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Detection of Infectious Tobamoviruses in Forest Soils

RONALD C. FILLHART, GEORGE D. BACHAND, AND JOHN D. CASTELLO*

Faculty of Environmental and Forest Biology, College of Environmental Science and Forestry, State University of New York, Syracuse, New York 13210-2788

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Our objectives were to evaluate elution and bait plant methods to detect infectious tobamoviruses in forest soils in New York State. Soils were collected from two forest sites: Whiteface Mountain (WF) and Heiberg Forest (HF). The effectiveness of four buffers to elute tomato mosaic tobamovirus (ToMV) from organic and mineral fractions of WF soil amended with ToMV was tested, and virus content was assessed by enzyme-linked immunosorbent assay (ELISA). The effectiveness of *Chenopodium quinoa* (Willd.) bait plants to detect the virus also was tested. Both methods then were utilized to detect tobamoviruses in 11 WF and 2 HF soil samples. A phosphate buffer (100 mM, pH 7.0) eluted more ToMV from soil than the other buffers tested. Mineral soil bound more virus than organic soil. Virus recoveries from virus-amended organic and mineral soils were 3 and 10%, respectively, and the detection sensitivity was 10 to 20 ng/g of soil. Roots of bait plants grown in all virus-amended soils tested positive by ELISA, and virus concentrations averaged 10 ng/g. Both ToMV and tobacco mosaic tobamovirus (TMV) were transmitted to *C. quinoa* by elution from one of two HF soil samples but not from the WF soil samples. A tobamovirus was detected by bait planting in 12 of 73 (16%) root extracts representing 5 of 13 soil samples (38%). Tobamovirus-like particles were seen by transmission electron microscopy in 6 of 12 infected root extracts. Tobamoviruses occur in forest soils in New York State. Abiotic soil transmission to trees may permit localized spread and persistence of these viruses in forest ecosystems.

Plant viruses occur in natural ecosystems (13) including the air (10), water (8), soil (7), and trees (28) of forests. Many of the viruses detected in forest ecosystems lack biotic vectors and are soilborne (abiotic soil transmission), e.g., tobamo-, potex-, and tombusviruses. However, the ecological and epidemiological significance of abiotic, soilborne virus transmission is unknown (23). Soilborne transmission of tomato mosaic tobamovirus (ToMV) and tobacco mosaic tobamovirus (TMV) in agricultural or greenhouse settings has been investigated (2, 5, 6, 12, 26, 31), but studies in natural ecosystems are lacking. Although such studies may help to explain how these and similar viruses spread into and/or within agricultural and forest ecosystems, very little research has been conducted on the development of sensitive methods for detecting infectious plant viruses in soils.

ToMV is one of 13 plant viruses that spread under natural conditions without a biotic vector (17, 23). ToMV is soilborne (17) and waterborne (19), has a wide host range (17), is extremely stable, and infects plants through roots (30, 31). Recently, it has been detected in red spruce (*Picea rubens* Sarg.) (21), stream water (19), and clouds (10) in high-elevation, montane, spruce-fir ecosystems. The virus infects red spruce by an airborne mechanism, and infected trees occur throughout the northeastern United States (15). Red spruce seedlings become infected when inoculated with purified virus (4, 20). However, the virus has not been detected in soil in which infected red spruce trees are found, and the specific infection mechanism is unknown.

ToMV infection affects both the growth and physiology of red spruce. Infection of dominant and codominant red spruce trees on Whiteface Mountain, N.Y. (WF), was positively correlated with the number of fine roots and negatively correlated with the length of the live crown (11). Infection of seedlings caused a 50% reduction in rate of increase of height, weight, and root volume compared with that of noninfected seedlings, but the freezing tolerance of the infected seedlings was greater than that of noninfected seedlings (4).

To our knowledge, only one study has attempted to detect soilborne plant viruses in forest ecosystems. Büttner and Nienhaus (7) transmitted plant viruses in the potex-, tobamo-, necro-, and potyvirus genera from German forest soils to herbaceous and/or woody hosts. About one-third of 284 soil samples tested were positive for one or more of the viruses. Because many European forests occur on what was once agricultural land, the viruses detected in these trees and soils may have originated from infected crop residues. As more forests develop on former agricultural land (3, 29), the incidence of virus diseases in forest tree species in the United States may increase. Alternatively, viruses detected in forest ecosystems with little or no prior history of agriculture may be indigenous or introduced by some other mechanism. In either case, knowing what viruses are present in forest ecosystems, how best to detect them, their origin, how they spread, and their effects on tree growth and development will be necessary to assess their impact and to develop effective disease management strategies. Thus, the objectives of this study were to evaluate methods to detect infectious tobamoviruses and to demonstrate their presence in forest soils. We hypothesize that infectious tobamoviruses can be detected by elution and bait plant methods in forest soils collected from stands where virus-infected trees occur.

MATERIALS AND METHODS

Soil collection. The research was conducted primarily with soil collected from WF (northwestern slope at an elevation of 1,070 m, 44°22′55″N and 73°54′30″W), because our interest lies in the epidemiology and ecology of this virus in high-elevation, montane, spruce-fir ecosystems. However, for comparative purposes, two soil samples also were collected from Heiberg Memorial Forest, Cortland County, N.Y. (HF) (42°46′45″N and 76°5′11″W), and assayed for ToMV. ToMV-infected red spruces also are present on the HF site (8a), and the virus has been detected in stream water there (19). In addition, TMV-infected white ash (*Fraxi-*

* Corresponding author. Mailing address: State University of New York, College of Environmental Science & Forestry, Faculty of Environmental & Forest Biology, 1 Forestry Dr., Syracuse, NY 13210. Phone: (315) 470-6789. Fax: (315) 470-6934. E-mail: jdcastel@syr.edu.

nus americana L.) can be found at this site (9). The two sites differ in disturbance history. WF was logged from the late 1800s to the early decades of this century and experienced slash fires and soil erosion until the 1930s. HF was logged earlier and was used for agriculture and pasture until the 1950s, at which time the site reverted to northern hardwood forest (e.g., American beech, yellow birch, and sugar maple). The two sites also differ in soil type (14). WF soil is a Spodosol of the Sisk-Glebe complex consisting of silt loam overlaid by an organic horizon. HF soil is a Mardin channery silt loam. Soil pH, cation-exchange capacity, texture, percent organic matter for HF soil, the organic and mineral fractions for WF soil, and Promix potting mixture (Premier Horticulture, Inc., Rivière-du-Loup, Quebec, Canada) were as reported by Fillhart (14).

Soil from WF was collected from three soil pits in June 1994 and February, April, August, September, and December 1995. WF soil was separated into mineral and organic fractions. Soil was collected from beneath infected red spruce at HF in February and September 1995, upslope from a small stream in which ToMV also was previously isolated (19). All soils were stored moist at 4°C because soil drying can inactivate tobamoviruses (2, 12, 36).

Elution methodology. Two methods to detect ToMV in soil were evaluated: elution and the use of bait plants. Both methods subsequently were utilized to detect the virus in forest soils.

For the elution methodology, the effectiveness of four buffers to elute a WF stream water isolate of ToMV (ToMV-38) from the mineral and organic fractions of WF soil was tested: 100 mM citrate buffer (pH 3.0), 100 mM citrate buffer (pH 5.0), 100 mM phosphate buffer (pH 7.0), and 100 mM borate buffer (pH 9.0). These buffers were selected to provide a range of pH values because virus elution is highly dependent on the pH of the elution buffer used (18). Two micrograms of purified ToMV in 1 ml of autoclaved, deionized water was added to organic (3 g) and mineral (5 g) soil samples in separate 125-ml flasks. The flasks were shaken by hand for 5 min. Approximately 25 ml of autoclaved, deionized water was added to each flask, shaken at 250 rpm for 1 h, and incubated overnight at 4°C. The solutions were filtered under a vacuum through Whatman no. 1 filter paper, and the filtrates were centrifuged for 2 h at 100,000 × *g*. Pellets were suspended in 500 μl of double-antibody sandwich, enzyme-linked immunosorbent assay (DAS-ELISA) extraction buffer (EEB) (20). A 25-ml aliquot of test buffer then was added to the soil in each flask, shaken, incubated, filtered, and centrifuged as described above. Pellet suspensions obtained from the water and buffer eluates were tested separately for ToMV by DAS-ELISA as described by Jacobi and Castello (20). Organic and mineral fractions without added virus were treated similarly to serve as negative controls for DAS-ELISA.

The protocol described above is comprised of elution and ultracentrifugation phases. The percentage of virus recovered from each phase of the protocol was determined as follows. For the elution phase, 1 g of air-dried mineral soil or 100 mg of organic soil was placed into separate 1.5-ml microcentrifuge tubes. Purified ToMV was added in amounts of 0, 1, 5, 10, 25, 50, 100, 250, 500, 1,000, or 1,500 ng in 1 ml of 100 mM phosphate buffer (pH 7.0). The suspensions were agitated on a vortex mixer for 1 min, shaken on a rotary shaker (250 rpm) for 30 min, incubated at 4°C overnight, and then centrifuged at 10,000 × *g* for 2 min. The supernatants were tested for ToMV by DAS-ELISA. The amount of added virus that was recovered was calculated by comparing the absorbance at 405 nm (A_{405}) with a dilution series of purified virus. The percent virus recovery was calculated as follows: (nanograms of virus recovered/nanograms of virus added) × 100.

For the ultracentrifugation phase, 1 ml of purified ToMV was added to separate flasks each containing 10 ml of 100 mM phosphate buffer (pH 7.0) for final concentrations of 1, 5, 10, 15, 20, 25, 50, and 100 ng of ToMV in 11 ml. The solutions were filtered under vacuum through Whatman no. 1 filter paper and washed with 15 ml of buffer for a total of 26 ml/flask. The virus-containing filtrates were centrifuged at 100,000 × *g* for 2 h. The resulting pellets were suspended in 1 ml of EEB and tested for ToMV by DAS-ELISA. The amount and percentage of virus recovered were calculated as described above. The percent virus recovery in both phases combined was determined as follows: (percent recovery in the elution phase × percent recovery in the centrifugation phase)/100.

The complete elution protocol was tested as follows: 5-g aliquots of WF mineral soil and 3-g aliquots of WF organic soil were placed into separate 125-ml flasks. A 2-ml aliquot of autoclaved, deionized water containing purified ToMV in amounts of 0, 50, 100, 250, 500, 1,000, or 1,500 ng was added to separate flasks and subjected to the complete elution protocol described above. The resultant water and buffer eluates were tested for ToMV by DAS-ELISA, and the percent virus recovery was determined as described above.

To determine the effect of soil extracts on the infectivity of ToMV, dilutions of purified virus were prepared in WF mineral and organic soil fractions as follows. One liter of 100 mM phosphate buffer (pH 7.0) was added to 1 kg of mineral soil and to 500 g of organic soil, and the slurries were shaken at 250 rpm for 1 h followed by incubation overnight at 4°C. The slurries were filtered through Whatman no. 1 filter paper under a vacuum, and the filtrates were centrifuged at 100,000 × *g* for 2 h. The pellets were then suspended in 1 ml of 10 mM phosphate buffer (pH 7.0). Dilutions of purified ToMV (0, 1, 10, 100, and 1,000 ng/ml) were prepared in the pellet resuspensions from mineral and organic soils and in 10 mM phosphate buffer (pH 7.0). Celite was added to each of the preparations, which were then used to inoculate leaves of *Chenopodium quinoa* Willd. Lesions were counted after a 5-day incubation in the greenhouse.

Bait plant methodology. To determine whether *C. quinoa* plants would become infected if grown in soil containing infectious virus, the basic method of Büttner and Nienhaus (7) was used, modified as described here. Seeds were planted in three 15-cm-diameter pots containing WF organic soil, WF mineral soil, or Promix. Ten grams of fresh, symptomatic tobacco (*Nicotiana tabacum* L. cv. Turkish) tissue systemically infected with ToMV was chopped, added to each pot, and mixed thoroughly. As a control, seeds were planted in pots containing soils amended with noninfected tobacco tissue. Upon germination, plants were thinned to 15 per pot and grown in the greenhouse with a day length of 15 h for 6 weeks. Roots were tested for ToMV by DAS-ELISA.

Detection of tobamoviruses in forest soils. The elution and bait plant techniques then were used to detect infectious tobamoviruses in forest soils. For the elution protocol, 1 liter of 100 mM phosphate buffer (pH 7.0) was added to 1 kg each of WF mineral and HF soils or to 500 g of WF organic soil obtained at each collection date. The resultant slurries were treated as described above. The pellets from high-speed centrifugation were suspended in a total volume of 1 ml of 10 mM phosphate buffer (pH 7.0) per soil sample. *C. quinoa* plants then were mechanically inoculated with each suspension and monitored for symptom development for 2 weeks. Thirteen soil samples (11 and 2 samples from WF and HF, respectively) were assayed for infectious virus in this manner.

The same 13 soil samples also were tested for infectious ToMV by the bait plant protocol modified as follows: soils were mixed 1:1 (vol/vol) with autoclaved Promix because plant growth was poor in unamended soils. To duplicate soil and water conditions on WF, plants were watered with water collected from Esther Brook on WF and autoclaved. The plants were thinned to 20 per pot at 1 week after germination and were harvested 8 weeks after planting. Roots of two to six plants per pot were collected and assayed separately for ToMV by DAS-ELISA. In addition, roots from the remaining 14 to 18 plants in each pot were combined, triturated in 10 mM phosphate buffer (pH 7.2), clarified by low-speed centrifugation (10,000 × *g* for 10 min), concentrated by ultracentrifugation (100,000 × *g* for 2 h), suspended in 500 μl of 10 mM phosphate buffer (pH 7.2), and used to inoculate the leaves of *C. quinoa*, which were then monitored for symptoms for 2 weeks. Approximately 250 g of soil from each pot then was leached with 600 ml of 100 mM phosphate buffer (pH 7.0) as described above, and the suspended pellets were used to inoculate the leaves of *C. quinoa.*

TEM. Transmission electron microscopy (TEM) was conducted on leaf extracts of *C. quinoa* plants that showed symptoms when inoculated with concentrated soil eluates and in root extracts of bait plants in which the virus was detected by DAS-ELISA. Formvar and carbon-coated grids for TEM were incubated for 15 min on crude tissue extracts, rinsed with 100 mM phosphate buffer

TABLE 1. Mean A_{405} values of water filtrates and buffer eluates of unamended and virus-amended WF soils

Elution treatment	Mean A_{405} ± SD[a] of:			
	WF organic soil		WF mineral soil	
	Not amended	ToMV amended	Not amended	ToMV amended
Deionized water	0.20 ± 0.02	>2.0	0.27 ± 0.06	1.1 ± 0.46
Citrate buffer (pH 3)	0.19 ± 0.02	0.18	0.18 ± 0.03	0.26
Citrate buffer (pH 5)	0.24 ± 0.03	0.26	0.22 ± 0.01	0.32
Phosphate buffer (pH 7)	0.27 ± 0.01	1.5	0.23 ± 0.03	>2.0
Borate buffer (pH 9)	0.64 ± 0.03	0.64	0.24 ± 0.02	0.33

[a] Virus presence was assessed by DAS-ELISA. Mean A_{405} values and standard deviations represent readings from four wells each. Mean values without standard deviations represent two wells. For comparison, the mean A_{405} values of dilutions of purified ToMV were 1.88, 0.98, 0.63, 0.34, 0.23, and 0.21 for 100, 50, 25, 10, 1, and 0 ng/ml, respectively.

TABLE 2. Amounts and percentages of ToMV recovered from unconcentrated eluates of virus-amended mineral and organic WF soils

Amt of ToMV added (ng)[a]	WF organic soil			WF mineral soil			Purified ToMV A_{405}[b]
	A_{405}[b]	ToMV recovered[c]		A_{405}[b]	ToMV recovered[c]		
		Amt (ng)	%		Amt (ng)	%	
1,500	1.88	>100		>2.00	>100		
1,000	1.28	80	8	1.43	83	8	
500	1.01	50	10	1.17	60	12	
250	0.68	40	16	0.85	5	18	
100	0.44	22	22	0.54	30	30	1.54
50	0.28	8	16	0.36	15	30	0.99
25	0.25	7	28	0.25	7	28	0.49
10	0.16	2	20	0.17	3	30	0.33
5	0.13			0.15			0.21
1	0.12			0.13			0.14
0	0.12			0.12			0.12
Mean			17			22	

[a] Purified ToMV was added at the concentrations indicated to 1 g of air-dried mineral soil or 100 mg of organic soil in a volume of 1 ml of 100 mM phosphate buffer (pH 7.0).
[b] A_{405} values obtained by DAS-ELISA represent the mean of two wells.
[c] The amount of virus recovered was determined by comparing DAS-ELISA A_{405} values of eluates to the absorbance of dilutions of purified ToMV (last column). The percentage of virus recovered was determined as follows: (amount [ng] of ToMV recovered/amount [ng] of ToMV added) × 100.

(pH 7.0) and distilled water, negatively stained with 2% aqueous uranyl acetate, air dried, and viewed on a Japanese Electron Optics Laboratory JEM 2000EX transmission electron microscope.

RESULTS

Elution methodology. More ToMV bound to WF mineral than to organic soil because water alone removed more than 100 ng of ToMV from virus-amended organic soil and approximately 50 ng from virus-amended mineral soil (Table 1). The 100 mM phosphate buffer (pH 7.0) eluted more ToMV from both mineral and organic soils than did the other buffers tested (Table 1). By comparison to the A_{405} values of a dilution series with purified virus, more than 100 ng and approximately 75 ng of ToMV were eluted from mineral and organic soils, respectively (Table 1). Increasing the molarity (0.2 M) or adding Tween 80 (0.1%) to phosphate buffer did not increase the amount of virus eluted from these soils (data not shown).

The percent recovery of ToMV from the elution phase ranged from 8 to 30%, with a mean of 17% for organic and 22% for mineral soils (Table 2). The percent recovery of ToMV from the centrifugation phase ranged from approximately 20 to 30%, with a mean of 26% (Table 3). When both phases of the protocol are combined, approximately 5% of the ToMV added to WF soils should be recoverable, which agrees well with the calculated recoveries of ToMV from virus-amended organic and mineral soils of 3 and 10%, respectively (Table 4). Based upon the ELISA results, the detection sensitivity of the elution method was approximately 10 to 20 ng of ToMV/g of soil (Table 4).

Dilution of purified ToMV in mineral and organic soil extracts reduced virus infectivity. *C. quinoa* plants inoculated with 1 ng of ToMV per ml prepared in phosphate buffer produced 23 local lesions, whereas no lesions were produced on plants inoculated with virus prepared in mineral or organic soil extracts. Plants inoculated with 10-ng/ml samples of virus prepared in buffer, organic soil, and mineral soil extracts produced 200, 30, and 3 lesions, respectively, a finding which corresponds to 85 and 98% inhibition of infectivity, respectively. All plants inoculated with 100- and 1,000-ng/ml samples of virus produced numerous local lesions.

Bait plant methodology. Plant survival and growth in the WF soils were poor, particularly in the mineral soil. Therefore, the roots of surviving plants grown in mineral and organic soils were combined by soil type to obtain sufficient tissue for DAS-ELISA. Roots collected from plants grown in all virus-amended soils tested positive by DAS-ELISA (the mean A_{405} values of infected and control root samples were 1.00 and 0.14, respectively). The composite root sample from plants grown in non-virus-amended organic soil, but not Promix or mineral soil, also tested positive for ToMV by DAS-ELISA (the A_{405} values of the infected root sample and the control root sample were 0.27 and 0.14, respectively). When the absorbances were compared with those of dilutions of purified virus, the mean virus concentration within infected roots was found to be approximately 10 ng/g.

Tobamovirus detection in forest soils. Eleven necrotic local lesions developed on *C. quinoa* leaves inoculated with the concentrated eluate from HF soil collected in February 1995 (Table 5). Many rigid rods approximately 250 to 300 nm in

TABLE 3. Mean A_{405} values of dilutions of purified ToMV tested prior to and following ultracentrifugation

Concn of purified ToMV (ng/ml)	A_{405} of purified ToMV[a]		% of ToMV recovered[b]
	Precentrifugation	Postcentrifugation	
100	>2.0	0.81	
50	1.40	0.42	24
25	0.74	0.30	28
20	0.60	0.29	30
15	0.47	0.20	20
10	0.34	0.16	
5	0.26	0.16	
1	0.17	0.11	
0	0.11	0.11	
Mean			26

[a] A_{405} values represent the mean of two wells per sample as determined by DAS-ELISA.
[b] The percentage of virus recovered was determined by comparing the A_{405} values of postcentrifugation dilutions of purified ToMV to those of precentrifugation virus dilutions.

TABLE 4. ToMV recovery from concentrated eluates of virus-amended WF organic and mineral soils

Amt of ToMV added (ng)[a]	WF organic soil[b]				WF mineral soil[b]				Purified ToMV A_{405}[b]
	A_{405} of:		ToMV recovered		A_{405} of:		ToMV recovered		
	Water filtrate	Buffer eluate	Amt (ng)	%	Water filtrate	Buffer eluate	Amt (ng)	%	
1,500	>2.0	1.04	25		0	>2.0			
1,000	>2.0	1.36	36		0	>2.0			
500	0.79	0.56	16	3	0	>2.0			
250	0.94	0.25	8	3	0	>2.0			
100	0.20	0.06	2	2	0	0.26	8	8	>2.0
50	0.09	0.05	2	4	0	0.16	6	12	1.84
25									0.92
20									0.71
15									0.54
10									0.33
5									0.15
1									0.02
0	0	0			0	0			0
Mean				3				10	

[a] Purified ToMV was added to 5 g of WF mineral soil or to 3 g of WF organic soil at the concentrations indicated in 25 ml of deionized water. To correct for background absorbance, the A_{405} values of soils to which virus was not added (organic = 0.36; mineral = 0.26) were subtracted from the A_{405} values of soils to which virus was added.

[b] Absorbances represent the mean of two wells per sample. The amount of ToMV recovered was calculated by comparing the A_{405} values of concentrated soil eluates, as determined by DAS-ELISA and corrected for background absorbance, to the A_{405} values of dilutions of purified ToMV corrected for the absorbance of EEB (0.12). The percentage of ToMV recovered was determined as follows: (amount [ng] recovered/amount [ng] added) × 100.

length and 18 nm in diameter were observed by TEM of infected leaf tissue. Black turtle beans (*Phaseolus vulgaris* L. cv. Black Turtle 1), a differential host for TMV and ToMV, were mechanically inoculated separately with several of the lesions that developed on this *C. quinoa* plant. Some bean plants developed red, necrotic, local lesions characteristic of TMV, and others did not produce symptoms, which indicated the presence of ToMV but not TMV. Symptoms were not observed on *C. quinoa* plants inoculated with other concentrated soil eluates.

A tobamovirus was detected by DAS-ELISA in the roots of *C. quinoa* plants grown in WF and HF soils (Table 5). There was no relationship between virus detection and the time of soil collection. Virus was detected in soils collected in June and August (WF organic), December (WF mineral), and February and September (HF) (Table 5). Several tobamovirus-like particles (250 to 300 nm by 18 nm) were detected by TEM in 6 of the 12 ELISA-positive *C. quinoa* root extracts. Tobamoviruses were not transmitted to *C. quinoa* from root or soil concentrates.

TABLE 5. Results of WF and HF soils tested for infectious ToMV by elution and bait plant methods

Soil type and collection date[a]	Eluate bioassay[b]	Mean A_{405} ± SD (n)[c] of:		No. of TEM$^+$/no. of DAS-ELISA$^+$ extracts[d]
		Noninfected extracts	Infected extracts	
WF mineral				
6/94	−	0.20 ± 0.02 (6)	—	
8/94	−	0.20 ± 0.02 (6)	—	
2/95	−	0.19 ± 0.01 (6)	—	
4/95	−	0.21 ± 0.02 (6)	—	
9/95	−	0.20 ± 0.02 (5)	—	
12/95	−	0.22 ± 0.02 (4)	0.31 ± 0.06 (1)	0/1
WF organic				
6/94	−	0.24 ± 0.02 (6)	0.32 ± 0.03 (2)	1/2
8/94	−	—	0.38 ± 0.07 (6)	4/6
4/95	−	0.21 ± 0.03 (6)	—	
9/95	−	0.20 ± 0.01 (5)	—	
12/95	−	0.21 ± 0.02 (2)	—	
HF				
2/95	+	0.22 ± 0.02 (4)	0.37 ± 0 (2)	1/2
9/95	−	0.20 ± 0.03 (5)	0.39 ± 0.01 (1)	0/1

[a] Month/year.

[b] *C. quinoa* plants were inoculated with concentrated soil eluates and monitored for the development of local lesions.

[c] A bait plant root extract was considered positive for ToMV if the mean A_{405}, as determined by DAS-ELISA, of the two sample wells was greater than the mean absorbance (plus three standard deviations) of roots of *C. quinoa* grown in Promix (A_{405} = 0.22 ± 0.01 [mean ± standard deviation]). For comparison, the A_{405} values of dilutions of purified ToMV were 1.7, 0.33, 0.30, and 0.21 for 100, 10, 5, and 1 ng/ml, respectively. —, no extracts.

[d] Root extracts that tested positive for ToMV by DAS-ELISA also were examined for tobamovirus-like particles by TEM.

DISCUSSION

A tobamovirus was detected in HF and WF soils by DAS-ELISA of bait plant roots and then confirmed by TEM in 50% of those samples (Table 5). Because of the cross-reactivity of ToMV antiserum, the ToMV DAS-ELISA cannot distinguish between TMV and ToMV in infected tissues. Therefore, the identity of the virus detected in soil cannot be determined. However, because ToMV has been detected in clouds (10), red spruce (21), and stream water (19) on WF, we believe that the virus detected in WF soil is ToMV and not TMV. However, based on particle morphology and the presence or absence of symptoms in the differential host bean cultivar Black Turtle 1, both TMV and ToMV were transmitted to *C. quinoa* from one concentrated HF soil eluate (Table 5). Both ToMV and TMV have been detected previously at the HF site, with ToMV being detected in stream and pond water (19) and in red spruce (8a) and TMV being detected in white ash (*F. americana* L.) (9). These results support our hypothesis that infectious tobamoviruses are present in soils of forest stands in which infected trees occur and, to our knowledge, represent only the second report of infectious plant viruses detected in forest soils.

Although both the elution and bait plant methods permitted detection of infectious tobamoviruses in forest soils, neither TMV nor ToMV was detected frequently. Both viruses were transmitted from only 1 of 13 concentrated soil eluates (Table 5), and they were detected by ELISA in only 12 of 73 (16%) bait plant root extracts representing 5 of 13 soil samples (38%) (Table 5). Apparently, the bait plant method is more sensitive than elution for the detection of tobamoviruses in forest soils. Although plant viruses in six different genera have been detected in soil using the bait plant technique (16), tobamoviruses were rarely (5%) detected by this method in forest soils in Germany (7). Tobamoviruses may not be common in forest soils or their concentration may be too low to permit reliable detection by elution-infectivity bioassay. Similarly, exposure of *C. quinoa* roots to low levels of virus inoculum in forest soil may be too short to generate more than a few localized root infections. Long-term exposure to a low-concentration inoculum may be required to initiate infection or to achieve a high virus concentration within infected tissues. However, because of its extremely long life span (400 years), red spruce may be more likely to become infected when growing in soil containing low levels of virus. Therefore, soilborne virus may be a source of inoculum for infection of this and other long-lived woody species. The duration and degree of host exposure to inoculum, as well as the inoculum concentration, directly influence abiotic, soilborne virus transmission (16). Likewise, the incidence of ToMV infection of tomato plants decreased with decreased inoculum concentration (31). Methods more sensitive than elution or bait plants followed by infectivity bioassay or ELISA are now available for virus detection. DAS-ELISA and PCR amplification can detect nanogram and femtogram quantities of virus, respectively, but these methods are problematic for direct use with soils that often contain interfering substances (1). We have recently developed a multiplex immunocapture reverse transcriptase PCR protocol (22), which should permit detection and differentiation of TMV and ToMV with great sensitivity directly in the roots of bait plants or clarified soil eluates.

Techniques to extract, concentrate, and enumerate infectious viruses from soils come primarily from elution studies with human and animal viruses. These techniques are marginally efficient for a few enteroviruses and inadequate for others (34). Elution followed by direct assay on tissue-cultured animal cell lines is used routinely to detect animal and human viruses in soils (18, 39). For example, recovery of poliovirus from estuarine sediments ranged from 0.1 to 71% (mean = 18% [39]), and the recovery of four enteroviruses from a loamy sand soil ranged from 0.1 to 88% (18). By comparison, the recoveries of ToMV by elution from organic and mineral soils were 19 and 22%, respectively (Table 2). Unfortunately, only 25% of the eluted virus was recovered during the subsequent centrifugation phase (Table 3). In the only study of which we are aware that utilized elution to detect plant viruses in soil, Cheo (12) eluted TMV from agricultural soil with 25 mM phosphate buffer (pH 7.5) followed by differential centrifugation and either infectivity bioassay or spectrophotometry to detect the virus. Detection sensitivity was low: 10 to 1,000 ng/ml for the infectivity bioassay and 50 to 300 μg/ml for spectrophotometry. The sensitivity of our infectivity bioassay to detect ToMV was approximately 10 ng/ml for virus prepared in soil extracts, which was 10-fold less than the sensitivity of the assay to detect virus prepared in phosphate buffer. Humic acids in organic soils are known to inhibit the infectivity of TMV in tobacco (33). Therefore, assuming a 10% recovery of virus by elution (Table 4), at least 100 ng of virus must be present in a soil sample in order for it to be detected by elution and infectivity bioassay.

Tobamoviruses persist in plant debris in soil (26). Therefore, their presence in forest soils and trees may relate to past land use history. Many European forests, in which viruses have been detected (7), were planted on former agricultural land. The HF site in this study was in agriculture and pasture prior to 1955. Interestingly, on WF ToMV but not TMV has been detected in trees (21), stream water (19), clouds (10), and possibly soil (Table 5). The apparent absence of TMV at this site may relate to the absence of a prior history of agriculture. Alternatively, the concentration and persistence of certain viruses in forest soils may relate to differences in soil or virus characteristics that affect mobility and persistence.

Many factors affect the mobility and persistence of viruses in soils, including soil pH and moisture content, concentration of ions, cation-exchange capacity, type and amount of clay, organic matter concentration, proteins, salt concentrations in soil and groundwater, hydraulic conditions, infiltration rate, virus type and strain, and isoelectric point (IEP) (24, 27, 37, 38). More ToMV adsorbed to WF mineral than to organic soils (Tables 1 and 4). Organic soils tend not to bind as much virus as mineral soils (34). Therefore, if virus input is high, ToMV is more likely to remain unbound in organic soils than in the mineral horizon where clay particles may bind the virus. Adsorption of some plant (23, 27, 32) and animal (27) viruses to clay particles may stabilize infectivity. However, adsorption of TMV to bentonite clay increased its degradation (27). The IEP of the WF stream isolate is 3.7 (14), which is lower than that of other ToMV strains (17) and equivalent to that of some TMV strains. pH determines both type and strength of virus surface charge (34), such that virions with no net or a weak surface charge are readily mobilized in soil (38). The IEP of the WF isolate of ToMV is equivalent to the pH of WF organic soil (3.7) and lower than that of WF mineral soil (4.4) and HF soil (4.8) (14). At soil pH values above the IEP of a virus, as in WF mineral soil and HF soil, adsorption to clay particles may be considerably reduced due to the repulsive forces of negatively charged particles (38). In conjunction with low soil substratum permeability and rapid surface runoff on the WF site, ToMV may readily leach from WF soils. In addition, because the IEP of the virus is identical to that of WF organic soil, slight changes in groundwater or rainwater pH may alter its mobility in organic soil. Organic soils contain little clay to bind virus,

and humic and fulvic acids interfere with potential binding sites (37). These factors may help to explain why tobamoviruses were not readily detected by direct elution of WF soils.

Tobamoviruses are present in forest soils in New York State, from which they may infect forest trees. Both TMV and ToMV have been detected in forest, shade, and ornamental tree species (9, 11, 25, 28, 35), which could then serve as reservoir hosts to maintain these viruses in forest ecosystems. Subsequent, abiotic soil transmission to trees may provide the mechanism for the localized spread of these viruses and their persistence in forest ecosystems.

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