Ecology and Epidemiology

Isolation of Tomato Mosaic Virus from Waters Draining Forest Stands in New York State

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ABSTRACT

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A survey for plant viruses in streams and lakes draining selected forest stands in central New York and in the Adirondack Mountains was conducted in 1989 to determine both the presence and diversity of plant viruses in forest ecosystems in New York. Viruses were concentrated from 29 20-L water samples by adsorption to Zeta Plus 50 S membrane filters, followed by elution, high speed centrifugation, resuspension of the pellets in phosphate buffer, and inoculation onto herbaceous virus indicator plants. Rod-shaped particles identified as tomato mosaic virus (ToMV) on the basis of electron microscopy, host range, symptomatology, and serological tests were transmitted from eight water concentrates to *Chenopodium quinoa*. Biological and serological differences, but no differences in immunoelectrophoretic properties among the isolates, were observed. ToMV was recovered from water during April through June but not from July through October. Antisera were produced to three ToMV isolates from three forest stands.

Additional keywords: ecology, epidemiology, forest decline, waterborne plant viruses

In recent years, plant viruses belonging to the potex-, tombus-, tobamo-, necro-, rhabdo-, and cucumo-virus groups, plus several ungrouped or as yet unidentified plant viruses have been detected in water (14,16,23–25), including waters draining forest ecosystems (5,7). The plant viruses detected in water share certain features. With the exception of cucumber mosaic virus (CMV), they are stable, possess wide host ranges, occur in high concentration in plant tissues, and can infect plants through their roots (14). Many do not have biological vectors.

In 1985–1986, as part of the research effort on forest decline in Germany, a survey was conducted for plant viruses in waters draining forest stands in North Rhine-Westphalia. The sampling sites were located in forested areas from which viruses were isolated previously from tree root and soil samples (7). Tomato bushy stunt virus (TBSV), carnation Italian ringspot virus (CIRV), potexviruses, and tomato mosaic virus (ToMV), among others, were recovered from more than 50% of the 66 water samples (7).

Based on the European results, we assume that plant viruses should also be recoverable from forested watersheds in the United States. We also recognize that virus isolation and identification is just a first step leading toward a better understanding of plant viruses in natural forest ecosystems, which is our ultimate goal. In this paper, we report the identification and characterization of ToMV isolates recovered from waters draining forest stands in central New York and in the high peaks region of the Adirondack Mountains. A preliminary report has been published (13).

MATERIALS AND METHODS

Water sampling sites. Three water sampling sites in central New York were located at the Heiberg Memorial Forest (HMF) in Tully. These sites were selected because they were easily accessible from Syracuse, and they permitted development and optimization of the virus recovery system. They consisted of forest stands representative of beech-hemlock and mixed northern hardwood forest types (Table 1), and they had not been recently disturbed or farmed. Five sites in the Adirondack Mountains at Whiteface Mountain (WF), Catamount Mountain (CAT), Mount Algonquin,

Cranberry Lake (Lk.), and Woods Lake. (Table 1) were selected because their forest stands were representative of high elevation red spruce-balsam fir, mixed conifer-northern hardwood, and northern hardwood forest types (Table 1); they have not been recently disturbed or farmed, and the upper elevation sites (WF and Mt. Algonquin) consisting of red spruce (*Picea rubens* Sarg.) have a recent history of dieback and decline.

Collection of water samples and virus concentration. The procedure of Tomlinson et al (25) was modified to concentrate viruses from water. Positively charged Zeta Plus 50 S membrane filters (90 mm, pore size range 0.3–0.75 μm) (AMF CUNO, Meriden, CT) were placed in an In-line-90 polysulfone holder (AMF CUNO). Twenty-liter water samples were collected from April through October in Nalgene polypropylene carboys (Nalge Company, Rochester, NY), brought to the laboratory, and stored at 2–4 C until processing. The water samples were either adjusted to pH 5.5, 6.0, or 6.5 (with 1 N NaOH or HCl) or unadjusted before filtration and pumped through the Zeta Plus 50 S filter disks with a micropump (Micropump, Concord, CA) at a flow rate of 250–500 ml/min. A new filter disk was used for each 5 L of water. Viruses were eluted from each filter (four filters per 20-L water sample) by soaking for 10 min in 30 ml of 50 mM arginine (pH 8.0) containing 1% beef extract. The four filters and eluate from one 20-L water sample were vacuum filtered, combined, and centrifuged for 2 h at 100,000 *g*. Pellets were suspended in a total volume of 1 ml of 10 mM phosphate buffer (pH 7.0). Concentrates were used to inoculate herbaceous virus indicator plants and examined for virus-like particles by transmission electron microscopy (TEM). Two 20-L water samples (one consisting of deionized water and one of tap water) were treated similarly as controls.

Infectivity bioassay and host range. Carborundum-dusted *Chenopodium quinoa* Willd., and tobacco (*Nicotiana tabacum* L. 'Turkish') were inoculated with the water concentrates and incubated in the greenhouse at 20–25 C for 3 wk. Virus isolates were passaged three times through a local lesion host (*N. glutinosa* L.) and propagated in Turkish tobacco. Infected leaf tissue was desiccated over anhydrous calcium chloride and stored at 4 C. *C. quinoa* and tobacco plants also were inoculated with concentrates from the control water samples.

The following indicator plants were mechanically inoculated with viruses contained in the water concentrates: *Beta vulgaris* L. 'Detroit Dark Red'; *Capsicum annuum* L. 'Crispy Hybrid,' 'Early Calwonder,' and 'Poblano Ancho'; *C. quinoa*; *Cucumis sativus* L. 'Straight Eight'; *Gomphrena globosa* L.; *Lycopersicon esculentum* L. 'Burpeeana Early,' 'Red Cherry,' and 'Super Beefsteak'; *Nicotiana benthamiana* Domin.; *N. clevelandii* Gray; *N. glutinosa*; *N. rustica* L.; *N. tabacum* L. 'Havana 38,' 'Turkish,' 'Windsor Shade-117,' 'Xanthi;' *Phaseolus vulgaris* L. 'Black Turtle'; *Physalis floridana* L.; and *Vigna unguiculata* (L.) Walp. 'Blackeye'.

Electron microscopy. Formvar-coated 400-mesh nickel grids were incubated for 5 min on drops of the water concentrates and on leaf extracts from symptomatic virus indicator plants

TABLE 1. Location and characteristics of water sampling sites and the characteristics of the water samples collected at those sites that were assayed for plant viruses by transmission electron microscopy and infectivity bioassay

Collection site	Collection date	Forest type	Water sample characteristics[a]	TEM[b]	Bioassay[c]	Site location[d]
Heiberg Memorial Forest	4/12	Beech-hemlock	s, 7.3, 5.5	R	HMF-32	4736600N 411100E
	4/19	N. hardwood	p, 7.0, 5.5	R	HMF-35	4735600N 411900E
	4/26	N. hardwood	s, 7.4, 5.5	R	HMF-36	4736700N 411300E
Whiteface Mt.	5/10	Spruce-fir	s, 5.3, 5.5	R	WF-38	4915600N 587750E
	5/10	Spruce-fir	s, 5.3, 5.5	R	WF-39	4915600N 587750E
	5/10	Spruce-fir	s, 4.9, 6.5	···	···	
	5/11	Spruce-fir	s, 5.0, 5.5	R	···	
	6/1	Spruce-fir	s, 4.9, 4.9	···	···	
	6/22	N. hardwood	p, 6.2, 6.2	R	WF-51	4917200N 586100E
	6/22	Spruce-fir	s, 5.2, 6.0	···	···	
	10/9	Spruce-fir	s, 5.3, 5.5	···	···	
Catamount Mt.	5/11	N. hardwood	p, 6.7, 6.7	R,F,S	CAT-46	4927100N 593200E
	5/11	N. hardwood	p, 6.5, 5.5	R,F	···	
	5/11	N. hardwood	p, 6.8, 5.5	R,F	···	
	5/11	N. hardwood	p, 6.7, 6.7	R,F	···	
	6/23	N. hardwood	s, 6.0, 6.0	R	CAT-49	4925400N 593000E
	7/21	N. hardwood	s, 6.4, 5.5	···	···	
	7/21	N. hardwood	s, 7.4, 5.5	···	···	
	7/21	N. hardwood	p, 6.6, 5.5	···	···	
	7/21	N. hardwood	p, 6.8, 5.5	···	···	
	10/9	N. hardwood	p, 6.7, 6.7	···	···	
Woods Lk.	7/8	Hardwood-conifer	s, 4.9, 5.5	···	···	
	8/15	Hardwood-conifer	p, 6.3, 5.5	R	···	
	8/15	Hardwood-conifer	s, 5.8, 5.5	R	···	
	9/28	Hardwood-conifer	p, 6.1, 6.1	···	···	
Cranberry Lk.	7/9	N. hardwood	p, 6.5, 5.5	···	···	
	7/17	Mixed conifer	p, 4.6, 5.5	···	···	
Mt. Algonquin	7/15	N. hardwood	s, 6.9, 5.5	···	···	
	7/15	Hardwood-conifer	s, 6.5, 5.5	···	···	

[a] Water source (s = stream, p = pond), pH of water sample unadjusted and adjusted, respectively.
[b] Virus-like particles observed in concentrate by transmission electron microscopy (··· = none, R = rigid rods, F = flexuous rods, S = spheres).
[c] Tomato mosaic virus isolate transmitted from water concentrate to *Chenopodium quinoa*; ··· = no virus transmitted.
[d] Universal Transverse Mercator system of location is provided for sampling sites from which viruses were transmitted. All sampling sites are located in zone 18.

inoculated with the water concentrates. Grids were negatively stained with 2% aqueous uranyl acetate (pH 4.0) and observed with an RCA-EMU 4 transmission electron microscope. Grids of the concentrates from the control water samples were also prepared and observed for virus particles by TEM.

Virus purification and serology. Viruses were propagated in Turkish tobacco, purified (12), and subjected to equilibrium density gradient centrifugation in cesium chloride (22).

Antisera to the three virus isolates WF-38, CAT-46, and HMF-32 were produced in female New Zealand white rabbits (10). Antisera were cross-absorbed with purified healthy tobacco protein (21) and titered by a microprecipitin test (3). Immunoglobulin G (IgG) was purified by ammonium sulfate precipitation and dialysis followed by anion exchange chromatography on DEAE cellulose (9).

Reciprocal agar-gel double-diffusion tests (3) were conducted with undiluted antisera to tobacco mosaic virus strain U1 (TMV-U1 and its antiserum were obtained from M. Zaitlin, Cornell University, Ithaca, NY), ToMV-dogwood strain (ToMV-DW and its antiserum were obtained from B. B. Reddick, University of Tennessee, Knoxville, TN), WF-38, CAT-46, and HMF-32. Plates were incubated at 37 C for 48 h.

Serological relationships among WF-38, CAT-46, and HMF-32 also were evaluated against antisera to each other and nine tobamoviruses by indirect enzyme-linked immunosorbent assay (ELISA) (19). The tobamoviruses were: Holmes' ribgrass mosaic virus (RMV-H), Primula strain of ribgrass mosaic virus (RMV-P), tobacco mild green mosaic virus (TMGMV), Frangipani mosaic virus (FMV), and bell pepper mottle virus (BPMV) (antisera listed above were obtained from C. Wetter, Universitaet Saarbruecken, Germany), ToMV-L (antiserum obtained from M. Zaitlin), ToMV-DW, ToMV-lilac strain (ToMV-R, isolated from lilac cv. Rutilant by C. R. Hibben, Brooklyn Botanic Garden Research Center, Ossining, NY. Antiserum prepared by J. D. Castello), and TMV-U1.

Electrophoresis. Whole virion electrophoresis in agarose gels has been used previously to separate tobamoviruses into subgroups (1,20) and to purify isolates of TMV (2). Immunoelectrophoresis combines this resolution with the specificity of serological analysis. Virus isolates, purified at the same time and stored at −20 C, were subjected to electrophoresis (50 μg/10 μl sample) in a MINNIE submarine agarose gel unit (Model HE 33, Hoefer Scientific Instruments, San Francisco, CA) in 0.8% low electroendosmosis agarose (BioRad, Richmond, CA). RS solution (20% Ficoll, 50 mM EDTA, 0.05% bromophenol blue) was mixed with each virus sample at 1:1 to facilitate sample application. Electrophoresis was performed at 4 C in 0.02 M Tris-phosphate buffer, pH 8.4 (26), for 30 min at 2.5 V/cm followed by 2 h at 5.0 V/cm. Ethidium bromide was added at 0.5 μg/ml to the melted agarose gel and the tank buffer to visualize virus bands under ultraviolet light (17). At the termination of electrophoresis, antiserum to virus isolate HMF-32 was diluted 1:5 in 0.15% sodium chloride, placed in lateral troughs cut in the gel, and the gels were incubated for 48–72 h at 25 C.

RESULTS

Virus recovery and electron microscopy. Rigid rods, 270–300 nm in length, were observed by TEM in the concentrates of 14 of the 29 water samples (Table 1) but not in the control concentrates. A virus was transmitted to *C. quinoa* plants from eight of 29 water sample concentrates (Table 1), but not from the control concentrate. Rigid rods of the same length also were detected by TEM in leaf dips of all infected and symptomatic *C. quinoa* plants, which suggested that the viruses were members of the tobamovirus group. Symptoms in *C. quinoa* consisted of yellow lesions with necrotic centers on the inoculated leaves followed by development of systemic chlorotic lesions and mottle.

Long flexuous rods (approximately 700 nm length) and icosahedral virus-like particles (50 nm diameter) also were detected by TEM in water concentrates from CAT, but these were not transmitted to herbaceous hosts (Table 1). No viruses were transmitted from concentrates of water collected from Cranberry Lk., Mt. Algonquin, or Woods Lk., although rigid rods, 300 nm in length, were observed in two water samples from Woods Lk (Table 1).

Symptomatology and host range. The eight virus isolates from water produced systemic symptoms in *C. annuum* 'Early Calwonder'; *C. quinoa*; *G. globosa*; *L. esculentum* 'Burpeeana Early,' 'Red Cherry,' and 'Super Beefsteak'; *N. benthamiana*; *N. clevelandii*; *N. tabacum* 'Havana 38,' 'Turkish,' and 'Xanthi'; and *P. floridana*. Only local symptoms were produced in the following hosts: *B. vulgaris* 'Detroit Dark Red'; *C. annuum* 'Crispy Hybrid'; *C. sativus* 'Straight Eight'; *N. glutinosa*; *N. rustica*; *N. tabacum* 'Windsor Shade-117'; *P. vulgaris* 'Black Turtle'; and *V. unguiculata* 'Blackeye.'

However, symptoms induced by three of the virus isolates from water differed from the other isolates in some indicator hosts (Table 2). The symptomatology of all the virus isolates from water, as well as the three strains of ToMV tested, were different from the symptoms induced by TMV-U1 on several indicator hosts (Table 2).

Serology. Antisera were produced to HMF-32, WF-38, and CAT-46 with titers of 1:512, 1:1,024, and 1:1,024, respectively. In reciprocal agar-gel double-diffusion tests, WF-38 and ToMV-DW reacted with some degree of identity against TMV-U1, ToMV-DW, and WF-38 antisera (Fig. 1A, B, and C, respectively). Spurs were produced against TMV-U1 with all three antisera (Fig. 1A, B, and C). HMF-32 and CAT-46 antisera produced reactions identical to that of WF-38 in agar gel tests (results not shown).

Differences in dilution end points of HMF-32, WF-38, and CAT-46 were tested against antisera to each other and to nine tobamoviruses in indirect ELISA tests (Table 3). All three isolates reacted with identity to antiserum of ToMV-DW and were closely serologically related to each other and to ToMV-L and R (differences in dilution end point of less than two fivefold dilution steps) (Table 3). Differences in dilution end point of three fivefold dilution steps were detected with all three water viruses against antiserum to TMV-U1. All three isolates were distantly serologically related (low dilution end points) to RMV-P and -H, TMGMV, BPMV, and FMV (Table 3).

Immunoelectrophoresis. Virus isolates HMF-32, HMF-35, HMF-36, WF-38, WF-39, WF-51, CAT-46, CAT-49, ToML-L, and ToMV-DW have similar migration patterns in agarose gels and show two distinct electrophoretic bands (Fig. 2). ToMV-R (from lilac) shows two electrophoretic bands, one that comigrates with the upper band from the 10 isolates above, and a lower band that migrates faster than the lower band of the 10 isolates above (Fig. 2).

DISCUSSION

Plant viruses are present in streams and lakes draining forest

TABLE 2. Comparison of symptom production[a] induced by tobacco mosaic virus strain U1 (TMV-U1), three strains of tomato mosaic virus (ToMV), and three virus isolates from water on selected virus indicator plants

	Virus isolates				
Indicator plant	HMF-32	WF-38	WF-39	ToMV[b] (L, R, DW)	TMV-U1
Lycopersicon esculentum cv. Red Cherry	m	m,sn	···	m,sn	···
Capsicum annuum cv. Poblano Ancho	l	m	m	m	···
cv. Early Calwonder	m	m	sn	m	···
Chenopodium quinoa	l,m	l,m	l,m	l,m	l
Cucumis sativus	l	l	l	l	0
Phaseolus vulgaris cv. Black Turtle	0	0	0	0	l

[a]Symptoms produced: m = systemic mosaic/chlorosis; sn = systemic necrosis; l = local lesions; 0 = no infection; ··· = not tested.
[b]Strains of ToMV: (L = L strain, R = lilac strain, and DW = dogwood strain).

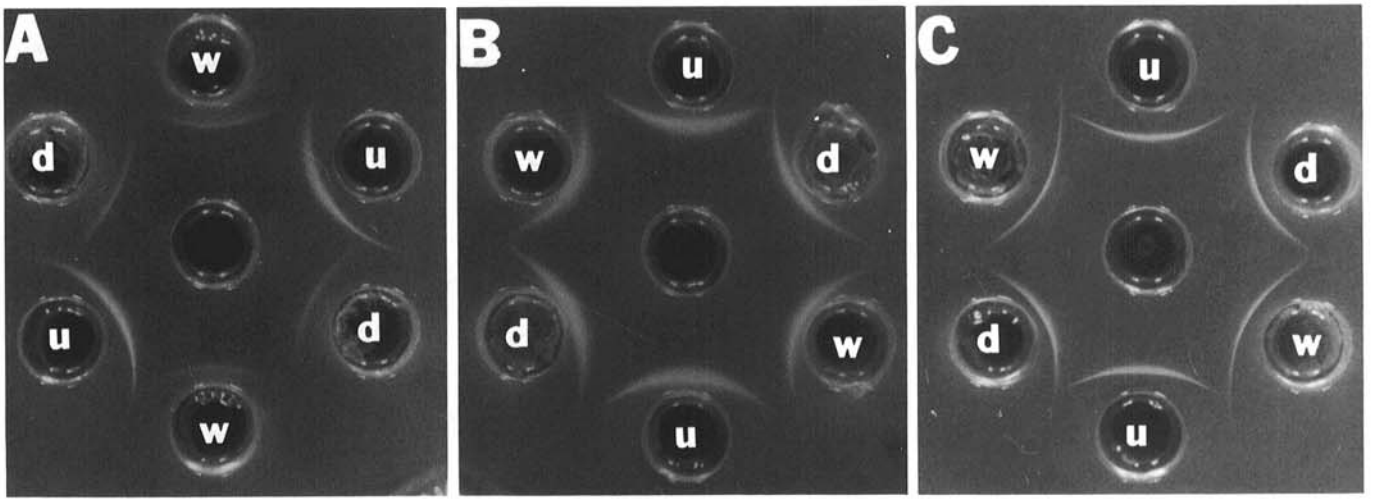

Fig. 1. Results of agar-gel double-diffusion tests of tobacco mosaic virus (TMV) strain U1 (u), tomato mosaic virus (ToMV) strain DW (d), and a water isolate of ToMV-WF-38 (w) in the peripheral wells against antisera (center wells) to **A,** TMV-U1, **B,** ToMV-DW, and **C,** ToMV-WF-38. Fused precipitation lines were produced between wells containing ToMV strains DW and WF-38; spurs were produced between these lines and precipitation lines from wells containing TMV-U1.

TABLE 3. Comparison of the dilution end points[a] of three purified virus isolates from water against various tobamovirus antisera in indirect enzyme-linked immunosorbent assay

	Dilution end point[b]		
Antisera	HMF-32	WF-38	CAT-46
TMV-U1 (6)[c]	3	3	3
ToMV-R (6)	5	6	7
ToMV-DW (7)	7	7	7
ToMV-L (6)	4	5	5
HMF-32 (7)	7	6	7
WF-38 (7)	5	7	6
CAT-46 (7)	5	5	7
Ribgrass mosaic virus-Primula	2	2	4
Ribgrass mosaic virus-Holmes	1	1	3
Tobacco mild green mosaic virus	2	2	3
Bell pepper mottle virus	4	2	4
Frangipani mosaic virus	2	2	4

[a] Mean of two determinations.

[b] Plate wells were coated with purified virus (1 μg/ml, 150 μl/well) in phosphate buffered saline (PBS) and incubated for 1 h at 37 C. After washing with PBS, wells were blocked by adding PBS containing Tween-20 and 0.1% nonfat dry milk powder to the wells and incubating the plate for 1 h at 25 C. After washing, a seven-step fivefold dilution series of antiserum (beginning at 1:500) was prepared and added to separate virus-coated wells (125 μl/well). The plates were incubated for 1 h at 37 C. After washing, goat-anti-rabbit IgG conjugated to alkaline phosphatase was diluted to 1:1,000 in blocking buffer, added to the wells, and the plates were incubated for 1 h at 37 C. Following the final rinse, substrate (*p*-nitrophenylphosphate at 1 mg/ml) was added and the absorbance at A_{405nm} was measured following a 1 h incubation at 25 C.

[c] Numbers in parentheses represent the dilution end point (number of fivefold dilution steps) of the homologous reaction.

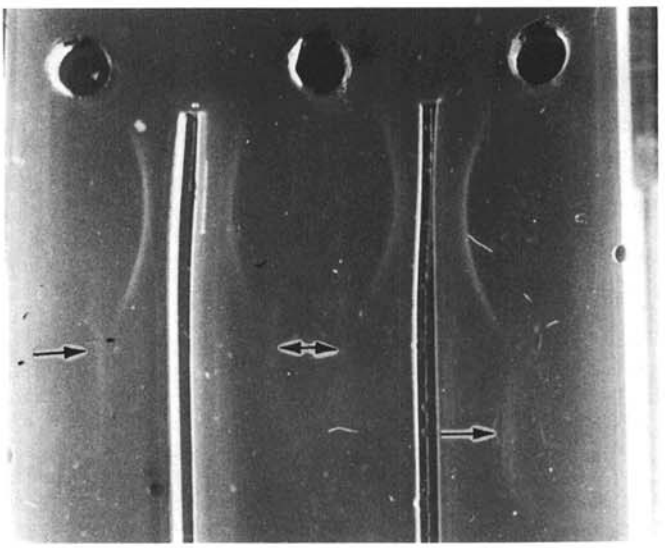

Fig. 2. Whole virion immunoelectrophoresis of tomato mosaic virus (ToMV) water isolate HMF-32 (left lane), ToMV-DW (dogwood strain, center lane), and ToMV-R (lilac strain, right lane). The remaining seven water isolates of ToMV showed electrophoretic patterns similar to that of HMF-32. Arrows highlight lower bands in each lane. Each well contained 50 μg of virus in 10 μl of 0.02 M Tris-phosphate buffer, pH 8.4, (TP) and containing an equal volume of RS solution (20% Ficoll, 50 mM EDTA, and 0.05% bromophenol blue). Electrophoresis was conducted in a MINNIE submarine gel unit containing 0.8% low electroendosmosis agarose at 4 C in TP for 30 min. at 2.5 V/cm followed by 2 h at 5.0 V/cm. At the termination of electrophoresis, two lateral troughs were cut in the gels, antiserum to ToMV (strain HMF-32) was diluted 1:5 in 0.15% saline, placed in the troughs, and the gels were incubated for 48–72 h at 25 C.

stands in New York and were recovered from these waters by adsorption to electropositive membrane filters (Table 1). Based on host range, agar-gel double-diffusion, and indirect ELISA results all virus isolates were identified as ToMV and were distinct from TMV-U1. At least one isolate (CAT-46) appears to be biologically and serologically identical to ToMV-DW. Because symptomatology (Table 2) and indirect ELISA (Table 3) indicate differences among the ToMV isolates from water and because ToMV was never detected in control concentrates or in plants inoculated with them, it is unlikely that the recovery of ToMV represents a greenhouse or laboratory contamination. Isolates HMF-32 and WF-39 are symptomologically distinct from each other and from the other isolates, which induce similar symptoms on the hosts tested (Table 2). Indirect ELISA detected slight differences in dilution end point among HMF-32, WF-38, and CAT-46 (Table 3). All ToMV isolates from water as well as ToMV-L and ToMV-DW are similar in their electrophoretic properties, but electrophoretically distinct from ToMV-R. Therefore, isolates of ToMV that differ in symptomological and serological characteristics were recovered from waters that drain forest stands in different geographic regions of New York State. Koenig et al (15) recognized symptomological, serological, and electrophoretic diversity among 26 South American isolates of Andean potato latent virus originating from different geographic regions of the Andes Mountains. Plant viruses with a wide geographic distri-

bution, such as ToMV, may exist as a mixture of distinct strains or strain groups in which the geographic distribution pattern of individual strains is patchy or confined to small areas.

ToMV was recovered from water draining forest stands in the beech-hemlock, northern hardwood, and spruce-fir forest types; from both streams and ponds (Table 1) and from water samples with pH values from approximately 5.0 to 7.0 (Table 1).

All water samples from which ToMV was recovered were collected from April through June (Table 1). Viruses were not transmitted to herbaceous hosts from water samples collected in July to October, even from the Catamount Mt. site from which they were recovered in May and June (Table 1). Viruses were not recovered from the Woods Lk., Cranberry Lk., or Mt. Algonquin sites; but these sites were not sampled in April, May, or June. Viruses may not be present in waters from these sites, or water samples may have been collected at the wrong time of year. Conversely, with the exception of one sample collected on October 9, 1989, the Heiberg Memorial Forest and Whiteface Mt. sites were not sampled from July through October 1989 (Table 1). However, ToMV was not detected or recovered in water samples collected monthly from June 1990 through January 1991 from the high elevation Whiteface Mt. site (J. D. Castello and V. Jacobi, *unpublished*). Seasonality in virus recovery has been noted in other systems. Bergh et al (4) by using TEM to make direct counts of femtoplankton (viruses of less than 0.2 μm in size) in coastal, open ocean, and freshwater systems found the highest concentration of viruses and bacteria in marine samples during the spring and summer months. Marine samples collected in the winter months showed very low virus counts, thus indicating a seasonal variation in the concentration of viruses in natural waters.

This study represents the first survey for plant viruses in water draining forest ecosystems in the United States. Microporous, positively charged Zeta Plus membrane filters were used to recover ToMV from more than 25% of the water samples collected from forest stands in New York. This method has been employed previously to recover bacteriophage (18), tobacco necrosis virus (25), and tomato bushy stunt virus (24) from large volumes of water. In a survey for plant viruses in waters draining forest stands in Germany, viruses of different taxonomic groups were recovered from more than 50% of the water samples tested (7). The plant virus load in European waters may be higher than that of waters draining New York forests. However, in the German study an Amicon hollow fiber system was used to recover plant viruses from 50-L water samples. The hollow fiber system is a molecular sieve, which retains all particles, including viruses, with a MW larger than the pore size of the hollow fiber (~100,000). Virus recovery in the membrane filtration system, however, depends on virus surface charge and is pH-dependent. Because our system was optimized for tobamoviruses, it is not surprising that viruses in other taxonomic groups were not recovered in high enough concentration to enable transmission to herbaceous hosts. Therefore, ToMV is probably not the only plant virus in these waters. Other viruses may not have been recovered because they were not mechanically transmissible, the indicator plants mechanically inoculated were not susceptible hosts, the filtration technique used was specific for tobamoviruses, or virus concentrations were below those necessary for mechanical transmission. Flexuous rods and spherical virus-like particles were observed in one water sample concentrate (Table 1), but because these virus-like particles were not transmitted to virus indicator plants they were not positively identified.

ToMV is a common inhabitant of forest ecosystems in both Europe and North America. Its host range among woody plant species may be larger than previously believed. ToMV was repeatedly recovered from soil in two forest districts of North Rhine-Westphalia, Germany, and from root-soil samples collected under pine, spruce, beech, and oak forests in three federal states of Germany (6). In the United States, a distinct isolate of ToMV (ToMV-DW) was recently transmitted from symptomless flowering dogwood trees (*Cornus florida* L.) used as nursery stock in Tennessee (20). Additionally, ToMV was mechanically transmitted to Xanthi-nc tobacco from symptomatic foliage of the lilac hybrid *Syringa* × *nanceiana* McKelvey (*S.* × *henryi* Schneid. × *S. sweginzowii* Koehne & Lingelsh.) 'Rutilant' growing at the Arnold Arboretum, Jamaica Plain, MA (8). The virus was back-transmitted to healthy seedlings of *S.* × *henryi* cv. Lutece and white ash (*Fraxinus americana* L.). No symptoms developed in lilac but a systemic chlorotic mottle and mosaic developed in white ash.

The epidemiological consequences of waterborne plant viruses may be significant. Gerik et al (11) recently identified tomato bushy stunt virus as the most probable etiologic agent of widespread tomato plant decline throughout the Imperial Valley of California. Tombusviruses have been detected frequently in river water throughout the world (5,24). The widespread incidence of this disease is best explained by virus spread in irrigation water because the Colorado River is the primary source of irrigation water in the valley (11).

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