

Tomato Mosaic Virus Associated with Shoestring Symptom in Chilean Tomatoes

O. ANDRADE, B. A. LATORRE, and O. ESCAFFI, Departamento de Sanidad Vegetal, Facultad de Agronomía, Universidad de Chile, Casilla 1004, Santiago

ABSTRACT

Andrade, O., Latorre, B. A., and Escaffi, O. 1981. Tomato mosaic virus associated with shoestring symptom in Chilean tomatoes. *Plant Disease* 65:761-762.

Severe outbreaks of tomato shoestring, varying in incidence from 5 to 42%, occurred in commercial tomato plantings in Chile. The causal agent was identified as tomato mosaic virus based on biological, physical, morphological, and serologic properties. Cucumber mosaic virus was not found in any of five field samples assayed.

Shoestring and fern-leaf symptoms in tomatoes (*Lycopersicon esculentum* Mill.) have been associated around the world with either cucumber mosaic virus (CMV) or tomato mosaic virus (ToMV) (1-4,8). Shoestring symptoms affecting 5-42% of the plants in individual fields were observed during 1979 and 1980 in northern Chile. The disease was found in fresh-market and processing tomatoes grown under greenhouse and field conditions. Leaves of affected plants were severely distorted and generally reduced to tendril-like structures (Fig. 1A). A mild mosaic was observed occasionally on diseased plants in the field. We report the characterization of the causal agent of the shoestring disease.

MATERIALS AND METHODS

Diseased leaves were collected from five fields during 1979-1980. Field samples 1 and 4 were tomato cultivar VF 6718, samples 2 and 3 were Earlypak, and sample 5 was Cal-Ace.

The agent was isolated from each sample by mechanical transmission to tomato cultivar Euromande. Unless stated otherwise, inoculum was prepared by grinding affected leaves in 0.05 M potassium phosphate buffer, pH 7.0 (4 g of tissue to 1 ml of buffer), and was applied to first true leaves that had been dusted with 400-mesh Carborundum. Plants were maintained in the greenhouse

as sources of inoculum during this study.

Tomato cultivars Ace 55 VF, Cal-Ace, Cal-J, Earlypak, Euromande, Extase, VF 6718, Limachino, Pakmor, Petomeech II, Ronita, Curabel, Florida MH-1, and Tropic were mechanically inoculated with each field isolate under greenhouse conditions. Indicator hosts *Cucumis melo* 'Honey Dew'; *C. sativus* 'National Pickling'; *Chenopodium amaranticolor*; *Datura stramonium*; *Nicotiana glutinosa*; *N. tabacum* 'Burley 21', 'Coker 86', 'Coker 347', and 'NC 95'; *Petunia hybrida*; and *Vigna sinensis* 'Black Eye' were mechanically inoculated.

Aphid transmissibility of field isolates 1 and 2 was tested with *Myzus persicae* reared on *Raphanus sylvestris*. Groups of 10 apterous adults were starved for 30 min, then given a 30-sec acquisition access on diseased tomato leaves and 30-min inoculation periods on cotyledons of healthy National Pickling cucumber seedlings.

Longevity in vitro was determined at 20 C with viral extracts prepared in phosphate buffer, pH 7.0, and maintained in sealed tubes until tested for infectivity every 10 days on *N. glutinosa* and Euromande tomato. The dilution end point and thermal inactivation point in crude extract were determined on *N. glutinosa*.

Diseased leaf tissues from each of the five field samples were crushed in 2% neutral phosphotungstic acid and examined in a Philips EM-300 electron microscope on collodion-coated grids. Leaf tissue sections (1 × 2 mm) from field sample 1 were fixed in 3% glutaraldehyde in phosphate buffer, pH 6.8, for 2 hr; rinsed; and postfixated overnight in 2% osmium tetroxide in the same buffer. After dehydration in acetone, samples were infiltrated in epoxy resins (11), and ultrathin sections were cut with a diamond knife mounted on a Porter Blum ultramicrotome. Sections were stained in uranyl acetate and lead citrate and examined in the electron microscope.

Virus from field sample 1 was purified from 65 g of systemically infected tomato

leaves (6). Infectivity of the purified extract was determined on *N. glutinosa* and Euromande tomato.

Virus extracts from each of the five field samples were tested for serologic relationships to ToMV, tobacco mosaic virus (TMV), and CMV with the double immunodiffusion technique as described for tobacco viruses (5).

RESULTS

All tomato cultivars except Curabel developed mild to severe mosaic symptoms followed by severe leaf distortion and stunting of the plants (Fig. 1A). Curabel contains the Tm-2² gene (3,9) and was immune to all five virus isolates.

The virus produced local lesions on *Chenopodium amaranticolor*, *D. stramonium*, *N. glutinosa*, *N. tabacum*, and *P. hybrida*. Both local lesions and systemic reactions occurred in Coker 347 tobacco. No symptoms were observed on *C. melo*, *C. sativus*, or *V. sinensis* with any of the five samples.

Isolates from all five inoculum sources showed longevity in vitro of more than 70 days, a dilution end point between 10⁻⁶ and 10⁻⁸, and a thermal inactivation point between 82 and 86 C.

Rod-shaped particles were easily detected in leaf dip preparations from diseased tomato plants. Modal lengths of particles ranged from 280 to 320 nm and widths from 16 to 20 nm (Fig. 1B). Ultrathin sections of mesophyll cells contained crystalline plates and X-bodies (Fig. 1C). Crystalline plates varied in size and consisted of rows of virus particles arranged in parallel arrays. X-bodies were elongated and vacuolated (Fig. 1C). No isometric viruslike particles were seen.

All attempts to transmit the virus from diseased tomatoes to cucumber by aphids were unsuccessful.

Double-diffusion tests with antiserum against CMV were negative. Patterns of total identity were obtained with antisera against ToMV and TMV.

The virus was readily purified from diseased tomato plants. Ultraviolet absorbance spectra of purified virus preparations were typical of a rod-shaped virus with a maximum at 260 nm, a minimum at 248 nm, and an A₂₆₀/A₂₈₀ ratio of 1.23. Purified preparations induced local lesions on *N. glutinosa* and systemic infection on Euromande tomatoes; shoestring symptoms developed within 15 days on the latter.

Portion of an Ingeniero Agrónomo thesis submitted to the Universidad de Chile by the senior author. Supported in part by Servicio de Desarrollo Científico, Artístico y de Cooperación Internacional, Universidad de Chile, Proyecto A1032-801. Present address of second author: Facultad de Agronomía, Pontificia Universidad Católica, Casilla 114-D, Santiago, Chile.

Accepted for publication 20 June 1981.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

0191-2917/81/09076102/\$03.00/0
©1981 American Phytopathological Society

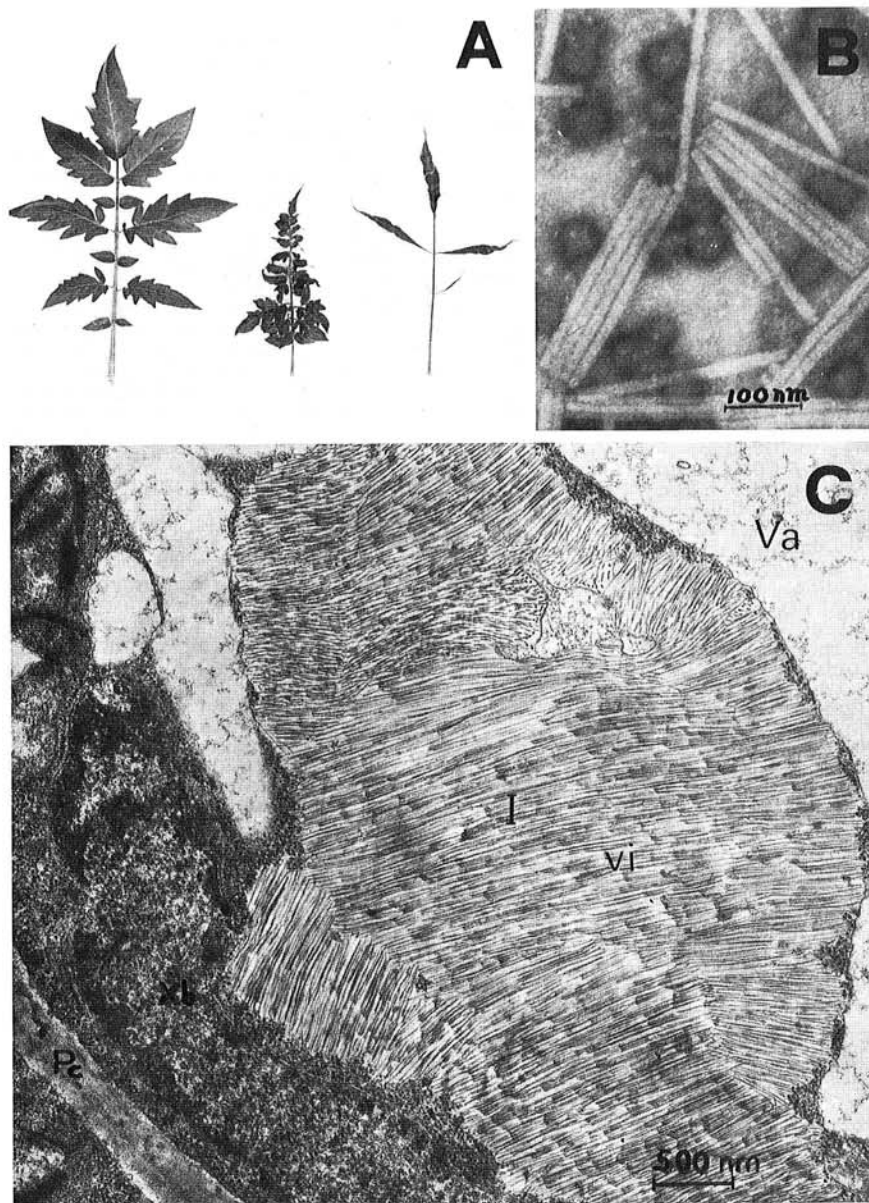


Fig. 1. (A) Diseased and healthy (left) Euromande tomato leaves. (B) Rod-shaped particles in leaf dip preparations from tomatoes with shoestring symptoms. (C) Ultrathin section of a diseased leaf, showing inclusion bodies (I) of parallel virus particles (vi), cell wall (Pc), vacuole (Va), and X-bodies (Xb).

DISCUSSION

The agent causing the shoestring symptoms on tomatoes was identified as ToMV on the basis of biological, physical, morphological, and serologic properties. Several reports (1,4,10) have attributed shoestring symptoms to CMV; however, we found no evidence of this virus in diseased samples. Tomato plants

mechanically inoculated with crude extracts heated at 86 C, and plants inoculated with purified viral extract always developed leaf distortion varying from mild fern-leaf appearance to the severe shoestring symptom.

The negative results obtained on Curabel tomato, which is immune to ToMV but susceptible to CMV, further

support our belief that ToMV was responsible for the symptoms observed. Most isolates of ToMV cause local lesions on tobacco cultivars, such as Coker 347, without systemic infection. Our isolates caused both local lesions and systemic infections. This reaction, and the serologic tests, where apparent reactions of identity were obtained with both ToMV and common TMV, could indicate a mixture of these two strains in our samples. However, we believe that only ToMV was involved, because 1) the immunodiffusion tests we conducted would not necessarily differentiate ToMV and common TMV, 2) strains of ToMV have been reported that cause both local lesions and systemic symptoms on tobacco (8), and 3) our isolates differed from a reference isolate of common TMV in their behavior on *P. hybrida* and tobacco cultivars Coker 347 and NC 95 (7). Further work is needed to clarify this point, however.

ACKNOWLEDGMENT

We thank G. V. Gooding, Jr., North Carolina State University, Raleigh, for providing the antisera and virus samples.

LITERATURE CITED

1. Barksdale, T., Good, J., and Danielson, L. 1972. Tomato diseases and their control. U.S. Dep. Agric., Agric. Handb. 203. 109 pp.
2. Chupp, C., and Sherf, A. 1960. Vegetable Diseases and Their Control. Ronald Press, New York. 693 pp.
3. Fletcher, J., and Griffin, M. J., eds. 1980. Control of diseases of protected crops. Tomatoes. Agric. Dev. Advis. Serv., Ministry of Agriculture, Fisheries and Food, England. Booklet 2243.
4. Gibbs, A. J., and Harrison, B. D. 1970. Cucumber mosaic virus. Descriptions of Plant Viruses, No. 1. Assoc. Appl. Biol./Commonw. Mycol. Inst., Kew, Surrey, England. 4 pp.
5. Gooding, G. V., Jr. 1975. Serological identification of tobacco viruses. Tob. Sci. 29:135-139.
6. Gooding, G. V., Jr., and Hebert, T. T. 1967. A simple technique for purification of tobacco mosaic virus in large quantities. Phytopathology 57:1285.
7. Gooding, G. V., Jr., and Suggs, E. G. 1976. Seedborne tobacco mosaic virus in commercial sources of tomato seed. Plant Dis. Rep. 60:441-442.
8. Hollings, M., and Huttinga, H. 1976. Tomato mosaic virus. Descriptions of Plant Viruses, No. 156. Assoc. Appl. Biol./Commonw. Mycol. Inst., Kew, Surrey, England. 6 pp.
9. Pelham, J. 1972. Strain-genotype interaction of TMV in tomato. Ann. Appl. Biol. 71:219-228.
10. Smith, K. M. 1972. A Textbook of Plant Virus Diseases. 3rd ed. Academic Press, New York. 684 pp.
11. Spurr, A. R. 1969. A low viscosity epoxy-resin embedding medium for electron microscopy. Gen. Ultrastruct. Res. 26:31-43.