

Isolation of Tomato Mosaic Virus from Lilac

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ABSTRACT

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Tomato mosaic virus (ToMV) was transmitted to tobacco on three occasions from young leaves without viruslike symptoms (VLS), but not from mature leaves with VLS collected from a Rutilant lilac growing at the Arnold Arboretum, Jamaica Plain, Massachusetts. The virus also was detected by enzyme-linked immunosorbent assay (ELISA) in young foliage but not in mature foliage, flowers, or seeds. ToMV also was detected by ELISA in leaves of young rooted Rutilant lilac cuttings, which displayed similar VLS, obtained from the Royal Botanical Garden, Hamilton, Ontario. Based upon host range, symptomatology, and serological tests, the virus was closely related to the dogwood strain of ToMV. The virus was mechanically transmitted to lilac cultivars Lutece and Royalty, in which no symptoms developed, and to white ash, in which foliar mosaic developed.

Several viruses have been identified in lilac (*Syringa* spp.) hosts (2,12,15,18,19). During a survey of mycoplasmal diseases in major collections of lilacs in the United States and Canada, foliar symptoms of mosaic and chlorotic mottle (Fig. 1) were observed on a specimen of *S. × nanceiana* McKelvey cv. Rutilant at the Arnold Arboretum, Jamaica Plain, Massachusetts. *S. × nanceiana* is an interspecific hybrid (*S. × henryi* Schneid. × *S. sweginzowii* Koehne & Lingelsh.) with landscape value as a late-blooming lilac. Because no virus had been reported for any cultivar of this lilac hybrid, we attempted to isolate and identify a virus from the symptomatic Rutilant lilac. This paper reports the identification of tomato mosaic tobamovirus (ToMV) in *S. × nanceiana* cv. Rutilant. Part of the findings have been reported in an abstract (3).

MATERIALS AND METHODS

Virus transmission from lilac. In August 1986, mature leaves with mosaic and chlorotic mottle and clusters of spindly shoots with young leaves (symptoms of mycoplasmalike organism [MLO] infection) were collected from a symptomatic Rutilant lilac located at the Arnold Arboretum. This lilac had previously been confirmed to be infected with MLOs (8), the cause of lilac witches'-broom (9). In preparation for transmission tests, both sets of leaves were triturated separately in 100 mM phosphate buffer (pH 7.1) containing 10 mM sodium diethyldithiocarbamate and

20 mM sodium thioglycollate (extraction buffer). Superfine Sephacryl S-300 (Pharmacia, Uppsala, Sweden) was added to the leaf extracts at 1:1 (v/v). The resulting slurries were vacuum-filtered in a Büchner funnel through Whatman No. 1 grade filter paper, and the filtrates were rubbed onto the leaves of the following carborundum-dusted indicator plants: *Chenopodium quinoa* Willd., *Cucumis sativus* L. cv. Chicago Pickling, *Vigna unguiculata* (L.) Walp. subsp. *unguiculata* cv. California Ramshorn, *Datura stramonium* L., *Nicotiana tabacum* L. cv. Xanthi-nc, *N. glutinosa* L., and *Physalis floridana* Rydb.

Two sets of leaves, as described above, were collected from the same Rutilant lilac in August 1987 and subjected separately to a second clarification scheme prior to transmission attempts. Coarse-grade Sephadex G-25 was added to the leaf extracts to achieve a thick slurry, which was centrifuged at 10,000 g for 10 min. The supernatant was separated on a 25-ml column packed with Controlled-Pore glass, GG-170-200 (Sigma Chemical, St. Louis, MO). One-ml fractions were collected and rubbed onto leaves of the indicator plants.

By a third clarification method, stem cuttings from the Rutilant lilac were collected in September 1988. Leaf buds were forced at 25 C, and the young, symptomless leaf tissue was triturated in the extraction buffer and centrifuged at low speed as above. The supernatant was separated on a 25-ml column packed with Sephadex G-25. Selected fractions were concentrated at 37,000 rpm for 3 hr in a Beckman Ti 60 rotor. The resulting pellet was resuspended in 10 mM phosphate buffer (pH 7.1) and rubbed onto the leaves of the indicator plants.

Transmission electron microscopy.

Virus-infected tobacco leaf tissue was triturated in 100 mM phosphate buffer (pH 7.0). Formvar-coated nickel grids were floated on drops of the extract for 5 min. After a rinse with distilled water, the grids were stained with 2% aqueous uranyl acetate (pH 4.0) and viewed with an RCA-EMU 4 transmission electron microscope. Upon virus purification, 50 particles were measured, and mean particle length determined.

Virus purification. The virus was purified from systemically infected Xanthi tobacco tissue according to the protocol of Gooding and Hebert (7). The virus was further purified by isopycnic density gradient centrifugation in cesium chloride (17).

Comparative host range determination. The following indicator plant species were inoculated with the lilac virus and the L (obtained from M. Zaitlin, Department of Plant Pathology, Cornell University, Ithaca, NY) and dogwood (DW) (obtained from B. Reddick, Department of Plant Pathology and Entomology, University of Tennessee, Knoxville) strains of ToMV by rubbing carborundum-dusted cotyledons or leaves with purified virus in 10 mM phosphate buffer (pH 7.1): *N. tabacum* cvs. Xanthi and Turkish, *N. glutinosa*, *Beta vulgaris* L., *Spinacia oleracea* L., *Lycopersicon esculentum* Mill. cvs. Burpeeana Early, Super Beefsteak, and Red Cherry, *C. quinoa*, *Pisum sativum* L., and *Helianthus annuus* L.

Serology. An antiserum to purified virus was prepared in female New Zealand White rabbits by administering four weekly subcutaneous and intramuscular injections of 1 mg/ml of purified virus in phosphate buffer mixed with an equal volume of Freund's complete adjuvant. Four weeks following the last injection an intravenous injection consisting of 0.5 mg of virus in 0.5 ml of phosphate buffer was administered into a marginal ear vein. One week later blood was collected and the serum fraction separated and stored at -20 C.

Agar gel double-diffusion tests (1) were conducted in 0.7% Noble agar containing 0.85% sodium chloride and 0.25% sodium azide. Plates were incubated for 48 hr at 37 C. The lilac virus and antiserum were tested against the following viruses and their antisera: tobacco mosaic virus (TMV) strain U1

and ToMV-DW (antiserum provided by B. Reddick). Antisera were undiluted, and virus antigen consisted of purified virus at 1 mg/ml in 0.85% sodium chloride.

Antiserum dilution endpoints against the lilac virus and ToMV-DW were determined by indirect enzyme-linked immunosorbent assay (ELISA) (13) for antisera to eight tobamoviruses: TMV-U1, ToMV-L (provided by M. Zaitlin), ToMV-DW, ribgrass mosaic virus from *Primula* sp., Holmes' ribgrass mosaic virus, tobacco mild green mosaic virus, bell pepper mottle virus (provided by C. Wetter, Universität des Saarlandes, Saarbrücken, Germany) and frangipani mosaic virus (provided by R. I. B. Francki, Waite Agricultural Research Institute, University of Adelaide, Australia).

ELISA of Rutilant tissue samples. In July 1991, leaves (see above), flowers, and seeds were collected from the same Rutilant lilac at the Arnold Arboretum. These tissues were indexed by direct double-antibody sandwich (DAS) ELISA (4) for the lilac virus.

In March 1991, we obtained five dormant, rooted cuttings of a Rutilant lilac from the Royal Botanical Garden, Hamilton, Ontario. When these cuttings were forced in April 1991, the foliage showed mosaic and chlorotic mottle (Fig. 2) similar to symptoms on Rutilant lilac at the Arnold Arboretum. Root and leaf tissues from these cuttings were tested for the lilac virus by direct DAS-ELISA in the summer of 1991.

Inoculation of lilac and white ash. We were unable to obtain virus-free Rutilant lilacs for inoculations with the lilac virus. Cuttings of Rutilant lilac from the Royal Botanical Garden were infected. This cultivar was unavailable from other lilac collections in North America and Europe (F. Vrugtman, Royal Botanical Garden, *personal communication*). Therefore, seedlings of the following lilac cultivars were inoculated with the purified lilac virus: *S. × henryi* cv. Lutece, *S. × josiflexa* cv. Royalty, *S. × prestoniae* McKelvey cvs. Coral and Maybelle Farnum, *S. villosa* Vahl, and *S. vulgaris* L. cvs. Charles Joly and Ellen Willmott. Seedlings of white ash (*Fraxinus americana* L.) in the same family (Oleaceae) also were inoculated with purified lilac virus. All lilac and ash plants used for inoculation studies were raised from seed and inoculated while very young. Therefore, because of their small size, the actual seedlings were not tested for the lilac virus prior to inoculation. However, a random infectivity bioassay of approximately 50 ash seedlings and 25 lilac seedlings did not reveal the presence of virus.

RESULTS

Virus transmission, electron microscopy, and purification. Necrotic local

lesions developed on Xanthi-nc tobacco inoculated with extracts from young Rutilant lilac leaf tissues prepared by all three methods. None of the other initial indicator species developed symptoms. Sap expressed from tobacco with lesions was infectious after passage through membrane filters with pore sizes of 0.22- and 0.45- μ m. No lesions were produced by preparations from mature symptomatic Rutilant lilac leaves.

Rigid, rod-shaped virus particles, representative of the tobamovirus group of plant viruses, were observed by transmission electron microscopy in leaf dip preparations of symptomatic Xanthi-nc

tissue. Measurement of 50 particles from a purified preparation of the virus gave a mean particle length of 325 ± 123 nm. End-to-end aggregation and half-length particles were common.

Approximately 275 mg of virus was purified from 100 g of systemically infected Xanthi tobacco leaf tissue. Following isopycnic density gradient centrifugation of 5-10 mg of purified virus the $A_{260/280}$ was 1.22, and the buoyant density was 1.324.

Host range and symptomatology. Symptoms of the lilac virus and the ToMV-L and ToMV-DW strains were identical on all of the herbaceous



Fig. 1. Viruslike symptoms on *Syringa × nanceiana* 'Rutilant' growing at the Arnold Arboretum in Jamaica Plain, Massachusetts.



Fig. 2. Foliar symptoms on forced Rutilant lilac cuttings obtained from the Royal Botanical Garden, Hamilton, Ontario.

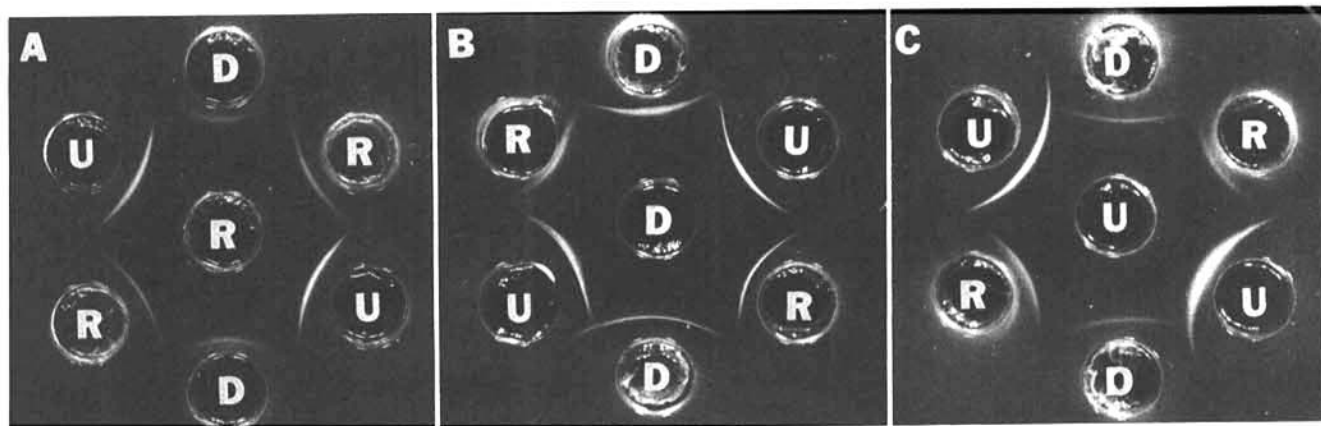


Fig. 3. Agar gel double-diffusion serology. Center wells contain undiluted antisera to (A) the lilac isolate of tomato mosaic virus (ToMV), (B) the dogwood isolate of ToMV, and (C) the U1 strain of tobacco mosaic virus (TMV). Virus antigen in outside wells: R = lilac virus, U = U1 strain of TMV, and D = dogwood isolate of ToMV.

Table 1. Reciprocal endpoint titers (mean of two determinations) of tobamovirus antisera reacted against purified virus from Rutilant lilac and an isolate of ToMV from dogwood in indirect enzyme-linked immunosorbent assay^a

Antiserum to:	Endpoint	
	ToMV from lilac	ToMV from dogwood
Tobacco mosaic virus strain U1 (1.5×10^6) ^b	6.2×10^4	1.2×10^4
Tomato mosaic virus from lilac	1.5×10^6	1.5×10^6
Tomato mosaic virus from dogwood	7.8×10^6	7.8×10^6
Tomato mosaic virus strain L (3.1×10^5)	3.1×10^5	3.1×10^5
Ribgrass mosaic virus from <i>Primula</i> sp.	2.5×10^3	5.0×10^2
Holmes' ribgrass mosaic virus	2.5×10^3	5.0×10^2
Tobacco mild green mosaic virus	2.5×10^3	2.5×10^3
Bell pepper mottle virus	1.2×10^4	1.2×10^4
Frangipani mosaic virus	2.5×10^3	2.5×10^3

^aPlate wells were coated with purified virus (1 μ g/ml, 150 μ l per well) in phosphate buffered saline (PBS) and incubated for 1 hr 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 hr 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 μ l per well). The plates were incubated for 1 hr at 37 C. After washing, goat anti-rabbit IgG conjugated to alkaline phosphatase was diluted to 1:1,000 in blocking buffer and added to the wells, and the plates were incubated for 1 hr at 37 C. Following the final rinse, substrate (p-nitrophenyl phosphate at 1 mg/ml) was added, and the absorbance at 405 nm was measured after a 1-hr incubation at 25 C.

^bNumbers in parentheses represent the endpoint titer of the homologous reaction.

indicator species tested. Symptoms were necrotic local lesions in *N. glutinosa*, chlorotic local lesions in *B. vulgaris*, chlorotic local lesions followed by systemic mottle in *C. quinoa*, and systemic mottle and mosaic in Turkish and Xanthi tobaccos and all three tomato cultivars. No symptoms were seen on *S. oleracea*, *H. annuus*, and *P. sativum*.

Serology. The microprecipitin titer of the antiserum was 1:1,000. In agar gel double-diffusion tests, the lilac virus and ToMV-DW reacted with identity against antiserum to each virus (Fig. 3A and B). Both ToMV-DW and the lilac virus produced spurs with TMV-U1 when reacted with dogwood and lilac virus antisera (Fig. 3A and B). With U1 virus antiserum, spur formation was formed with U1 antigen by both the lilac virus and ToMV-DW, but not to each other (Fig. 3C).

The results of indirect ELISA tests against nine tobamovirus antisera are presented in Table 1. The dogwood and

lilac viruses reacted with low but identical reciprocal endpoint titers to antisera of frangipani mosaic virus, bell pepper mottle virus, and tobacco mild green mosaic virus. One fivefold dilution step difference in endpoint titer was detected in reactions of the viruses with antisera to Holmes' ribgrass mosaic virus and ribgrass mosaic virus from *Primula* sp. Both viruses reacted with identical and high endpoint titers to each other's antiserum as well as antiserum to ToMV-L. Both the lilac and DW isolates of ToMV reacted to antiserum of TMV-U1 with a low endpoint titer (6.2×10^4 vs. 1.2×10^4 , respectively, Table 1). A fivefold difference in endpoint titer between the two viruses and U1 antiserum was observed.

Detection of virus in Rutilant lilac tissues by ELISA. ToMV was detected by ELISA in young leaves from spindly shoots but not in mature and symptomatic leaves or in flower and seed tissues obtained from the Arnold Arboretum

Rutilant lilac. The virus was also detected in the foliage, but not in the roots, of three of the five symptomatic Rutilant lilac cuttings obtained from the Royal Botanical Garden.

Infection of lilac cultivars and white ash. No leaf symptoms developed on any lilacs inoculated with the lilac virus, but the virus was mechanically transmitted to *N. glutinosa* from new leaves of Lutece and Royalty lilacs 6 and 18 mo post-inoculation, respectively. A mosaic developed on new leaves of inoculated ash, and the lilac virus was mechanically transmitted from symptomatic ash leaves to Xanthi-nc tobacco 6 mo postinoculation.

DISCUSSION

Because of its host range and symptomatology and results of agar gel double-diffusion and indirect ELISA, the virus transmitted from *S. × nanceiana* cv. Rutilant was identified as ToMV and is closely related to the dogwood isolate of ToMV (16). Several viruses have been reported in *S. vulgaris*, including lilac ring mottle virus (18), lilac chlorotic leafspot virus (2), elm mottle virus (12), arabis mosaic virus (15), tomato bushy stunt virus (15), and cherry leafroll virus (15). Lilac mottle virus was identified in *S. oblata* cv. *alba* Rehder (*S. oblata* cv. *affinis* (L. Henry) Lingelsh.) (19). Ours is the first report of a virus in the lilac hybrid *S. × nanceiana*.

The repeated transmissions of the virus from Rutilant lilac and detection of the virus by ELISA in Rutilant lilacs from two lilac collections indicate that this lilac cultivar is naturally infected with ToMV. Symptoms did not develop in lilacs infected with the lilac virus by mechanical inoculation. Apparently ToMV causes no foliar symptoms in the lilac cultivars that we tested, and ToMV infection of lilac may be latent, as in other woody hosts (14,16). ToMV was transmitted only from nonsymptomatic leaves of Rutilant lilac, and detection by DAS-ELISA was most often in nonsympto-

matic leaves.

We have not determined the geographical range of ToMV in Rutilant lilacs or the host range in other lilac taxa. However, like other tobamoviruses, the lilac isolate of ToMV has the potential of becoming widespread. In recent studies, ToMV was recovered from natural waters draining forested sites in central and northern New York State (10) and from red spruce (11) and symptomless dogwood trees (16). In addition, there is considerable exchange of lilac propagative material among collections within the United States and Canada, as well as between these sources and collections in Europe (Belgium, Czechoslovakia, Denmark, France, Germany, Hungary, Netherlands, Norway, Poland, United Kingdom, and the former Soviet Union) and Asia (Japan and Korea) (F. Vrugtman, *personal communication*). Indeed, the infected Rutilant lilac at the Arnold Arboretum originated as an import in 1932 from Victor Lemoine and Sons, Nancy, France (J. Alexander, Arnold Arboretum, *personal communication*). As far as we can determine, the Arnold Arboretum is the sole source of Rutilant lilac in present lilac collections in North America. In addition, if the virus is symptomless in many other lilac cultivars, it will be impossible to detect by visual inspection alone. The standard methods for propagating lilac cultivars have been by grafting and softwood cuttings (6). Tissue culture is rapidly

becoming the method of choice for commercial propagators of lilacs and the principal source of own-root lilacs for the home gardener (5). The standard methodology for lilacs is to culture explants of actively growing shoot tips on growth-promoting media and to use the resulting proliferation of shoots for the production of plantlets (5). Explants derived from ToMV-infected lilacs may allow for the rapid dissemination of diseased plants.

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