Detection of Infectious Tomato Mosaic Tobamovirus in Fog and Clouds

J. D. Castello, D. K. Lakshman, S. M. Tavantzis, S. O. Rogers, G. D. Bachand, R. Jagels, J. Carlisle, and Y. Liu

First, fourth, fifth, and eighth authors: professor, associate professor, and graduate research assistants, respectively, State University of New York, College of Environmental Science & Forestry, Faculty of Environmental & Forest Biology, 1 Forestry Dr., Syracuse 13210-2788; second and third authors: associate scientist and professor, respectively, University of Maine, Department of Plant Biology & Pathology, 5722 Deering Hall, Orono 04469-5722; sixth and seventh authors: professor and chair, and assistant scientist, respectively, University of Maine, Department of Forest Ecosystem Science, 5755 Nutting Hall, Orono 04469-5755.

Supported in part by grants from the McIntire-Stennis Cooperative Forestry Research Program of the USDA and USDA-NRICGP award 93-37101-8837 to J. D. Castello, the Hatch CSRS Program of the USDA to S. M. Tavantzis, and The Andrew W. Mellon Foundation to R. Jagels.

We thank D. Wolfe and S. Roychowdhury, Atmospheric Sciences Research Center, Whiteface Mountain Field Station, for collection of cloud samples; C. Long and J. O'Day for technical assistance; D. Leopold, J. Hamacher, V. Jacobi, W. Powell, M. Schaedle, and S. Silbiger for reviews of earlier drafts of the manuscript.

Accepted for publication 17 August 1995.

ABSTRACT

Castello, J. D., Lakshman, D. K., Tavantzis, S. M., Rogers, S. O., Bachand, G. D., Jagels, R., Carlisle, J., and Liu, Y. 1995. Detection of infectious tomato mosaic tobamovirus in fog and clouds. Phytopathology 85:1409-1412.

Tomato mosaic tobamovirus (ToMV) was detected by enzyme-linked immunosorbent assay and reverse transcription-polymerase chain reaction-blot hybridization (RT-PCR-BH) in cloud samples collected from the summit of Whiteface Mountain, NY, and in fog samples from two collection sites along the coast of Maine. The virus was subsequently transmitted to *Chenopodium quinoa* from a composite of RT-PCR-BH–positive concentrates. There was no apparent relationship between the presence of ToMV and sample collection date, volume, or pH. The RT-PCR products of three cloud and fog samples and one deionized water sample were identical to one another and to a stream water isolate of ToMV from Whiteface Mountain based on nucleotide sequencing of a 347-bp fragment within the coat protein gene and 99.1 and 72.9% similarity to ToMV-L and tobacco mosaic virus-vulgare, respectively.

Additional keywords: atmosphere, red spruce, waterborne virus.

Plant viruses (25), enteric viruses (17), and bacteriophage (4) have been detected in surface waters. Aerosolization and recovery of enteric viruses from spray irrigation of wastewater (32) and phage from ocean surf (3) have been demonstrated, as well as aerosol transmission of some animal viruses (16). To our knowledge, however, long-range atmospheric spread of infectious plant viruses in the absence of biological vectors has not been reported.

To investigate the potential for atmospheric spread of plant viruses, we selected the red spruce (*Picea rubens* Sarg.)-tomato mosaic tobamovirus (ToMV) pathosystem (1,9,20,21). The virus is a stable, soil- and waterborne RNA virus that is widespread in agricultural (15,27) and forest ecosystems (5,6,19,21) and that lacks an invertebrate vector (15). Virus infection of red spruce is associated with an adverse impact on the growth of this ecologically and economically valuable tree species (1,9). Incidence and ToMV concentration in roots of red spruce on Whiteface Mountain, NY, are site related (9). Virus-infected red spruce have been detected in other sites in New York, as well as in Vermont, New Hampshire, and Maine (J. D. Castello, *unpublished data*). A common feature of both coastal and high-elevation red spruce forests is their exposure to wet aerosol, either as advective fog or orographic clouds (10,12,24,28). We contend that virus incidence is related to exposure of spruce to virus-laden clouds or fog. In support of this contention, we hypothesize that infectious ToMV is present in clouds and fog and propose that such meteorological phenomena provide a mechanism for virus spread and transmission to red spruce. A preliminary report of this study has been published (8).

Corresponding author: J. D. Castello; E-mail address: jdcastel@mailbox.syr.edu

MATERIALS AND METHODS

Collection and concentration of cloud, fog, and water. To determine whether waterborne ToMV was present in our laboratory water, 12 deionized water samples (ranging in volume from 33 to 140 ml) from our laboratories at the State University of New York, Syracuse, and the University of Maine, Orono, 6 diethylpyrocarbonate (DEPC)-treated/autoclaved deionized water samples from our laboratories and DEPC-treated/autoclaved phosphate buffer were concentrated by ultracentrifugation at 100,000 × *g* for 2 h and tested for ToMV as described below. The resulting pellets were suspended in 1 ml of 10 mM DEPC-treated/autoclaved phosphate buffer (pH 7.2) and subsequently stored at –20°C (referred to as concentrates).

Between June and August 1992, 22 cloud samples from 11 cloud events were collected using an Atmospheric Sciences Research Center (ASRC) passive collector located above the tree line on the summit (1,483 m) of Whiteface Mountain, NY (44°23′N, 73°59′W). Cloud samples were collected as part of the ongoing Mountain Cloud Chemistry Project conducted at the ASRC, Wilmington, NY. Concurrently, 22 fog samples were collected from one CWP active collector (11) each on Mount Desert Rock (43°59′N, 68°08′W) and Isle Au Haut (44°01′N, 68°38′W), ME. Mount Desert Rock is an unforested 1.2-ha rock located 34 km south of Mount Desert Isle. Isle Au Haut is a 2,400-ha island, forested primarily with red spruce in various stages of dieback, located 54 km to the west-northwest of Mount Desert Rock (22). The fog samples were collected as part of a separate research project at the University of Maine on the movement of air pollutants along the northeastern Atlantic Coast. Collection date, volume, and pH (where available) were recorded for each sample. Samples collected on the same day were collected at different times from the same collector and

represent a cloud or fog event of long duration. All fog and cloud samples were stored in high-density polyethylene bottles and shipped to the State University of New York at Syracuse where they were stored at 4°C until concentrated by ultracentrifugation as described above. Concentrates were stored at –20°C until tested for ToMV.

Several steps were undertaken to minimize the likelihood of virus contamination. Fog and cloud samples were collected and stored in either new or thoroughly washed and rinsed bottles. Ultracentrifugation was conducted in new or autoclaved centrifuge tubes. All buffers were prepared in DEPC-treated/autoclaved deionized water. DEPC treatment was very efficient in inactivating tobacco mosaic virus (TMV) RNA by reaction with adenine residues (26) and was used in conjunction with autoclaving to denature viral proteins from all buffers and treated water samples. Autoclaving destroyed the antigenicity of ToMV coat protein (J. D. Castello, *unpublished data*). All glassware was thoroughly washed, rinsed, and then autoclaved. Pipette tips also were autoclaved. To determine if virus in non–DEPC-treated deionized rinse water might have adsorbed to collection bottles and leached into the fog/cloud samples during storage, three bottles were rinsed with DEPC-treated/autoclaved water that was concentrated and tested for ToMV as described below.

TABLE 1. Collection date, volume, pH, and results of reverse transcription-polymerase chain reaction-blot hybridization (RT-PCR-BH) assays for tomato mosaic tobamovirus in cloud and fog concentrates

Sample	Collection date (1992)	Volume (ml)[a]	pH	RT-PCR-BH
Whiteface Mountain, NY				
1	6/18	138	3.5	+
2	6/24	140	3.6	–
3	6/24	110	3.3	+
4	6/24	50	3.3	–
5	6/24	138	3.5	+
6	6/24	118	3.7	–
7	6/25	140	4.4	+
8	6/25	130	4.0	+
9	7/9	134	nd[b]	–
10	7/9	132	nd	+
11	7/13	141	nd	–
12	7/13	142	nd	+
13	7/17	84	nd	–
14	7/18	92	nd	–
15	7/18	138	nd	–
16	7/18	140	nd	–
17	7/18	134	nd	+
18	7/18	140	nd	+
19	7/26	116	nd	–
20	7/28	106	nd	+
21	8/16	96	nd	+
22	8/17	106	nd	+
Mount Desert Rock, ME				
11	6/22	125	4.6	+
32	7/15	26	4.5	–
24	7/18	33	4.1	+
26	7/21	228	3.7	–
36	8/1	92	3.1	–
42	8/4	134	3.7	+
48	8/10	130	3.6	–
51	8/20	138	2.7	+
58	8/27	129	3.3	+
59	8/28	139	4.6	+
60	8/29	123	2.9	–
66	9/8	126	4.6	–
Isle Au Haut, ME				
33	7/21	134	3.5	+
34	8/1	185	3.0	+
40	8/5	186	3.6	–
41	8/13	181	3.5	+
44	8/19	174	3.7	–
47	8/20	132	2.8	+
53	8/27	290	3.3	+
54	8/28	261	3.3	+
55	8/30	92	3.1	+
71	10/14	750	3.3	–

[a] Cloud and fog samples were concentrated by ultracentrifugation in new or autoclaved centrifuge tubes at 100,000 × *g* for 2 h, and the pellets were suspended in 1 ml of 10 mM diethylpyrocarbonate-treated/autoclaved phosphate buffer (pH 7.2).

[b] nd = not determined.

To determine the best method for virus detection, cloud, fog, and deionized water concentrates were tested for ToMV by infectivity bioassay, direct double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA), and reverse transcription-polymerase chain reaction-blot hybridization (RT-PCR-BH) as described below.

DAS-ELISA and RT-PCR-BH. The cloud and fog concentrates, as well as several non–DEPC-treated deionized water concentrates, were tested individually for ToMV by DAS-ELISA as described by Jacobi et al. (21). For a conservative evaluation, samples were considered positive for ToMV only if their mean absorbance at 405 nm (A_{405}) was greater than both the mean A_{405} of wells that contained phosphate buffer and those that contained purified ToMV at 1 ng/ml.

Cloud, fog, DEPC-treated, and non–DEPC-treated deionized water concentrates, collection bottle rinsate, and phosphate buffer were tested for ToMV by RT-PCR-BH as follows. Nucleic acids were purified by proteinase-K digestion and phenol-chloroform extraction (33). RT-PCR was performed in a Perkin-Elmer thermal cycler (model 480) using a GenAmp RNA PCR kit (Perkin-Elmer Cetus, Norwalk, CT) and specific primers (5′-GAGTGCGGGCTACTGCCCTTTG, identical to bases 5349–5370, and 5′-TGGGCCCCAACCGGGGGT, complementary to bases 6367–6384 of ToMV-L [29]). PCR was done at 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min, for 35 cycles, with a final extension at 72°C for 10 min. RT-PCR products were electrophoresed on 1% agarose gels in 0.5× TBE (45 mM Tris-borate, 1 mM EDTA) and stained with ethidium bromide. The gels then were capillary blotted to Hybond-N nylon membrane (Amersham Corp., Arlington Heights, IL) and hybridized with a ^{32}P-labeled oligo-primed DNA probe (13,30) synthesized from an 820-bp *Hin*dIII-*Eco*RI cloned cDNA fragment containing the coat protein gene, the 3′ end of the 30-kDa protein gene, and the intergenic region (2) (corresponding to bases 5536–6356 of ToMV-L [29]). Autoradiography was done with Kodak XAR-5 or XK-1 film (Eastman-Kodak Co., Rochester, NY).

Infectivity bioassay. *Chenopodium quinoa* Willd. plants, a local lesion host for ToMV, were inoculated mechanically with each cloud and fog concentrate and several non–DEPC-treated deionized water concentrates. The plants were kept in the greenhouse for 2 weeks and monitored for the development of local lesions.

A composite of all cloud and fog concentrates that tested positive for ToMV by RT-PCR-BH was further concentrated 25-fold by additional ultracentrifugation, as described above, and inoculated onto *C. quinoa*. A composite of cloud and fog concentrates that tested negative for ToMV was treated similarly. Several plants were mock-inoculated with buffer to serve as controls.

Nucleotide sequencing. The RT-PCR products of three cloud and fog concentrates, one from each collector as well as one deionized water concentrate from the University of Maine, were compared to each other and to ToMV-38 (a stream water isolate of the virus from Whiteface Mountain), the type strain of ToMV from Japan (ToMV-L, GenBank accession X02144), and the type strain of TMV (TMV-vulgare, GenBank accession J02415) by nucleotide sequencing. A 347-bp PCR product was generated using two nested primers within the coat protein region (5′-TTCAACAGCAGTTCAGCGAG, position 5836–5855, and 5′-TTAAGATGCAGGTGCAGAGG, position 6163–6182 of ToMV-L) under the conditions mentioned above. Both DNA strands were sequenced (7) using a dye-labeled cycle sequencing kit and an ABI373A DNA sequencer (Applied Biosystems, Foster City, CA). Sequences were aligned by the CLUSTAL program of PC/Gene, version 6.5 (IntelliGenetics, Inc., Mountain View, CA).

RESULTS

ELISA, RT-PCR-BH, and nucleotide sequencing. The mean absorbance (and standard deviation) of eight wells containing phosphate buffer was 0.112 ± 0.005 and of eight wells containing 1 ng of purified ToMV per ml was 0.182 ± 0.019. Only two concentrates, one from Whiteface Mountain and another from Mount Desert Rock, had an A_{405} that approximated the positive/negative threshold with values of 0.186 and 0.177, respectively. The range of A_{405} values for the fog/cloud samples that tested negative for ToMV was 0.097 to 0.143 (0.119 ± 0.011, mean and standard deviation). None of the non–DEPC-treated/autoclaved deionized water samples tested positive by DAS-ELISA.

ToMV-specific hybridization signals were detected in 10 of 12 non–DEPC-treated deionized water concentrates from both laboratories but not in any of the 6 DEPC-treated/autoclaved water concentrates, phosphate buffer, or the rinsate of sample collection bottles.

Twenty-five of forty-four cloud and fog concentrates tested positive for ToMV by RT-PCR-BH (Table 1). RT-PCR products often were visible when resolved on 1% agarose gels stained with ethidium bromide. The RT-PCR-BH products from representative ToMV-positive cloud and fog concentrates from each collector are presented in Figure 1. Although sample volumes ranged from 26 to 750 ml and pH values ranged from 2.7 to 4.6, there was no apparent relationship between sample collection date, volume, or pH and detectable ToMV (Table 1).

The RT-PCR products from the fog, cloud, and deionized water concentrates that were sequenced were identical to ToMV-38 and were 99.1 and 72.9% similar to ToMV-L and TMV-vulgare, respectively, within a 347-bp sequence of the coat protein gene (Fig. 2).

Infectivity bioassay. No lesions developed on *C. quinoa* plants inoculated with individual cloud, fog, or non–DEPC-treated deionized water concentrates. However, approximately 400 typical ToMV lesions developed within 3 days on leaves inoculated with the composite of cloud/fog concentrates that tested positive for ToMV by RT-PCR-BH and were augmented 25× by ultracentrifugation. No lesions appeared on mock-inoculated leaves or on leaves inoculated with a composite of all RT-PCR-BH–negative concentrates prepared similarly. The virus was confirmed as ToMV in agar-gel double-diffusion tests conducted in agar containing 0.5% sodium dodecyl sulfate, in which the cloud/fog isolate of ToMV produced a contiguous precipitin line with ToMV-38 when tested against antiserum to this virus (data not shown).

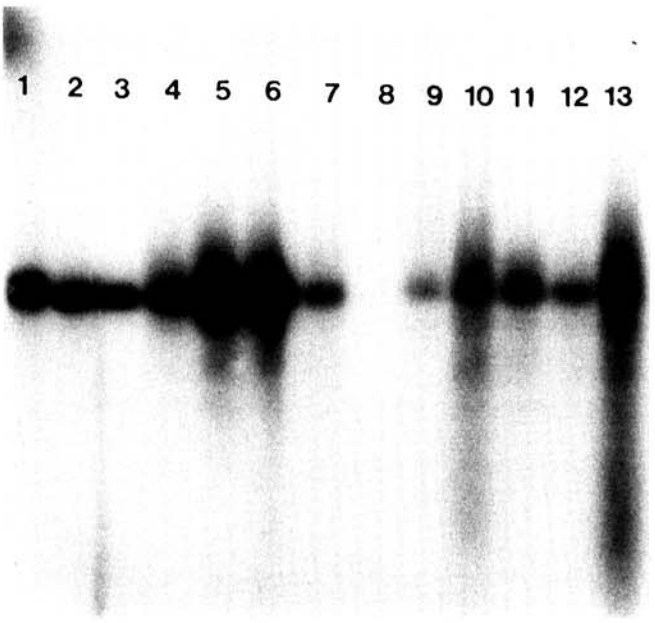

Fig. 1. Autoradiogram of representative cloud and fog concentrates after reverse transcription-polymerase chain reaction-blot hybridization. Lane 1, 1.0-kb tomato mosaic tobamovirus (ToMV) cDNA band; lanes 2–4, ToMV-positive cloud and fog concentrates from Mount Desert Rock, ME; lanes 5–7, ToMV-positive cloud and fog concentrates from Isle Au Haut, ME; lane 8, blank; and lanes 9–13, ToMV-positive cloud and fog concentrates from Whiteface Mountain summit, NY. Concentrates were prepared by ultracentrifugation at 100,000 × *g* for 2 h, and pellets were suspended in diethylpyrocarbonate-treated/autoclaved phosphate buffer.

DISCUSSION

Because ToMV is waterborne, we suspected that the virus might be present in our laboratory water supplies. This suspicion was confirmed by RT-PCR and blot hybridization, in which the virus was detected in non–DEPC-treated deionized water from both university laboratories. To eliminate the chance of contamination, all glassware was autoclaved, and all buffers were treated with DEPC and autoclaved prior to use in this study. This virus most likely occurs in water supplies in other laboratories, but DEPC treatment combined with autoclaving can eliminate it.

The virus was detected in at least one fog and cloud concentrate by ELISA and in many others by RT-PCR-BH. Its infectious nature was confirmed by mechanical transmission to *C. quinoa*. Theoretically, PCR can detect a single target molecule (18) and is more sensitive than either ELISA or infectivity bioassay for detecting viruses. However, ultracentrifugation and resuspension of pellets into a smaller final volume may permit the routine detection of this virus in fog and clouds by ELISA and, perhaps, also by infectivity bioassay.

Nucleotide sequencing results within the coat protein gene confirm the identity of the virus from clouds and fog as ToMV (Fig. 2). Atmospheric isolates of this virus from the northeastern United States are closely related to a Japanese isolate of the type strain of ToMV. Sequencing of less conserved regions of the genome will

```
TMV          TTCAAAGACA ATTCAGTGAG GTGTGGAAAC CTTCACCACA AGTAACTGTT   50
ToMV-L       .....CAG.. G.....C... .......... ...TC..T.. GAGC..C..C
ToMV-fog,    .....CAG.. G.....C... .......... ...TC..T.. GAGC..C..C
cloud,&
water

TMV          AGGTTCCCTG ACAGTGACTT TAAGGTGTAC AGGTACAATG CGGTATTAGA  100
ToMV-L       ..A..T.... G.GA..TT.A .......... .......... .A..T.....
ToMV-fog,    ..A..T.... GTGA..TT.A .......... .......... .A..T.....
cloud,&                  *
water

TMV          CCCGCTAGTC ACAGCACTGT TAGGTGCATT CGACACTAGA AATAGAATAA  150
ToMV-L       T..T...A.T ..T..GT..C .G..G..T.. T..T.....G ..........
ToMV-fog,    T..T...A.T ..T..GT..C .G..GA.T.. ...T.....G ..........
cloud,&                                 *     *
water

TMV          TAGAAGTTGA AAATCAGGCG AACCCCACGA CTGCCGAAAC GTTAGATGCT  200
ToMV-L       .C.....A.. ...C...CA. .GT..G..A. .A..T..... ..........
ToMV-fog,    .C.....A.. ...C...CA. .GT..G..A. .A..T..... ..........
cloud,&
water

TMV          ACTCGTAGAG TAGACGACGC AACGGTGGCC ATAAGGAGCG CGATAAATAA  250
ToMV-L       ..C..C..G. .......... T.....T..A ..TC..TCT. .T........
ToMV-fog,    ..C..C..G. .......... T.....T..A ..TC..TCT. .T........
cloud,&
water

TMV          TTTAATAGTA GAATTGATCA GAGGAACCGG ATCTTATAAT CGGAGCTCTT  300
ToMV-L       ....G.TAAT ...C.AG.A. ....T..T.. .CTG..C... .A..ATA...
ToMV-fog,    ....G.TAAT ...C.AG.A. ....T..T.. .CTG..C... .A..ATA...
cloud,&
water

TMV          TCGAGAGCTC TTCTGGTTTG GTTTGGACCT CTGGTCCTGC AACTTGA     347
ToMV-L       .T..A..TAT G.....G... ..C..... ...CA..... .T...A.
ToMV-fog,    .T..A..TAT G.....G... ..C..... ...CA..... .T...A.
cloud,&
water
```

Fig. 2. Nucleotide sequence of a 347-bp fragment within the coat protein gene of the type strain of tobacco mosaic virus (TMV) (GenBank accession J02415), the type strain of tomato mosaic tobamovirus (ToMV-L) from Japan (GenBank accession X02144), and amplification products of three cloud and fog concentrates, one deionized water concentrate, and one Whiteface Mountain, NY, stream water isolate of the virus (ToMV fog and water). The fog, cloud, and water ToMV reverse transcription-polymerase chain reaction-blot hybridization products were identical to each other and were 99.1 and 72.9% similar to ToMV-L and TMV, respectively. Asterisks indicate differences between the fog and water isolates and ToMV-L.

be needed to assess further the relatedness of ToMV isolates from cloud, fog, water, soil, red spruce, and other sources around the world.

In another study, we demonstrated airborne transmission of ToMV to spruce seedlings on Whiteface Mountain (14), but the mechanism was unknown. Clouds are at least one airborne source of this virus for infection of red spruce on Whiteface Mountain and potentially for red spruce in coastal or other areas with frequent exposure to cloud or fog, (e.g., Isle Au Haut, ME). Because fog droplets are very small (radii of 0.5 to 50 μM) with a very large surface-to-volume ratio, they are very efficient particle collectors (31). Conifer needles are efficient fog collectors, and foliar surfaces are exposed throughout the year. Compared with balsam fir (*Abies balsamea*) and eastern white pine (*Pinus strobus*), red spruce has the highest fog collection efficiency under experimental conditions (23). In addition, the physicochemical wax characteristics of the surface of red spruce needles cause them to be wetted much more easily by water than the needles of many other conifers in the northeastern United States (31). Although the infection site of this virus in native red spruce is unknown, two mechanisms are plausible: direct entry through the needles or root infection from virus-laden condensate dripping into the soil from the canopy. Root infection of red spruce has been demonstrated (1,20). The potential for needle infection is currently under investigation (J. D. Castello and G. D. Bachand, *unpublished data*). Jagels (23) has shown that fog collects primarily around stomates in red spruce (less so in balsam fir and not in white pine); thus, stomatal pores could be possible virus infection sites.

Atmospheric spread of infectious plant viruses without invertebrate vectors represents a potentially significant long-distance transport mechanism for stable plant viruses. A cloud/fog mechanism may function for the transmission of other stable viruses to plant hosts in similar ecosystems. How this virus becomes airborne remains unknown. Because ToMV occurs in agricultural soils (15), it may become airborne on minute soil particles that serve as cloud condensation nuclei. If so, infection of red spruce in montane forest ecosystems (9,21), and the associated adverse effects on growth of this species (1,9), may be related to agricultural practices that release virus-contaminated soil into the air. Alternatively, this virus may be endemic to forest ecosystems from which it spreads via water to agricultural areas and back to montane forests in clouds. The discovery of plant viruses in clouds and fog raises the possibility that viruses of other hosts also might spread in this manner.

LITERATURE CITED

1. Bachand, G. D., and Castello, J. D. 1994. Effects of tomato mosaic virus infection on the growth and development of red spruce seedlings. (Abstr.) Phytopathology 84:541.
2. Bachand, G. D., Castello, J. D., and Rogers, S. O. 1994. cDNA cloning of the coat protein and a portion of the 30K protein region of tomato mosaic virus. (Abstr.) Phytopathology 84:541.
3. Baylor, E. R., Baylor, M. B., Blanchard, D. C., Syzdek, L. D., and Appel, C. 1977. Virus transfer from surf to wind. Science 198:575-580.
4. Bergh, O., Borsheim, K. Y., Bratbak, G., and Heldal, M. 1989. High abundance of viruses found in aquatic environments. Nature (Lond.) 340:467-468.
5. Büttner, C., and Nienhaus, F. 1989. Virus contamination of soils in forest ecosystems of the Federal Republic of Germany. Eur. J. For. Pathol. 19: 47-53.
6. Büttner, C., and Nienhaus, F. 1989. Virus contamination of waters in two forest districts of the Rhineland area (FGR). Eur. J. For. Pathol. 19:206-211.
7. Caporale, D. A., and Kocher, T. D. 1994. Sequence variation in the outer-surface-protein genes of *Borrelia burgdorferi*. Mol. Biol. Evol. 11:51-64.
8. Castello, J. D., Lakshman, D. K., Tavantzis, S. M., Rogers, S. O., Bachand, G. D., Jagels, R., Carlisle, J., and Liu, Y. 1995. Detection of infectious tomato mosaic tobamovirus in fog and clouds. Phytopathology (Abstr.) 85:1138.
9. Castello, J. D., Wargo, P. M., Jacobi, V., Bachand, G. D., Tobi, D. R., and Rogers, M. A. M. 1995. Tomato mosaic virus infection of red spruce on Whiteface Mt., New York: Prevalence and potential impact. Can. J. For. Res. 25:1340-1345.
10. Castillo, R. A., Jiusto, J. E., and McLaren, E. 1983. The pH and ionic composition of stratiform cloud water. Atmos. Environ. 17:1497-1505.
11. Daube, B., Jr., Kimball, D. D., Lamar, P. A., and Weathers, K. C. 1987. Two new groundlevel cloud water sampler designs which reduce rain contamination. Atmos. Environ. 21:893-900.
12. Falconer, R. E., and Falconer, P. D. 1980. Determination of cloud water acidity at a mountain observatory in the Adirondack Mountains of New York State. J. Geophys. Res. 85:7465-7470.
13. Feinberg, A. P., and Vogelstein, B. 1984. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. 132:6-13.
14. Fillhart, R. C., Castello, J. D., and Bachand, G. D. 1995. Airborne transmission of tomato mosaic tobamovirus to red spruce seedlings on Whiteface Mountain, NY. (Abstr.) Phytopathology 85:1138.
15. Gibbs, A. J. 1977. Descriptions of Plant Viruses. Commonwealth Mycological Institute/Association of Applied Biologists, Kew, England.
16. Grant, R. H., Scheidt, A. B., and Rueff, L. R. 1994. Aerosol transmission of a viable virus affecting swine: Explanation of an epizootic of pseudorabies. Int. J. Biometeorol. 38:33-39.
17. Hejkal, T. W., Keswick, B., LaBelle, R. L., Gerba, C. P., Sanchez, Y., Dreesman, G., Hafkin, B., and Melnick, J. L. 1982. Viruses in a community water supply associated with an outbreak of gastroenteritis and infectious hepatitis. J. Am. Water Works Assoc. 74:318-321.
18. Henson, J. M., and French, R. 1993. The polymerase chain reaction and plant disease diagnosis. Annu. Rev. Phytopathol. 31:81-109.
19. Jacobi, V., and Castello, J. D. 1991. Isolation of tomato mosaic virus from waters draining forest stands in New York State. Phytopathology 81:1112-1117.
20. Jacobi, V., and Castello, J. D. 1992. Infection of red spruce, black spruce, and balsam fir seedlings with tomato mosaic virus. Can. J. For. Res. 22: 919-924.
21. Jacobi, V., Castello, J. D., and Flachmann, M. 1992. Isolation of tomato mosaic virus from red spruce. Plant Dis. 76:518-522.
22. Jagels, R. 1986. Acid fog, ozone and low elevation spruce decline. Int. Assoc. Wood Anat. Bull. 7:299-307.
23. Jagels, R. 1991. Biophysical aspects of fog deposition on the needles of three conifers. J. Exp. Bot. 42:757-763.
24. Kimball, K. D., Jagels, R., Gordon, G. A., Weathers, K. C., and Carlisle, J. 1988. Differences between New England coastal fog and mountain cloud water chemistry. Water Air Soil Pollut. 39:383-393.
25. Koenig, R. 1986. Plant viruses in rivers and lakes. Adv. Virus Res. 31: 321-333.
26. Leonard, N. J., McDonald, J. J., Henderson, R. E. L., and Reichmann, M. E. 1971. Reaction of diethyl pyrocarbonate with nucleic acid components: Adenosine. Biochemistry 10:3335-3342.
27. Matthews, R. E. F. 1992. Fundamentals of Plant Virology. Academic Press, San Diego, CA.
28. Mohnen, V. A. 1992. Atmospheric deposition and pollutant exposure of eastern U.S. forests. Pages 64-124 in: Ecology and Decline of Red Spruce in the Eastern United States. Vol. 96. C. Eagar and M. B. Adams, eds. Springer-Verlag, New York.
29. Ohno, T., Aoyagi, M., Yamanashi, Y., Saito, H., Ikawa, S., Meshi, T., and Okada, Y. 1984. Nucleotide sequence of the tobacco mosaic virus (tomato strain) genome and comparison with the common strain genome. J. Biochem. 96:1915-1923.
30. Sambrook, J., Fritsch, E. F., and Maniatis, T. 1989. Molecular Cloning: A Laboratory Manual. 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
31. Schier, G. F., and Jensen, K. F. 1992. Atmospheric deposition effects on foliar injury and foliar leaching in red spruce. Pages 271-294 in: Ecology and Decline of Red Spruce in the Eastern United States. Vol. 96. C. Eagar and M. B. Adams, eds. Springer-Verlag, New York.
32. Shuval, H. I., Guttman-Bass, N., Applebaum, J., and Fattal, B. 1989. Aerosolized enteric bacteria and viruses generated by spray irrigation of wastewater. Water Sci. Technol. 21:131-135.
33. Tavantzis, S. M. 1983. Improved purification of two potato carlaviruses. Phytopathology 73:190-194.