# Tomato Scorch, a New Virus Disease of Tomatoes

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Joint contribution of Agricultural Research, Science and Education Administration, U. S. Department of Agriculture, and the Iowa Agriculture and Home Economics Experiment Station. Journal Series Paper J-9386 and Projects 1018 and 2135 of the latter.

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The authors thank Ray Guthrie, Montana State University, for a gift of antiserum against potato virus X; Dan Purcifull, University of Florida, for gifts of antiserum against potato virus Y and the G. Gooding isolate of potato virus Y; and Barbara Stitt Lucas for performing aphid transmission tests. The microscopy for this work was done in the Bessey Microscopy Facility, Department of Botany and Plant Pathology, Iowa State University.

Accepted for publication 24 August 1979.

## ABSTRACT

CLARK, R. L., J. H. HILL, and M. D. ELLIS. 1980. Tomato scorch, a new virus disease of tomatoes. Phytopathology 70:131-134.

A new virus disease of tomato, with symptoms characterized by mosaic, epinasty, and terminal necrosis of branches and leaflets, was found to be caused by coinfection of plants by tobacco mosaic and potato Y viruses. Identification of the viruses was based on host range, serology, electron microscopy, physical properties, and aphid transmission.

*Additional key words:* epidemiology, plant introductions, *Lycopersicon pimpinellifolium*, water stress.

In early July 1977, a previously undescribed disease began to develop on tomato plants in the seed increase plantings at the North Central Regional Plant Introduction Station at Ames, Iowa. By late July, most plants in the 6-ha field were affected. Initial observations of affected plants led us to suspect the disease was double virus streak, caused by the coinfection of tomato plants by tobacco mosaic virus (TMV) and potato virus X (PVX) (2,9). Symptoms consisted of necrotic areas on stems and leaves which progressed to death of individual branch tips and often the entire plant. These symptoms led us to propose the name "tomato scorch" for this disease (3).

Before necrosis appeared, individual leaflets had chlorotic mottle and epinasty and the entire plant was stunted. The lack of fruit symptoms as well as the different leaf and stem symptoms seemed to preclude the tip blight disease (8). The lack of purple striations on stems and the presence of stem necrosis suggested that the disease was different from that described in Florida by Conover and Fulton (4). Furthermore, no fungal or bacterial pathogens could be observed under the light microscope or isolated on agar media.

Electron microscopic observations indicated the presence of two distinct kinds of rod-shaped viruslike particles in leaflets from diseased plants; none was seen in leaflets from healthy plants. The particle length for the longer rod did not match that reported for PVX.

This paper reports the results of our tests to determine the identity of the viruses involved and to establish their role in the production of the disease syndrome.

## MATERIALS AND METHODS

Initial inoculations from diseased tomato plants collected from the field were made to greenhouse-grown *Lycopersicon esculentum* Mill. 'Rutgers'. Infected tissue was ground in 0.01 M potassium phosphate, pH 7.0, with mortar and pestle, and the sap was rubbed on plants previously dusted with 1,524 gr/cm Carborundum. Long, flexuous rod-shaped particles were separated from the particles resembling tobacco mosaic virus (TMV) in the greenhouse-inoculated plants by aphid transmission. Apterous *Myzus persicae* Sulz. were starved for 2 hr in petri dishes lined with moist filter paper. Aphids were transferred with a camel's-hair brush to diseased Rutgers tomato plants and allowed to feed for 1 min. The aphids then were transferred to healthy Rutgers tomato plants, which were caged for 24 hr. Plants were sprayed with nicotine sulfate and placed on the greenhouse bench for symptom development. The TMV-type particles were separated from the flexuous rod-shaped particles by mechanical inoculation of sap

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from diseased tomatoes to *Datura stramonium* L.

Inoculations for host-range studies were made as previously described for tomato. Attempts were made to recover virus from all inoculated plants to either *Chenopodium amaranticolor* Coste and Reynier, or *Physalis floridana* Rydb., or to both.

Thermal inactivation point (TIP) and dilution end point (DEP) were determined for the flexuous virus from inoculated leaves of Rutgers tomato and *Nicotiana glutinosa* L. Infectious sap was prepared by grinding tissue in 0.01 M potassium phosphate, pH 7.0 (1 g of tissue/2ml) and squeezing the homogenate through two layers of cheesecloth. For DEP, sap was diluted in the same buffer, and for longevity in vitro (LIV) it was maintained at room temperature. For TIP, sap was heated for 10 min and then rapidly cooled in an ice bath. Immediately after treatment, infectivity was assayed on leaves of *C. amaranticolor, P. floridana*, or both.

Leaf-dip preparations of virus from plants infected with one virus or both were stained with 2% potassium phosphotungstate, pH 6.5, and observed with the Hitachi HU 11C electron microscope. The microscope was calibrated by use of a carbon diffraction grating with 2,160 lines/mm.

**Fig. 1.** Rutgers tomato plants mechanically inoculated with sap from tomato scorch diseased plants in the field. Note flagging of leaflets and entire leaves.

Samples for microprecipitin tests were prepared by grinding infected tissue in 0.05 M sodium borate, pH 7.2, with a mortar and pestle. The sample was squeezed through two layers of cheesecloth and centrifuged for 10 min at 3,090 *g* and used for tests as previously described (1). Alternatively, double-diffusion tests were done in 0.9% Noble agar prepared in 0.05 M sodium borate, pH 7.2, containing 0.1% $NaN_3$ and 0.85% NaCl or in immunodiffusion media containing sodium dodecyl sulfate (SDS), as described by Purcifull and Batchelor (10). Antigens for double-diffusion tests were prepared from infected tissue as described for the microprecipitin tests. In the Ouchterlony test with PVX antiserum, the sample was treated with an equal volume of 5% pyrrolidine in borate buffer (11). Antigens used in diffusion tests involving SDS were prepared from infected tissue as described previously (10).

## RESULTS

**Particle morphology.** Leaflets from tomato plants with mosaic, epinasty, and necrosis (Fig. 1) were used to prepare leaf dips. Examination of these preparations under the electron microscope revealed two morphologically distinct kinds of particles (Fig. 2). One was a stiff rod with a modal length of 293 nm (Fig. 3) and width of 15 nm, morphologically similar to viruses in the tobamovirus group (6). The other particle, with a modal length of 746 nm (Fig. 3) and width of 11 nm, was morphologically similar to viruses in the potyvirus group (6).

**Separation of viruses.** Viruses in the potyvirus group often are aphid transmitted and those in the tobamovirus group are not (6); we separated the flexuous rod-shaped particle (potyvirus) from the stiff rod (tobamovirus) by transmission with *M. persicae*. Isolation of the potyvirus enabled further study. Host-range studies (Table 1) indicated that *D. stramonium* was not a host of the potyvirus; upon the supposition that the tobamovirus was TMV, *D. stramonium* was inoculated with sap from tomato plants containing both particle types. Leaves that developed local lesions caused by the tobamovirus were used for inoculation back to tomato. Electron-microscope and indicator-plant studies showed that the two viruses had been separated. When they were combined to again coinfect tomato plants, a disease syndrome similar to that observed in the field was produced. Symptoms, especially stunting, induced by either virus alone were much less severe than those induced by infection with both viruses simultaneously. TMV induced more stunting than did the potyvirus.

**Host range and symptoms.** Results of host range studies of the potyvirus are shown in Table 1. Virus was recovered on assay to *C. amaranticolor, P. floridana*, or both from all plants showing symptoms except *D. metel*. Inoculated plants of *D. metel* flowered

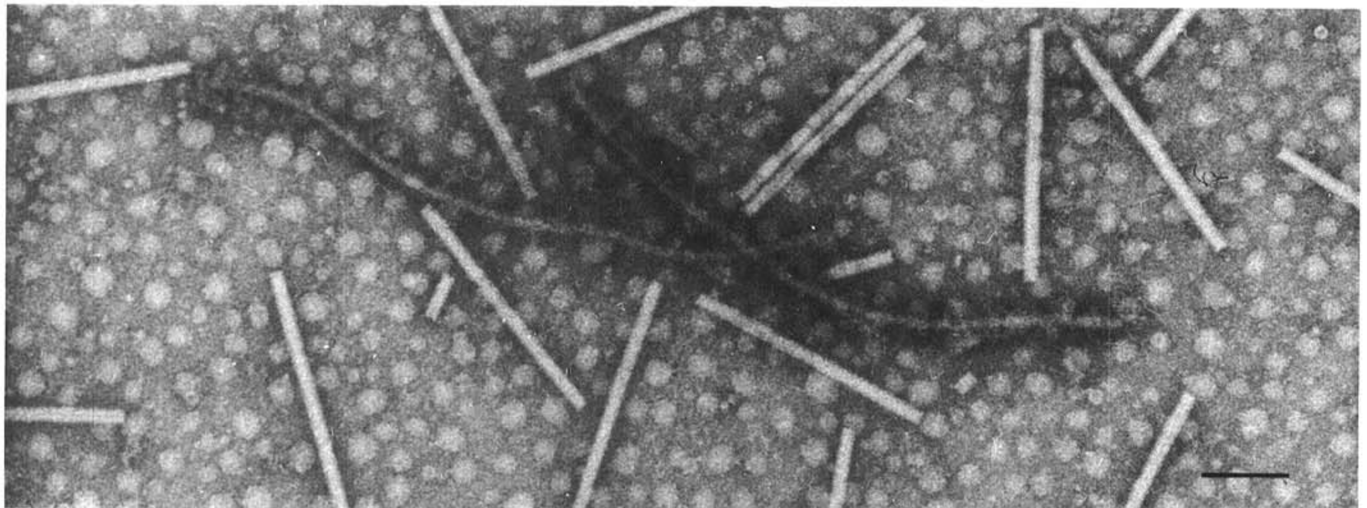

**Fig. 2.** Electron micrograph of leaf-dip preparation stained with 2% potassium phosphotungstate, pH 6.5, showing two morphologically distinct virus-particle types. Bar = 100 nm.

earlier than the controls and produced leaves with a grayish cast, but virus could not be recovered from these plants.

Leaves of *D. stramonium* and *N. glutinosa* inoculated with the tobamovirus had necrotic local lesions characteristic of those caused by TMV.

**Physical properties.** In heat-inactivation studies of the potyvirus, local lesions were produced when *C. amaranticolor* was inoculated with sap treated at 50 C, but not by inoculation with sap treated at 55 C. Lesions were obtained at dilutions of $10^{-4}$, but not at $10^{-5}$. The LIV was 1 day in sap prepared from Rutgers tomato, but 6 days in sap prepared from *N. glutinosa*.

**Serology.** Double-diffusion tests with antiserum against cucumber mosaic virus (ATCC PVAS 60) were negative when antigen was prepared from plants coinfected with both viruses. Tests with antisera against pyrrolidine-degraded PVX also were negative when sap containing both virus particles was treated with pyrrolidine and used as the antigen. Sap treated with SDS and containing only the potyvirus produced a strong reaction in SDS immunodiffusion tests when antiserum to potato virus Y (PVY) was used. Patterns of total identity were obtained when PVY antiserum was placed in the center well and adjacent peripheral wells contained SDS-treated antigens from plants infected with the potyvirus from tomatoes and PVY isolated by G. Gooding from tobacco (Fig. 4). Microprecipitin tests with antisera against tobacco etch virus (7) and PVX (ATCC PVAS 47) were negative, but strong positive reactions were obtained with serum to PVY (ATCC PVAS 50) when antigens were prepared from plants containing the potyvirus.

Antigens prepared from plants infected with the tobamovirus alone gave strong positive reactions with antiserum against TMV (ATCC PVAS 1) in microprecipitin and double-diffusion tests. Control antigens prepared from healthy plants gave no reactions in any tests.

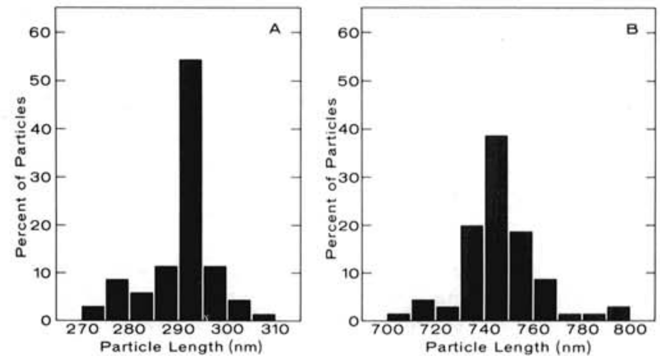


**Fig. 3.** Size distribution of two morphologically distinct kinds of virus particles. Measurements of 70 particles of each type were made from leaf-dip preparations stained with 2% potassium phosphotungstate, pH 6.5. **A.** Virus similar to viruses in the tobamovirus group. **B.** Virus similar to viruses in the potyvirus group.

TABLE 1. Symptom expression in host-range test plants of a potyvirus isolated from tomato

| Family | Genus | Species and, if applicable, cultivar | Symptoms[a] |
|---|---|---|---|
| Amaranthaceae | *Gomphrena* | *globosa* L. | 0 |
| Apiaceae | *Daucus* | *carota* L. | 0 |
| | *Petroselinum* | *crispum* (Mill.) Nym. ex A. W. Hill | 0 |
| Chenopodiaceae | *Beta* | *vulgaris* L. 'Iowa' | 0 |
| | *Chenopodium* | *amaranticolor* Coste & Reyn. | NLL |
| | *C.* | *quinoa* Willd. | 0 |
| Cucurbitaceae | *Cucumis* | *sativus* L. 'Select National Pickling' | 0 |
| Fabaceae | *Glycine* | *max* (L.) Merr. 'Williams' | 0 |
| | *Pisum* | *sativum* L. 'Green Giant #549' | 0 |
| | *Vicia* | *faba* L. | 0 |
| | *Vigna* | *unguiculata* (L.) Walp. subsp. *unguiculata* 'California Blackeye' | 0 |
| Phytolaccaceae | *Phytolacca* | *americana* L. | 0 |
| Solanaceae | *Capsicum* | *frutescens* L. 'Tobasco' | 0 |
| | *Datura* | *metel* L. | GL, EF |
| | *D.* | *stramonium* L. | 0 |
| | *Lycopersicon* | *esculentum* Mill. 'Rutgers' | M, LP, EP |
| | *L.* | *hirsutum* Humb. & Bonpl. | 0 |
| | *L.* | *peruvianum* (L.) Mill. | 0 |
| | *L.* | *pimpinellifolium* (L.) Mill. | LP, M, EP, EF |
| | *Nicandra* | *physalodes* (L.) Gaertn. | NLL, RU, M |
| | *Nicotiana* | *clevelandii* A. Gray | ST, RS, LC, M, VC |
| | *N.* | *glutinosa* L. | CS, VB, M, ST, LC, RU |
| | *N.* | *rustica* L. | M |
| | *N.* | *sylvestris* Speg. & Comes | VC, M |
| | *N.* | *tabacum* L. 'Havana 425' | VC, M |
| | *N.* | 'NN' | CRS, VC, M |
| | *N.* | 'Turkish' | CRS, VC, M |
| | *N.* | 'White Burley' | CRS, VC, M |
| | *Physalis* | *floridana* Rydb. | NLL, BL |
| | *Solanum* | *demissum* Lindl. P.I. 186551 | 0 |
| | *S.* | P.I. 230579 | 0 |
| | *S.* | *tuberosum* L. 'Katahdin' | 0 |
| | *S.* | 'Saco' | 0 |

[a]Key: BL (leaf blight and plant death), CRS (chlorotic ringspot), CS (chlorotic stippling), EF (early flowering), EP (epinasty), GL (leaf had graying cast but virus could not be recovered), LC (leaf curling), LP (local leaf purpling), M (mosaic), NLL (necrotic local lesions), 0 (no symptoms), RS (rosetting), RU (rugosity), ST (stunting), VB (vein banding), and VC (vein clearing).

## DISCUSSION

After initial separation of the two morphologically distinct viruses by aphid transmission and by inoculation of *D. stramonium*, we devoted more time to identification of the potyvirus than to identification of the tobamovirus because the potyvirus did not seem to be entirely similar to any of the known strains of PVY (or other potyviruses), as demonstrated by host-range reactions. The lack of infection of *Solanum* spp. (two PI [Plant Introduction] lines of *S. demissum* and the cultivars Saco and Katahdin of *S. tuberosum*) represents a deviation from reactions normally associated with strains of PVY (5). Physical properties, however, were similar to those reported for PVY (12), and the virus was immunologically indistinct from a strain of PVY isolated by G. Gooding, as evidenced by lack of spur formation when antiserum to his isolate was used in Ouchterlony tests. Symptom development in the greenhouse was slow, with 21 days usually required for the first evidence of symptoms on all systemically infected plants.

Particle length of the potyvirus (Fig. 3) and its transmission by *M. persicae* are consistent with reports for various strains of PVY (12). Seemingly identical virus particles were observed in leaf dips from diseased nightshade, *Solanum nigrum* L., plants growing in the same field as the infected tomatoes. This or other solanaceous weeds may have been the source of this strain of PVY. Attempts to demonstrate seed transmission as a possible source of the virus by use of two highly susceptible PI lines of tomato were unsuccessful. This constitutes the first report of the natural occurrence of PVY in Iowa.

The tobamovirus particle showed no characteristics that deviated from those expected of the common strain of TMV. Its presence is expected whenever large numbers of tomatoes are grown and handled by many workers (14).

The appearance of necrotic areas, often involving entire terminal branches and leaflets, on the tomato plants in the fields may have been accentuated by the unusual climatic conditions that prevailed in 1977. From July 1976 to June 1977, only 31 cm of precipitation fell in the Ames area. Normal for that period is 81 cm (13). Most of July 1977 continued to be extremely dry, and temperatures were high (33–38 C). The combination of low soil moisture and high ambient temperatures induced severe moisture stress in the plants. Those infected with TMV and PVY were seemingly unable to take up or transport enough water to prevent necrosis of the succulent tissues. Several Rutgers tomato plants infected with both PVY and TMV in the growth chamber developed similar terminal necrosis when water was withheld for several days, even at moderate (25 C) temperature. Uninoculated control plants exhibited no such symptoms.

The severe drought of 1976–1977 caused the early death of many native weeds, which in turn induced early movement of aphids from noncultivated areas to cultivated ones in central Iowa. Our seed-increase plots, except the tomato plot, were irrigated during June and early July 1977. In June 1977, lush vegetation existed within 300 m of the tomato seed-increase plots while the surrounding native vegetation was drying up. Such conditions may not reoccur in this area for many years. The disease was not observed during summer 1978.

There were more than 300 PI tomato accessions in the 1977 planting, and a wide range of reaction types was noted. In general, the *L. pimpinellifolium* (currant tomato) accessions showed less severe symptoms than the *L. esculentum* lines. The cultivar Rutgers was one of the most severely affected lines. Field tests in 1978 demonstrated yield reductions of 50–60% caused by this disease (authors', *unpublished*), even under adequate moisture conditions, where necrosis was not prevalent.

We conclude that tomato scorch, a new disease of tomato, is caused by the coinfection of tomato plants by TMV and PVY. The viruses were separated and identified, and the disease was reproduced by inoculating plants with both viruses. The disease has not reoccurred, and its initial occurrence may have been caused by the effects of unusually dry weather.

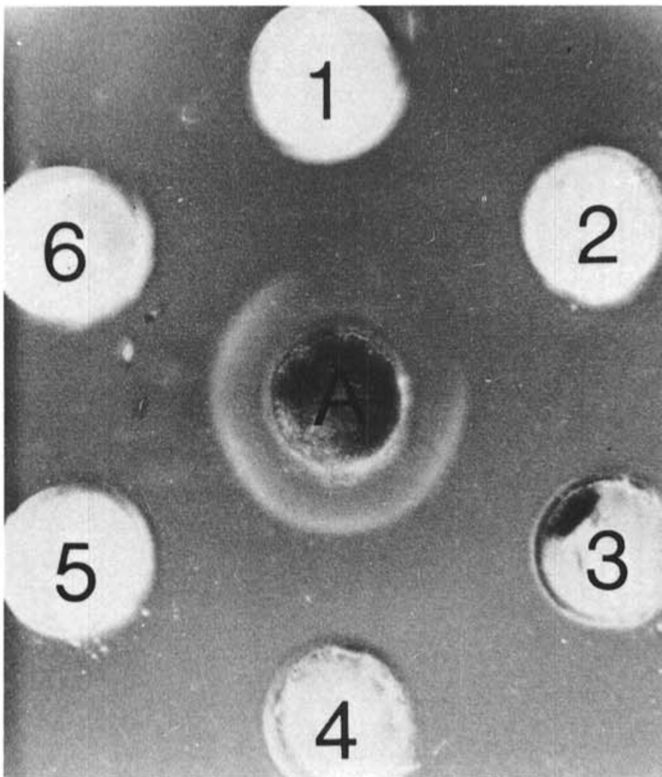


**Fig. 4.** An SDS double-diffusion test with the potato virus Y component of "tomato scorch" disease. Wells contain (A), antiserum to potato virus Y; (1) and (2), SDS-treated sap of healthy Rutgers tomato; (3) and (4), SDS-treated sap of Rutgers tomato inoculated with the potato virus Y component of "tomato scorch"; (5) and (6), SDS-treated sap of Rutgers tomato inoculated with potato virus Y isolated by G. Gooding.

## LITERATURE CITED

1. BALL, E. M. 1974. Serological tests for identification of plant viruses. American Phytopathological Society, St. Paul, MN. 31 pp.
2. BLOOD, H. L. 1928. A "streak" of tomatoes produced by a disturbing principle from apparently healthy potatoes in combination with tomato mosaic virus. Phytopathology 18:311.
3. CLARK, R. L., and J. H. HILL. 1978. A new tomato virus disease induced by co-infection with tobacco mosaic and potato Y viruses. Phytopathol. News 12:171.
4. CONOVER, R. A., and R. W. FULTON. 1953. Occurrence of potato Y virus on tomatoes in Florida. Plant Dis. Rep. 37:460-462.
5. EDWARDSON, J. R. 1974. Host range of viruses in the PVY-group. Fla. Agric. Exp. Stn. Monogr. Ser. 5. 225 pp.
6. FENNER, F. T. 1976. The classification and nomenclature of viruses. Intervirology 6:1-12.
7. McLAUGHLIN, M. R., J. H. HILL, and H. I. BENNER. 1975. Serological relationships among potyviruses: Maize dwarf mosaic virus, tobacco etch virus, and turnip mosaic virus. Phytopathology 65:334-335.
8. MILBRATH, J. A. 1939. Tomato tip-blight virus. Phytopathology 29:156-168.
9. PHILLIPS, M. J., S. HONMA, and H. H. MURAKISHI. 1967. The effect of mixed infection with tobacco mosaic virus and potato virus X on flowering and yield of field-grown tomatoes. Mich. Agric. Exp. Stn. Quart. Bull. 50:25-30.
10. PURCIFULL, D. E., and D. L. BATCHELOR. 1977. Immunodiffusion tests with sodium dodecyl sulfate (SDS)-treated plant viruses and plant viral inclusions. Fla. Agric. Exp. Stn. Tech. Bull. 788. 39 pp.
11. SHEPARD, J. F. 1972. Gel-diffusion methods for the serological detection of potato viruses X, S, and M. Mont. Agric. Exp. Stn. Bull. 662. 72 pp.
12. SMITH, K. M. 1972. A textbook of plant virus diseases. Academic Press, New York. 684 pp.
13. UNITED STATES DEPARTMENT OF COMMERCE, NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION. 1973. Monthly normals of temperature, precipitation, and heating and cooling degree days, 1941–1970. Climatography of the United States No. 81 (Iowa). National Climatic Center, Asheville, NC.
14. WALKER, J. C. 1952. Diseases of Vegetable Crops. McGraw-Hill, New York. 529 pp.