# Infection of Cultivated Gesneriads by Two Strains of Tobacco Mosaic Virus

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**ABSTRACT**

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The $U_1$ and $U_2$ strains of tobacco mosaic virus (TMV) were implicated as being widespread in cultivated gesneriads (family Gesneriaceae: *Achimenes, Aeschynanthus, Chirita, Codonanthe, Episcia, Gloxinia, Kohleria, Nematanthus, Streptocarpus, Smithiantha,* and *Sinningia* spp.). Infected plants were found in all six collections surveyed but not in 73 herbarium specimens representing plants collected from their native habitats in 16 South and Central American countries. Foliage symptoms of most infected plants were inconspicuous. Based on serological results, the $U_2$ strain was much more common than the $U_1$ strain (83 and 17% of 133 infected plants, respectively). In addition to the rigid rod-shaped TMV particles, flexuous rods with a modal length of 486 nm were seen in negatively stained leaf extracts of *Smithiantha* but not in any of the other gesneriads examined.

Additional key words: × *Achimenantha,* × *Codonatanthus,* × *Coltrichantha*

The family Gesneriaceae consists of about 126 genera (as defined by H. Wiehler, *unpublished*) and more than 2,000 species, many of which are popular flowering or foliage ornamentals such as *Saintpaulia* (African violet), *Sinningia* (florist's gloxinia), *Streptocarpus, Columnea,* and *Episcia*. Viral diseases of gesneriads have received relatively little attention. Previous reports of tobacco mosaic virus (TMV) infecting these plants are sporadic and appear to be limited to the genera *Achimenes* (6), *Gesneria* (13), *Kohleria* (12), and *Sinningia* (5). In addition, an unidentified viroid (9) infects *Columnea*, a bacilliform virus infects *Saintpaulia* (4), and *Sinningia* is susceptible to tomato spotted wilt (8), tomato ringspot (2), dahlia ringspot (7) viruses.

This study identifies two strains of TMV infecting various cultivated gesneriads and assesses their incidence and potential significance as pathogens of these plants. A preliminary report of this work was published previously (21).

## MATERIALS AND METHODS

**Sampling.** Leaf tissues of cultivated plants were obtained for testing through cooperators in California, Connecticut, Florida, and Ohio. Noncultivated gesneriads collected from their native habitats were herbarium specimens of the Department of Botany, Smithsonian Institution, Washington, DC. These samples represent specimens collected from their native habitats in the following countries (numbers in parentheses are the number of specimens sampled): Argentina (1), Bolivia (1), Brazil (12), British Honduras (1), Colombia (4), Costa Rica (3), Ecuador (1), Guatemala (6), Guyana (1), Honduras (2), Jamaica (1), Mexico (16), Nicaragua (1), Panama (17), Peru (4), and Venezuela (2).

**Serotesting and purification of immunogens.** Samples were tested serologically by the Ouchterlony agar double-diffusion method using a medium consisting of 0.8% Noble agar, 1.0% $NaN_3$, and 0.5% sodium dodecyl sulfate (SDS) and antiserum to SDS-dissociated virus (10). Antigens from living specimens were usually prepared by extracting leaf sap with a garlic press and diluting it 1:2 (w/v) with either deionized water or 1.5% SDS. Dried leaves of herbarium specimens were cut with a paper punch (17), soaked either in water or 1.5% SDS, and placed into antigen wells (about three disks per well). As a control, leaf tissue of Oneidan *Columnea* and *Achimenes* infected, respectively, with a $U_2$ strain isolated during this study from Oneidan *Columnea* (TMV-C) and an isolate of the $U_1$ strain of TMV were processed for herbarium storage and later returned to Gainesville for serological assay. These samples reacted strongly in immunodiffusion tests against the respective antisera.

The TMV immunogens used for routine serological tests were single isolates of the $U_1$ (common) strain provided by C. Wetter (Botany Department, Universität des Saarlandes, Saarbrücken, West Germany) and TMV-C. Other TMV antisera used for comparisons were: 1) degraded capsid protein of American Type Culture Collection isolate of PV-135 of the $U_1$ strain (provided by D. E. Purcifull and E. Hiebert, Plant Pathology Department, University of Florida, Gainesville), 2) $U_1$, $U_2$, and Dahlemense strains of TMV (provided by C. Wetter), and 3) the Reben isolate of the $U_1$ strain studied by Bercks (1) (provided by R. Koenig, Institut für Viruskrankheiten der Pflanzen, Braunschweig, West Germany). Antigens homologous to all antisera except the Reben isolate were included for comparison in this study.

The TMV-C and $U_1$ isolates compared in this study were purified from systemically infected *Nicotiana tabacum* 'Samsun Turkish' leaves by 1) clarification in chloroform and *n*-butanol, 2) two precipitations using, respectively, 4 and 8% polyethylene glycol 6000 (final concentration), and 3) differential centrifugation. The final pellets were resuspended in water and lyophilized. Just before each immunization, 5 mg of lyophilized virus was resuspended in 1 ml of 1% SDS containing 20 µl 2-mercaptoethanol and heated for 4 min in a boiling water bath to degrade the virions (11).

Immunizations consisted of three injections of SDS-treated purified capsid protein administered intramuscularly into thighs of rabbits at weekly intervals. Beginning 1 wk after the last injection, serum was collected weekly for several months. For each initial injection, the immunogen was emulsified with an equal volume of Freund's complete adjuvant, whereas incomplete adjuvant was used in all subsequent injections.

Antisera and antigens were titered in immunodiffusion tests using a twofold dilution series. Antisera were diluted in normal serum and antigens (TMV-C and $U_1$) were purified preparations at concentrations of 1 mg/ml (70 µl/antigen well) (10).

In one experiment involving *Smithiantha*, antisera and homologous antigens of certain potexviruses were used. These were papaya mosaic, clover yellow mosaic, and potato X viruses (provided by D. E. Purcifull), foxtail mosaic virus (provided by A. Q. Paulsen, Division of Biology, Kansas State University, Manhattan 66506), cymbidium mosaic virus (17), and nandina mosaic virus (19).

**Electron microscopy.** For observations

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of virus particles, leaf extracts were negatively stained in 2% aqueous uranyl acetate and examined with a Hitachi model H-600 electron microscope. Particles were measured by using a diffraction grating with linear spacing of 462.9 nm or with TMV particles as standards.

## RESULTS

Reciprocal reactions of identity were noted in immunodiffusion tests between all combinations of $U_1$ antisera and antigens tested. In similar reciprocal tests, TMV-C and Wetter's $U_2$ antisera reacted homologously against either the $U_2$ or TMV-C isolate. A similar reaction of identity between the $U_2$ and the TMV-C isolate against $U_2$ antiserum was noted in immunodiffusion tests by Wetter (*personal communication*).

When any of the $U_1$ antisera were tested, homologous precipitin lines spurred over those formed against antigens to either the TMV-C or Wetter's $U_2$ isolate. Conversely, when antisera to either the Wetter's $U_2$ or TMV-C isolates were tested, homologous reaction lines spurred over those of each of the $U_1$ isolates (Fig. 1). No cross-reaction lines were seen between the $U_2$ or TMV-C isolate and Bercks' Reben ($U_1$) antiserum, however (Fig. 1). Although antiserum to the Dahlemense strain reacted homologously in immunodiffusion tests, it reacted very weakly, if at all, to TMV-C, $U_2$, or any of the $U_1$ isolates tested. No reactions between normal serum and any of the antigens were seen (Fig. 1).

In immunodiffusion tests, the TMV-C and $U_1$ antisera used each had respective maximum homologous titers of at least 1/16 and heterologous titers of at least 1/8. Although strong homologous reactions were noted for each of the potexviruses tested serologically, no reactions between any of the antisera and *Smithiantha* leaf tissue were seen.

Tobacco mosaic virus was detected in all six collections of cultivated gesneriads surveyed; infection rates ranged from about 11 to 81%. In contrast, none of the 73 herbarium specimens collected from their native habitats were infected (Table 1).

Among the cultivated gesneriads, TMV was detected in 14 of 20 genera surveyed, including 10 not previously reported (Table 1). Based on comparative serological assay results, the TMV-C strain was much more prevalent than the $U_1$ strain (84 and 16%, respectively, of 116 samples) in all genera except *Achimenes* (Table 2). Whereas high virus incidences were recorded for the genera *Achimenes, Codonanthe, Columnea, Kohleria,* and *Smithiantha* (31–89%), they were much lower for *Aeschynanthus, Episcia,* and *Nematanthus* (2–8%).

There was agreement between serological and electron microscopic assays. All 39 samples determined by electron microscopy to be infected also reacted positively in serological tests. Likewise, virus particles were not seen in 56 leaf extracts of specimens determined healthy based on serological results.

Of 228 particles measured in Oneidan *Columnea* leaf extracts, 69% were 284–306 nm long, with a modal length of 298 nm. These particles were rigid in outline (Fig. 2). Similar rigid particles also were detected in extracts of other gesneriads as follows (the ratios in parentheses are the number of specimens with particles to number examined): *Columnea* (23/29), *Codonanthe* (2/4), *Episcia* (2/4), *Gloxinia* (1/1), *Kohleria* (2/4), *Nematanthus* (2/13), *Sinningia* (1/3), *Smithiantha* (5/6), and *Streptocarpus* (1/2).

A second type of particle, a flexuous rod, was also seen in four of the nine *Smithiantha* samples examined (Fig. 2B): Capistrano, *S. fulgida*, Santa Clara, and Vespers. Of 63 particles measured, 79% were between 450 and 510 nm long, with a

**Table 1.** Incidence of tobacco mosaic virus (TMV) in cultivated and wild Gesneriaceae

| Genus | Grower[a] | | | | | | Wild[b] |
|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | |
| *Achimenes* | 2/2 | 19/26 | – | – | – | – | 0/23 |
| *Codonanthe**[c] | 0/1 | 6/11 | 3/16 | – | – | – | 0/5 |
| *Columnea** | 16/42 | 23/97 | – | 10/17 | – | 1/5 | 0/16 |
| *Episcia** | 0/38 | 0/43 | – | 2/2 | – | – | 0/5 |
| *Gloxinia* | 0/1 | – | – | 1/1 | 1/1 | – | 0/5 |
| *Kohleria* | – | 7/13 | – | – | 1/1 | 3/4 | 0/6 |
| *Nematanthus** | 2/19 | 1/31 | 1/19 | 0/1 | – | – | 0/5 |
| *Sinningia* | 1/3 | – | – | – | 2/4 | – | 0/5 |
| *Smithiantha** | – | – | – | – | 8/9 | – | 0/3 |
| Miscellaneous[d] | 4/40 | 6/83 | – | 0/1 | 1/1 | – | – |

[a] Ratios are number of infected plants per total number tested; – = no samples tested. Results are based on serological and/or electron microscopy assays.
[b] All samples represent herbarium specimens.
[c] * = Not previously reported as susceptible to TMV.
[d] This category includes the following: *Aeschynanthus** 5/64, *Streptocarpus** 3/22, *Eucodonia* 0/3, *Niphaea* 0/1, *Chirita** 1/2, *Chrysothemis* 0/1, *Saintpaulia* 0/16, *Drymonia* 0/6, × *Achimenantha* 1/3, × *Coltrichantha** 1/2, and × *Codonatanthus* 0/5.

**Table 2.** Relative incidence of the $U_1$ and $U_2$ strains of tobacco mosaic virus in cultivated gesneriads

| Genus | No. plants infected[a] | | Total no. samples | Total infection (%) |
|---|---|---|---|---|
| | $U_1$ | $U_2$ | | |
| *Achimenes* | 12 | 8 | 28 | 71 |
| *Aeschynanthus* | 0 | 6 | 64 | 9 |
| *Chirita* | 0 | 1 | 2 | 50 |
| *Codonanthe* | 1 | 7 | 28 | 29 |
| *Columnea* | 4 | 46 | 160 | 31 |
| *Episcia* | 0 | 2 | 83 | 2 |
| *Gloxinia* | 0 | 1 | 3 | 33 |
| *Kohleria* | 2 | 6 | 18 | 44 |
| *Nematanthus* | 0 | 4 | 70 | 6 |
| *Sinningia* | 0 | 3 | 7 | 43 |
| *Streptocarpus* | 0 | 3 | 22 | 14 |
| *Smithiantha* | 0 | 8 | 9 | 89 |
| Hybrids[b] | 0 | 2 | 10 | 20 |
| Others[c] | 0 | 0 | 27 | 0 |

[a] All results based on serological assays.
[b] Intergeneric hybrids (ratios are number of infected per total number tested): × *Achimenantha* 1/3, × *Coltrichantha* 1/2, and × *Codonatanthus* 0/5.
[c] Numbers in parentheses are total number of specimens assayed: *Chrysothemis* (1), *Eucodonia* (3), *Drymonia* (6), *Niphaea* (1), and *Saintpaulia* (16).

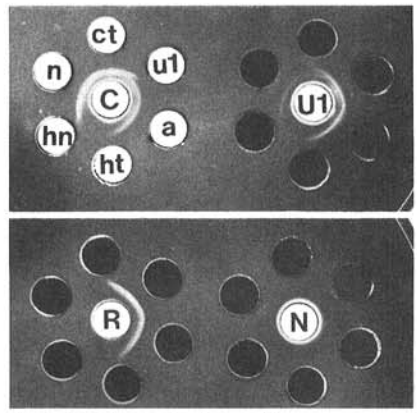


**Fig. 1.** Serological evidence for occurrence of two TMV strains in cultivated gesneriads. Center wells contained antisera to: the TMV-C isolate from Oneidan *Columnea* (C), normal serum (N), and $U_1$ isolates from tobacco (U1) and Reben (R); antisera C and U1 were to immunogens degraded by treatment with sodium dodecyl sulfate and boiling. Peripheral wells were the same for all sets and contained antigens to the $U_1$ strain in *Achimenes* (a) and tobacco (ul) leaf extracts and to the TMV-C strain in tobacco (ct) and *Nematanthus* (n) leaf extracts. Peripheral wells hn and ht contained leaf extracts of healthy *Nematanthus* and tobacco, respectively.

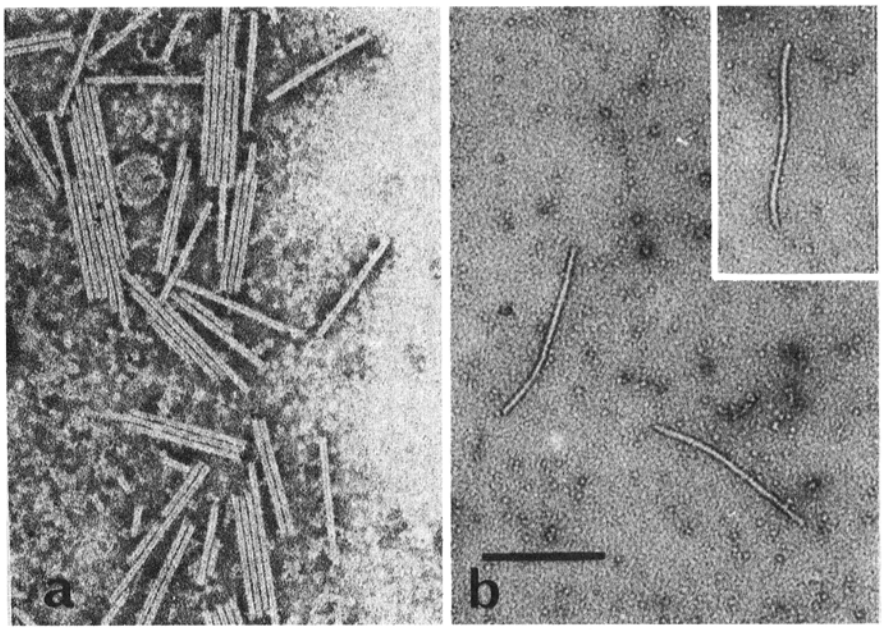


**Fig. 2.** Negatively stained virus particles in leaf extracts of (A) TMV-infected Oneidan *Columnea* and (B) *Smithiantha* infected with an unidentified flexuous-rod virus. Scale bar = 300 nm.

main maximum length of 486 nm. Five particles, assumed to be dimers, were 950–1,030 nm long. Such particles were not seen in extracts of any of the other gesneriads examined, however.

## DISCUSSION

Infection by TMV appears to be widespread in gesneriads cultivated in the United States. Diseased plants were detected in all collections from four states and in 14 of the 20 genera represented. None of the 16 *Saintpaulia* specimens were infected; based on previous studies involving inoculation attempts with TMV (3,5,14), it is possible that this genus differs by being resistant.

Two TMV strains were detected during this survey. The $U_1$ strain predominated only in the genus *Achimenes*; the $U_2$ strain was more common in all the other genera. Based on reciprocal reactions of identity, the second strain appears identical to $U_2$ strains studied by Wetter and Bernard (15,16). The isolate described by Koenig and Lesemann (6) infecting *Achimenes* appears to be $U_1$, based on a strong serological relationship with Bercks' Reben isolate (R. Koenig, *personal communication*). In our work, Bercks' Reben antiserum reacted homologously against $U_1$ antigens from other sources. No evidence for strains other than $U_1$ or $U_2$ was found in this study.

Because both the $U_1$ and $U_2$ strains occur naturally in tobacco (15,16) and because none of the wild gesneriads were infected, it is probable that some commercial tobacco product was the primary source of TMV inoculum for cultivated gesneriads. Once the virus is in these plants, secondary spread could be facilitated because most gesneriads are vegetatively propagated and TMV is exceptionally stable and easily transmitted manually. Moreover, the inconspicuous foliage symptoms would make it difficult for growers to detect and rogue infected plants. Thus, in many respects, TMV incidence in cultivated and noncultivated gesneriads closely parallels that previously noted for orchids, which are susceptible to the odontoglossum ringspot and cymbidium mosaic viruses (17,18,20). Although the economic damage caused by TMV in cultivated gesneriads is not yet known, growers have recently complained of declining productivity in such genera as *Columnea*. Based on our study, it seems possible that TMV is responsible for this decline.

The flexuous rod-shaped virus noted exclusively in *Smithiantha* has not been identified or characterized, but the particle length is of the same magnitude as a potexvirus. Whether or not other gesneriads are also susceptible to this virus is not known.

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