM). The isolate was indistinguishable from an isolate of ToMV recovered from water on Whiteface Mountain in 1989. The virus also was detected in needle concentrates and/or crude needle and root extracts from 13 of 19 red spruce trees by IEM and/or direct enzyme-linked immunosorbent assay (ELISA). IEM was five- to 10-fold more sensitive for detection of ToMV in conifer tissue than direct ELISA. This is the first report of the isolation of a plant virus from red spruce.

Additional keywords: forest decline

In Europe, regionwide surveys for viruses in forest ecosystems have been conducted in an effort to investigate the possible involvement of viruses in "forest decline" (22,23,26). Although many viruses have been detected in the forest environment (5-7), comparatively few have been isolated directly from forest trees (25,30). Even fewer have been transmitted from coniferous trees to herbaceous hosts (11,22,23). The ability to demonstrate a cause and effect relationship between virus infection and specific symptoms in conifers has been hampered by the general inability to detect viruses in conifers and to transmit them to herbaceous hosts in which they can be characterized, identified, purified, and back-transmitted. A few studies have been conducted in Europe (12,24,29). Until recently, research to investigate the prevalence and potential importance of viruses in American coniferous forest tree species has not been conducted.

In the early 1960s, red spruce (*Picea rubens* Sarg.) in the northern Adirondack Mountains of New York began to experience growth reduction and dieback (19). Several researchers have advanced hypotheses regarding the cause for this, including natural stand maturation, climatic factors, and atmospheric pollution, among others. Because viruses have been detected in declining forest ecosystems in Europe, we decided to determine if viruses were present in declining red spruce forests in New York.

In 1989, we transmitted tomato mosaic virus (ToMV) from stream water draining a deteriorating red spruce/balsam fir (*Abies balsamea* (L.) Mill.) forest on Whiteface Mountain in the Adirondacks (17). In addition, initial attempts to detect the presence of viruses in red spruce, black spruce (*P. mariana* (Mill.) B. S. P.), and white spruce (*P. glauca* (Moench) Voss) trees growing near Whiteface Mountain revealed rod-shaped viruslike particles in all three species. These results suggested that red spruce may be infected with ToMV and encouraged us to conduct further investigations. In this paper, we report the detection of ToMV in red spruce on Whiteface Mountain and its transmission to herbaceous hosts. A preliminary report has been published (18).

MATERIALS AND METHODS

Sampling sites. A spruce-fir stand on the northwestern slope of Whiteface Mountain was selected for this study because ToMV was recovered from a stream draining this stand in 1989 (17) and because high elevation spruce-fir forests on this site have a history of dieback and decline (19). The first sampling site, designated Whiteface 1 (WF-1) (UTM coordinates: 587400 E and 491570 N, zone 18), is located at an elevation of 900 m approximately 200 m downstream of a water sampling site from which ToMV previously was recovered. The canopy is dominated by balsam fir and paper birch (*Betula papyrifera* Marsh. var. *cordifolia* (Regel) Fernald). Twelve red spruce trees located within 30 m on either side of the stream were selected for sampling. Four trees were apparently healthy, vigorous saplings with a diameter at breast height (dbh)

ranging from 2 to 5 cm. The remaining eight trees were either intermediate or codominant in canopy position and ranged from 7 to 17 cm at dbh. The crown of each tree was rated for the degree of dieback on a scale of 0-4 where 0 = no crown dieback, 1 = <25%, 2 = >25% but <50%, 3 = >50% but <75%, and 4 = >75% dieback.

The second sampling site, designated Whiteface 2 (WF-2) (UTM coordinates: 587400E and 491470N, zone 18), is located at an elevation of approximately 1,100 m, 300 m upstream of the WF-1 site. Trees on this site are located beneath a canopy of declining red spruce and balsam fir and vigorous paper birch. Seven vigorous red spruce saplings, with no evidence of crown dieback, were selected for sampling at this site.

In total, 19 trees were selected for sampling. They ranged in diameter from 2 to 17 cm, were suppressed to codominant in canopy position, and ranged in crown condition from healthy to severe dieback.

Tissue sampling and processing. Crude and/or concentrated needle extracts from the 19 trees on the two sites were indexed for ToMV by enzyme-linked immunosorbent assay (ELISA) and immunoelectron microscopy (IEM). In addition, crude root extracts from each of the 12 trees on the WF-1 site also were indexed for ToMV by ELISA and IEM. Tissue collection and processing were conducted as described below.

Approximately 5-15 g of needles was collected from each of the seven trees on the WF-2 site in January and July and from five of the trees in December 1990 (two trees were not relocated because of deep snow). Approximately 40 g of needles from the January collection was rinsed thoroughly for 30 min in running tap water, frozen at -20 C, and subjected to the purification protocol of Flachmann et al (12) as described. All steps were carried out at 4 C. The frozen needles were triturated in a Waring blender with liquid nitrogen and then homogenized in 225 ml of 0.5 M Tris-HCl (pH 8.2) containing 4% Polyclar-AT, 0.5% bentonite, 1% Triton X-100, and 0.2% mercaptoethanol. The slurry then was centrifuged at 3,500 g for 15 min, and the supernatant was expressed through cheesecloth and centrifuged again at 5,000 g for 30 min. The supernatant then was centrifuged at

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90,000 g for 2 hr through a 20% sucrose cushion in 0.1 M Tris-HCl (pH 8.2). The pellet was resuspended in Tris-HCl buffer containing 10 mM MgCl₂. The pellet resuspension then was stirred for several minutes in precooled chloroform (1:1) and centrifuged for 30 min at 1,000 g. The aqueous phase was centrifuged at 90,000 g for 2 hr and the pellet was resuspended in 10 mM phosphate buffer (pH 7.0). To serve as a control, a 40-g needle sample was collected from 3-yr-old red spruce seedlings growing in the greenhouse, bulked, and purified similarly. These concentrates were indexed for ToMV by IEM and infectivity bioassay as described below. In addition, 1- to 2-g subsamples of needles collected in July and December from these trees were triturated to powder in liquid nitrogen in a mortar with pestle, extracted in ELISA extraction buffer (phosphate-buffered saline, pH 7.4, containing 2% polyvinylpyrrolidone-10,000 MW, and 0.05% Tween 20) at a rate of 1:6 (w/v), and stored frozen at -20 C until indexed for ToMV by direct ELISA and IEM.

In July 1990, approximately 5-25 g of needles and 2-3 g of young, fine roots were collected from each of the 12 trees on the WF-1 site. After a thorough rinsing in tap water, needle concentrates were prepared from 20-g subsamples from each of nine of the 12 trees (three trees either were too small or too tall to collect sufficient needles for concentration) according to the protocol of Flachmann et al (12) and indexed for ToMV by direct ELISA, IEM, and infectivity bioassay. As a control, a composite 20-g needle subsample was collected from red spruce seedlings growing in the greenhouse and treated similarly. Approximately 1- to 2-g subsamples of needles and roots (also rinsed thoroughly in tap water to remove soil particles) collected from these trees were prepared for ELISA testing, as described above, and stored frozen at -20 C until indexed for ToMV by direct ELISA and IEM.

In total, 12 needle concentrates (nine from the WF-1 trees, one from the WF-2 trees, and two control concentrates), 24 crude needle extracts (seven in July and five in December from the trees on the WF-2 site and 12 from the trees on the WF-1 site), and 12 crude root extracts (from the trees on the WF-1 site) were indexed for ToMV by ELISA and IEM.

Infectivity bioassay. The final high-speed pellets of each needle concentrate were suspended in 1 ml of 10 mM phosphate buffer (pH 7.0), and Carborundum-dusted leaves of *Chenopodium quinoa* Willd. plants were inoculated with 0.5-ml aliquots of the 12 needle concentrates. The plants were incubated in the greenhouse at 20-25 C for 3 wk and monitored for symptom development.

ELISA. An antiserum prepared to an

isolate of ToMV previously recovered from water on Whiteface Mountain (17) was used for all serological tests. The 36 crude needle and root extracts from the 19 trees on the two sites, as well as the nine needle concentrates from the trees on the WF-1 site, and the respective control concentrate were indexed for ToMV by direct ELISA (10). All ELISA tests were conducted in April 1991. To ensure a conservative interpretation of ELISA results, a sample was considered positive for ToMV only if the mean A_{405nm} of the two sample wells was greater than both the mean A_{405nm} of the corresponding control extracts (prepared from tissues collected from 3-yr-old red spruce seedlings growing in the greenhouse) plus three SD, and was greater than the mean A_{405nm} of two wells containing ToMV at 5 ng/ml.

Immunoelectron microscopy. IEM was used to corroborate ELISA and infectivity bioassay results. All needle concentrates and crude tissue extracts were tested for ToMV by IEM according to Milne and Luisoni (20). Initial experiments to test the concentrates prepared from needles collected from the trees on the WF-2 site in January 1990 and the control seedlings were conducted as follows. Piloform-carbon coated grids were incubated for 5 min on a drop of ToMV-specific antiserum diluted 1:500, washed with 20 drops of 100 mM phosphate buffer (pH 7.0), incubated overnight on a drop of concentrate, and rinsed again with 20 drops of 100 mM phosphate buffer (pH 7.0). Grids then were incubated on a drop of ToMV-specific antiserum diluted 1:50 for 15 min. After rinsing with 40 drops of distilled water, grids were negatively stained with five drops of 2% aqueous uranyl acetate (pH 4.0). All steps were performed at 22 C.

To improve virus detection sensitivity in the remaining concentrates and to

permit the detection of ToMV in the crude tissue extracts, the IEM protocol was modified and conducted as follows. Aliquots of crude needle and root extracts prepared for ELISA indexing were diluted further to 1:12 in ELISA extraction buffer and then clarified by centrifugation for 1 min at 13,000 g. Formvar-coated, carbon-fronted, 400-mesh nickel grids were floated on 20- μ l drops of ToMV-specific antiserum diluted 1:4,000 in ELISA coating buffer (10) and incubated for 10 min at 22 C. Grids were rinsed with 20 drops of 100 mM phosphate buffer (pH 7.0), incubated on a 20- μ l drop of sample for 3 hr at 22 C in a moist chamber (two grids per sample), rinsed with 20 drops of 100 mM phosphate buffer (pH 7.0), and incubated on a 20- μ l drop of ToMV-specific antiserum diluted 1:400 in 100 mM phosphate buffer (pH 7.0) for 15 min. The grids then were rinsed with 40 drops of distilled water and stained with seven to eight drops of 2% aqueous uranyl acetate (pH 4.0). Grids were viewed with an RCA-EMU 4 transmission electron microscope at a magnification of $\times 8,960$ with a $\times 10$ ocular. The number of virus particles detected in 50 fields of view (FOV) per sample (25 FOV on each of two grids) was counted. All subsequent IEM tests using the modified protocol were conducted in April 1991.

The sensitivity of the IEM protocol and the direct ELISA system for detection of ToMV were compared by adding purified ToMV at 1, 5, 10, and 100 ng/ml to seedling red spruce needle and root extracts, measuring the absorbance at 405 nm, and counting the number of particles observed in 50 FOV.

RESULTS

Infectivity bioassay. A tobamovirus was transmitted to *C. quinoa* from the 40-g composite red spruce needle concentrate prepared from needles collected

Table 1. Comparative sensitivity of ELISA and IEM for detection of tomato mosaic virus (ToMV) in crude needle and root extracts from uninoculated 3-yr-old red spruce seedlings seeded with purified ToMV at 0, 1, 5, 10, and 100 ng/ml

ToMV (ng/ml)	A_{405nm} and SD of ^a		Particles in 50 FOV ^b	
	Needle extracts	Root extracts	Needle extracts	Root extracts
100	0.555 \pm 0.088	0.608 \pm 0.057	6,476	5,850
10	0.136 \pm 0.011	0.184 \pm 0.021	800	675
5	0.108 \pm 0.009	0.137 \pm 0.006	400	350
1	85	75
0	0.094 \pm 0.006	0.129 \pm 0.008	0	0

^aMean absorbance at 405 nm (A_{405nm}) and standard deviation (SD) is based on 20 wells per sample. For this test, the sample was considered positive for virus if the mean A_{405nm} of the sample wells was greater than the mean A_{405nm} of the control extracts (prepared from tissues collected from 3-yr-old red spruce seedlings growing in the greenhouse) plus three SD. Coating antibody and alkaline phosphatase conjugate were used at a concentration of 2 μ g/ml and a dilution of 1:500, respectively. Substrate incubation time was 30 min.

^bThe number of decorated particles detected in 50 fields of view (FOV) in a 20- μ l drop of clarified extract diluted 1:12 in buffer. Particle counts were corrected for volume and dilution to make the results comparable to ELISA test results (sample volume of 50 μ l and dilution of 1:6). Trapping and decorating antibody were diluted 1:4,000 in ELISA coating buffer and 1:400 in 0.1 M phosphate buffer (pH 7.0), respectively. Sample incubation was for 3 hr at 22 C in a moist chamber (two grids per sample).

in January 1990 from trees on the WF-2 site. Twenty-two chlorotic local lesions with necrotic centers developed on the inoculated leaves 5 days postinoculation and were followed by a systemic mottle approximately 3 wk postinoculation. These symptoms are characteristic of the

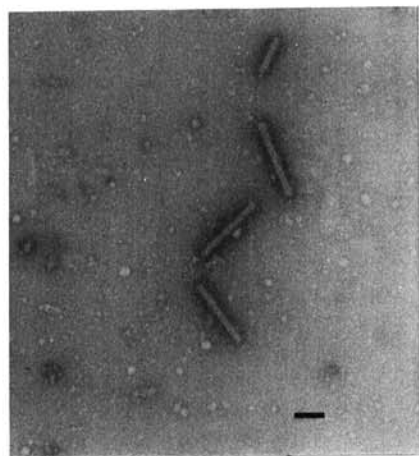


Fig. 1. Tomato mosaic virus (ToMV) particles detected in a composite, purified extract prepared from 40 g of needles collected from seven red spruce trees on the Whiteface-2 site in January 1990. Particles were trapped and decorated with ToMV-specific antiserum at dilutions of 1:500 and 1:50, respectively. Bar = 100 nm.

isolates of ToMV that we transmitted to *C. quinoa* from water in New York State (17). The virus was then passed three times through *Nicotiana glutinosa* L. and propagated in Turkish tobacco (*N. tabacum* L. 'Turkish'). The red spruce isolate reacted with identity to the Whiteface Mountain water isolate of ToMV in agar-gel double diffusion tests with antisera to TMV and the water isolate of ToMV, whereas spur formation was observed between TMV to both the water and red spruce isolates of ToMV. The red spruce isolate of ToMV also produced chlorotic local lesions on *Cucumis sativus* L.; a systemic mottle or mosaic in *Gomphrena globosa* L., *N. benthamiana* L., *N. clevelandii* L., *N. tabacum* 'Xanthi', and *Physalis floridana* L.; and did not produce symptoms in *Vigna unguiculata* (L.) Walp. 'Blackeye'. Identical symptoms on these hosts were produced by the water isolate of ToMV (17). The identification of the virus as ToMV also was supported by IEM results; hundreds of decorated, rod-shaped virus particles were detected in systemically infected Turkish tobacco tissue when incubated on ToMV antiserum-sensitized grids and decorated with ToMV antiserum.

ToMV was not transmitted from either of the two seedling red spruce control

concentrates or from the nine concentrates prepared from 20-g needle samples collected in July 1990 from trees on the WF-1 site.

Comparative sensitivity of ELISA and IEM for detection of ToMV. The sensitivity of direct ELISA for detection of ToMV in both needle and root extracts was between 5 and 10 ng/ml (Table 1). The sensitivity of the modified IEM protocol for ToMV detection in both needle and root extracts was approximately 1 ng/ml (Table 1).

ELISA and IEM of needle concentrates. Decorated, rod-shaped virus particles were detected by IEM in the 40-g concentrate of needles from Whiteface Mountain (Fig. 1) but not in the control concentrate. ToMV was not detected by ELISA in the nine 20-g needle concentrates prepared from needles collected in July 1990 from trees on the WF-1 site (Table 2). However, decorated particles were detected in three of the nine concentrates by IEM (Table 2).

ELISA and IEM of crude needle and root extracts. None of the crude needle extracts prepared from trees on the WF-1 site tested positive for ToMV by either ELISA or IEM. However, ToMV was detected by ELISA in six of 12 root extracts and confirmed by IEM (Table

Table 2. Diameter breast height (dbh), canopy position, crown condition, and tomato mosaic virus (ToMV) status of 12 red spruce trees located at the Whiteface-1 sampling site determined by enzyme-linked immunosorbent assay (ELISA) and immunoelectron microscopic (IEM) examination of needle concentrates and crude extracts prepared from roots collected in July 1990

Tree no.	dbh (cm)	Canopy position ^a	Crown condition ^b	Needle concentrates ^c			Crude root extracts		
				ELISA ^d		IEM ^e	ELISA		IEM
				$A_{405nm} \pm SD$	ng/g		$A_{405nm} \pm SD$	ng/g	
9	7	I	4	0.083 ± 0.004	ND ^f	0	0.169 ± 0.005	45	25
11	5	S	2	0.099 ± 0.004	ND	20
3	11	I	2	0.093 ± 0.002	ND	23	0.155 ± 0.009	38	10
8	13	C	1	0.084 ± 0.001	ND	0	0.140 ± 0	30	10
7	7	S	0	0.076 ± 0.002	ND	0	0.135 ± 0.010	28	10
12	17	C	1	0.081 ± 0.001	ND	5	0.130 ± 0	25	5
5	12	I	4	0.079 ± 0.002	ND	3	0.139 ± 0.003	28	5
1	2	S	0	0.097 ± 0.003	ND	5
2	3	S	0	0.091 ± 0.001	ND	0	0.091 ± 0.004	ND	0
4	9	I	3	0.080 ± 0.007	ND	0	0.099 ± 0.002	ND	0
6	5	S	0	0.074 ± 0.001	ND	0	0.104 ± 0.002	ND	0
10	13	C	3	0.103 ± 0.002	ND	0
Needle control				0.079 ± 0.001	ND	0
Root control				0.096 ± 0.002	ND	0
ToMV (5 ng/ml)				0.093 ± 0.003	5	...	0.124 ± 0.003	5	...

^aS = suppressed, I = intermediate, and C = codominant.

^b0 = No crown dieback, 1 = < 25%, 2 = > 25% but < 50%, 3 = > 50% but < 75%, and 4 = > 75% dieback.

^cNeedle concentrates were prepared according to the protocol of Flachmann et al (12) from 20-g needle samples collected from individual trees in July 1990. Final pellets were suspended in 1 ml of 0.01 M phosphate buffer (pH 7.0) and stored frozen at -20 C. Sufficient needles for concentration could not be collected from trees 1, 10, and 11.

^dMicrotiter plates were coated with ToMV-specific antibody at 2 µg/ml and alkaline phosphatase conjugated antibody was used at a dilution of 1:500. Substrate incubation time was 30 min. A sample was considered positive for virus if the mean A_{405nm} of the sample wells was greater than both the mean A_{405nm} of the corresponding control extract (prepared from 3-yr-old red spruce seedlings growing in the greenhouse) plus three SD and the mean A_{405nm} of the two wells containing 5 ng/ml of purified ToMV. ELISA tests on needle concentrates and crude extracts were conducted in April 1991. Virus concentration was estimated by interpolation of mean A_{405nm} values of two sample wells on a log plot of A_{405nm} values of purified ToMV standards and correcting for tissue dilution factor.

^eThe number of decorated particles in 50 fields of view detected in a 20-µl drop of undiluted concentrate or clarified crude extract diluted 1:12 in buffer and corrected for volume and dilution to make counts comparable to ELISA results (well volume of 50 µl and sample dilution of 1:6). Trapping and decorating antibody were 1:4,000 in ELISA coating buffer (10) and 1:400 in 0.1 M phosphate buffer (pH 7.0), respectively. Sample incubation was for 3 hr at 22 C in a moist chamber (two grids per sample). Extracts prepared from needles collected from 3-yr-old red spruce seedlings growing in the greenhouse served as negative controls. IEM tests were conducted in April 1991.

^fND = Not detected.

2). In addition, decorated particles were detected by IEM in two root extracts in which virus was not detected by ELISA (Table 2).

ToMV was not detected by ELISA in the crude needle extracts prepared from needles of trees on the WF-2 site collected in either July or December 1990 (Table 3). However, ToMV was detected by IEM in the crude needle extracts from two of seven trees in July and in the needle extracts of four of five trees sampled in December (Table 3).

In total, ToMV was detected in eight of 12 trees on the WF-1 site (Table 2), predominantly in the roots, and in five of seven trees on the WF-2 site (Table 3). The ToMV status of trees on the WF-1 site was not correlated either with diameter, relative tree health (as judged by the crown condition of individual trees), or canopy position (Table 2).

DISCUSSION

To our knowledge, this is the first North American report of the detection of a plant virus in a coniferous forest tree species in the field and its transmission to herbaceous hosts. In Europe, Ebrahim-Nesbat and Heitefuss (11) reported the transmission of potato virus Y (PVY) from one Norway spruce tree with needle yellowing in the Bavarian Forest in Germany. However, several European researchers have detected viruses and viruslike particles in conifers (25). In 1961, Cech et al (9) described a systemic virosis of Norway spruce (*P. abies* (L.) Karst.) in Czechoslovakia that was aphid- and graft-transmissible to spruce. Rod-shaped viruslike particles were detected by electron microscopy in needle extracts and twig exudates of spruce with yellowing symptoms but not in healthy trees. Both rod-shaped and flexuous rod-shaped viruslike particles were observed in needles of pine seedlings with ultrastructural abnormalities in Finland (29). In the United Kingdom, Biddle and Tinsley (2) observed rod-shaped viruslike particles in sap exudates of Sitka spruce (*P. sitchensis* (Bong.) Carrière) with needle chlorosis and defoliation, and rod-shaped viruslike particles were observed in western white pine (*Pinus monticola* Douglas ex D. Don) and Scots pine (*P. sylvestris* L.) as well. In Switzerland and southern Germany, viruslike particles were observed by electron microscopy in Norway spruce and silver fir (*A. alba* Mill.) (13-15). Flachmann et al (12) used IEM to detect two types of isometric viruslike particles in needle extracts of European silver fir, Nikko fir (*A. homolepis* Sieb & Zucc.), and five other *Abies* species including three species indigenous to North America (*A. amabilis* Douglas ex Forbes, *A. grandis* (Douglas ex D. Don) Lindl., and *A. procera* Rehd.), but the viruslike particles were not transmitted from fir to

herbaceous plants. Three types of double-stranded RNA (dsRNA) were detected by means of anti-dsRNA monoclonal antibodies in nucleic acid extracts of needles collected from silver fir and Nikko fir in the Black Forest (1). Nienhaus et al (24) reported the infection of Norway spruce seedlings with an oak isolate of tobacco necrosis virus (TNV); to date, however, TNV has not been detected in older Norway spruce in the field.

Tomato mosaic virus was transmitted from the 40-g composite needle concentrate prepared from the trees on the WF-2 site but not from subsequent needle concentrates prepared from trees on the WF-1 site. There are several plausible explanations, not mutually exclusive, that relate to virus concentration. First, only 20 g of needles was concentrated from the WF-1 trees because most of these trees were either too small, too tall, or had too much crown dieback for collection of 40-g needle samples. Second, virus concentration in red spruce needles may be related to the season. Needles from the WF-2 trees were collected in early winter, whereas needles from the WF-1 trees were collected in mid summer (Table 3). Third, distribution of ToMV in red spruce tissues may be uneven as has been reported with the majority of viruses that infect fruit trees (21). And fourth, ToMV concentration in the foliage of red spruce may be very low, as it is in the foliage of infected fruit

trees (21). Low virus concentration in conifer needles has been reported by others and has hampered transmission of viruses from conifers to herbaceous hosts (1,11,12,29).

The virus was detected in red spruce roots by both ELISA and IEM, but in both concentrated and crude needle extracts, it was detected only by IEM. Apparently, ToMV occurs in higher concentration in roots than in needles of red spruce. Indeed, ToMV occurs primarily in the roots of agricultural crops as well (3,4,16,28).

Because there was no apparent relationship between virus presence and dbh, canopy position, or crown condition (Tables 2 and 3), it is possible that ToMV is merely a surface contaminant and does not actually infect red spruce. Several lines of indirect evidence, however, support our contention that ToMV infects red spruce and is not merely a surface contaminant on the sampled tissues. First, to reduce the likelihood of surface contamination, all needle samples were thoroughly rinsed for 30 min in running tap water before virus purification. Second, only young, current season root tissues were sampled and these also were thoroughly rinsed in running tap water to remove all soil particles before ELISA and IEM tests. Third, ToMV was not detected by direct ELISA in young roots of red spruce seedlings 4.5 mo after the roots had been submersed in purified ToMV (10 µg/ml

Table 3. Canopy position, crown condition, and tomato mosaic virus (ToMV) status of seven red spruce trees located at the Whiteface-2 sampling site determined by enzyme-linked immunosorbent assay (ELISA) and immunoelectron microscopic (IEM) examination of crude extracts prepared from needles collected in July and December 1990

Tree no.	Canopy position ^a	Crown condition ^b	July 1990		December 1990	
			ELISA ^c ($A_{405nm} \pm SD$)	IEM ^d	ELISA ($A_{405nm} \pm SD$)	IEM
17	S	0	0.101 ± 0	10	0.093 ± 0.004	10
18	S	0	0.090 ± 0.002	0	0.085 ± 0.001	10
16	S	0	0.096 ± 0	0	0.084 ± 0.002	5
19	S	0	0.097 ± 0.003	0	0.088 ± 0.002	5
15	S	0	0.091 ± 0	0	0.088 ± 0.002	0
13	S	0	0.089 ± 0.001	5	... ^e	...
14	S	0	0.093 ± 0.003	0
Needle control			0.091 ± 0.004	0	0.091 ± 0.004	0
ToMV (5 ng/ml)			0.093 ± 0.003	...	0.093 ± 0.003	...

^aS = suppressed.

^b0 = no crown dieback.

^cMicrotiter plates were coated with ToMV-specific antibody at 2 µg/ml and alkaline phosphatase conjugated antibody was used at a concentration of 1:500. Substrate incubation time was 30 min. A sample was considered positive for ToMV if the mean A_{405nm} of the two sample wells was greater than the mean plus three standard deviations of corresponding control extracts (prepared from tissues collected from 3-yr-old red spruce seedlings growing in the greenhouse), and the mean A_{405nm} of two wells that contained 5 ng/ml of purified ToMV. All ELISA tests were conducted in April 1991.

^dThe number of decorated particles in 50 fields of view detected in a 20-µl drop of clarified crude extract diluted 1:12 in ELISA extraction buffer (10) corrected for volume and dilution factors to make counts comparable to ELISA results (well volume of 50 µl and sample dilution of 1:6). Trapping antibody and decorating antibody were diluted 1:4,000 in ELISA coating buffer (10) and 1:400 in 0.1 M phosphate buffer (pH 7.0), respectively. Sample incubation was for 3 hr at 22 C in a moist chamber (two grids per sample). Extracts prepared from needles collected from 3-yr-old red spruce seedlings growing in the greenhouse served as negative controls. IEM tests were conducted in April 1991.

^eTrees could not be relocated because of deep snow.

for 48 hr), rinsed in tap water, and potted in Promix (V. Jacobi and J. D. Castello, *unpublished*). Fourth, the concentration of ToMV in 12- to 20-L water samples collected from the stream draining our sampling sites on Whiteface Mountain from June 1990 through May 1991 was 10,000- to 64,000-fold lower (range of 0.5-3.4 pg/ml) (V. Jacobi and J. D. Castello, *unpublished*) than the concentration of ToMV detected in red spruce roots on this site (mean of approximately 32 ng of ToMV/g root tissue, Table 2). Because red spruce roots apparently do not retain adsorbed virus for very long and because the virus concentration in the surrounding surface waters is so low, we believe it is improbable that spruce roots adsorbed and retained ToMV from the soil/water environment. Rather, the comparatively high levels of virus detected in the roots is most likely attributable to virus replication within them. And fifth, ToMV has a wide woody plant host range (8,21,27), and in many of these hosts, virus infection is latent (21,27).

Red spruce trees growing at high elevation on Whiteface Mountain in the Adirondack Mountains of New York are infected with ToMV. ToMV may be widespread in forested ecosystems in both Europe (6,7) and North America (17). At this time, the potential involvement of ToMV in the current problems of red spruce at high elevation in the Adirondacks is unknown.

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