# Isolation of Tomato Spotted Wilt Virus from Hydrangea and Four Weed Species

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**ABSTRACT**

Allen, T. C., McMorran, J. P., and Locatelli, E. A. 1983. Isolation of tomato spotted wilt virus from hydrangea and four weed species. Plant Disease 67:429-431.

Field selections of *Hydrangea macrophylla* 'Imaculata,' tansy ragwort (*Senecio jacobea*), puncture vine (*Tribulus terrestris*), purslane (*Portulaca oleracea*), and cutleaf nightshade (*Solanum triflorum*) were infected with tomato spotted wilt virus (TSWV). Hydrangea exhibited ring spot symptoms in the field, and tansy ragwort from the field developed ring patterns when transplanted and maintained in a greenhouse. Both TSWV and hydrangea ringspot virus (HRSV) are associated with ring spots on hydrangea and local lesions on *Gomphrena globosa*, the most sensitive and commonly used indicator plant for HRSV. Therefore, special attention must be paid to correctly identify the virus involved in symptom formation in these plants.

In 1973, 40 seedlings of tansy ragwort (*Senecio jacobea*) were transplanted from a field near Corvallis, OR, to a greenhouse. After 1 mo, leaves of seven plants developed mosaic and yellow concentric rings. Mechanical inoculation onto *Nicotiana tabacum* 'Samsun NN' produced sunken gray to tan lesions, stem necrosis, collapse, and death of plants in the five-leaf stage.

Similar necrotic symptoms were expressed by Samsun NN inoculated in 1974 with sap from three of 22 weed species in 687 samples associated with potato fields in central Oregon. Symptoms were incited by inoculum from one of five purslane (*Portulaca oleracea*), two of five cutleaf nightshade (*Solanum triflorum*), and two of 18 puncture vine (*Tribulus terrestris*) samples (5).

In 1980, ring spots typical of virus infection were observed on leaves of about 50 plants of *Hydrangea macrophylla* 'Imaculata' in a field near the Oregon coast. This represented the total planting of Imaculata undergoing increase for future sales. Sap inoculation from leaves of these plants to *Gomphrena globosa* produced local lesions, supposedly diagnostic of hydrangea ringspot virus (HRSV) (4). Rod-shaped virus particles characteristic of HRSV, however, were not detected within leaf extracts examined with an electron microscope. Also, symptoms on Samsun NN tobacco resembled those produced by weed extracts in 1973 and 1974 and HRSV is differentiated from other viruses infecting hydrangea by its inability to infect tobacco. Therefore, serological tests, Ouchterlony agar double-diffusion, and enzyme-linked immunosorbent assay with antisera to other viruses reported to infect hydrangea were performed. No positive serological reactions were obtained between extracts of Imaculata and antisera to alfalfa mosaic virus, cucumber mosaic virus, tobacco necrosis virus, tobacco ringspot virus, or tomato ringspot virus.

Evidence that tomato spotted wilt virus (TSWV) was the virus associated with the hydrangea and weed samples is presented.

## MATERIALS AND METHODS

Leaves of field-grown *H. macrophylla, P. oleracea, S. jacobea, S. triflorum*, and *T. terrestris* were ground in 0.1 M phosphate buffer, pH 7.0, then inoculated with a pestle to Carborundum-dusted *N.*


**Fig. 1.** Local lesions on *Gomphrena globosa* inoculated with **(A)** tomato spotted wilt virus (TSWV) and **(B)** hydrangea ringspot virus (HRSV).

Present address of third author: The World Bank, Washington, DC 20433. Technical Paper No. 6492, Oregon Agricultural Experiment Station, Corvallis 97331. Supported in part by the Oregon Propagating Company.

Accepted for publication 8 November 1982.

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*tabacum* Samsun NN grown in a greenhouse. Thereafter, inoculated Samsun NN tobacco served as a virus source for symptomatology, host range determination, in vitro stability experiments, and electron microscopy.

Infected tobacco leaves and extracts were examined with a Philips EM 300 electron microscope operating at 60 KV. For in situ studies, 2-mm$^2$ pieces of leaves were cut, evacuated, and fixed for 3 hr in 2.5% glutaraldehyde in 0.2 M cacodylate buffer, pH 7.0. After overnight washing in buffer, postfixing in 1% cacodylate buffered $OsO_4$ for 1 hr, and initial dehydration in 50% acetone for 15 min, leaf pieces were stained for 20 min in 70% acetone saturated with uranyl acetate. Three 15-min changes in 100% acetone preceded tissue embedment in Spurr solution. Sections were cut on an MT-2 Servall Porter-Blum ultramicrotome with a diamond knife and stained with lead citrate.

Extracts of infected tobacco leaves were obtained by cutting leaves and dipping the cut surfaces into a drop of 10% formalin on a carbon-coated electron microscope grid, then passing a strip of the leaf upper epidermis through the formalin. After 5 min, grid surfaces were blotted and negatively stained with 2% sodium phosphotungstate, pH 7.0.

Virus particle measurements were made on electron micrographs magnified 2.6 times the original scope magnification. Magnification calibration was determined by measuring electron micrographs of diffraction-grating replicas of 2,160 lines per millimeter.

**Fig. 2.** Concentric rings that developed in tomato spotted wilt virus-infected greenhouse-grown tansy ragwort.

## RESULTS

Nine of 31 plant species inoculated with the TSWV isolates became infected. Local and systemic symptoms were incited in *Datura stramonium, G. globosa, Lycopersicon esculentum, Nicotiana glutinosa, N. tabacum, Petunia hybrida*, and *Vinca rosea*. Only local symptoms were expressed on *Cucumis sativus* and *Lathyrus odoratus*. Local symptoms consisted of chlorotic or necrotic lesions. Local lesions on *G. globosa* inoculated with TSWV appeared 7–15 days after inoculation; they increased to about 5 mm in diameter and had large white necrotic centers within purple borders. Inoculated leaves became chlorotic and then necrotic. In contrast, HRSV incited lesions 14–25 days after inoculation that were about 2.5 mm in diameter with pinpoint white necrotic centers surrounded by purple and showed no additional symptom development (Fig. 1).

Systemic symptoms generally were severe, consisting of mosaic, malformation, necrotic spots and ring spots, and general necrosis and wilting. An exception was seen in *L. odoratus* that expressed no systemic symptoms, but the virus could be recovered from leaves until plant death about 6 mo after inoculation.

Extracts of tobacco plants infected with TSWV from field samples of hydrangea, purslane, or tansy ragwort were used to inoculate greenhouse-grown seedlings or cuttings in order to record symptoms. Eleven of 27 inoculated seedlings of tansy ragwort developed mosaic and concentric rings (Fig. 2), three purslane seedlings showed black shoot tip necrosis and distorted leaves and wilting, and the 15 tissue-cultured virus-free Merit Supreme and Rose Supreme hydrangeas remained symptomless. Back-inoculations from purslane and tansy ragwort to tobacco produced many necrotic local lesions followed by necrosis, wilting, and death. The virus was not recovered from the inoculated hydrangeas.

The virus isolates were highly unstable in vitro, inactivating at 4–6 hr at room temperature, between 40 and 45 C/10 min, and had a dilution end point between $1 \times 10^{-2}$ and $1 \times 10^{-3}$ in crude extracts of tobacco.

In initial attempts, viruslike particles were not found in negatively stained extracts of naturally infected field plants or in extracts of inoculated Samsun NN with severe symptoms. Consequently, small pieces of infected and uninfected tobacco leaves were embedded, sectioned, and examined for virus particle aggregates or other cellular phenomena associated with virus infection. Numerous leaf cells contained groups of dark spheroid viruslike particles 70–80 nm in diameter within membranes (Fig. 3).

Because such large virus particles do not retain their integrity in routine extract preparations, leaf extracts were fixed in 10% formalin before negative staining with 1% sodium phosphotungstate, pH 7.0. Particles 80–90 nm in diameter then were observable (Fig. 4). Most of the particles were enclosed within membranes.

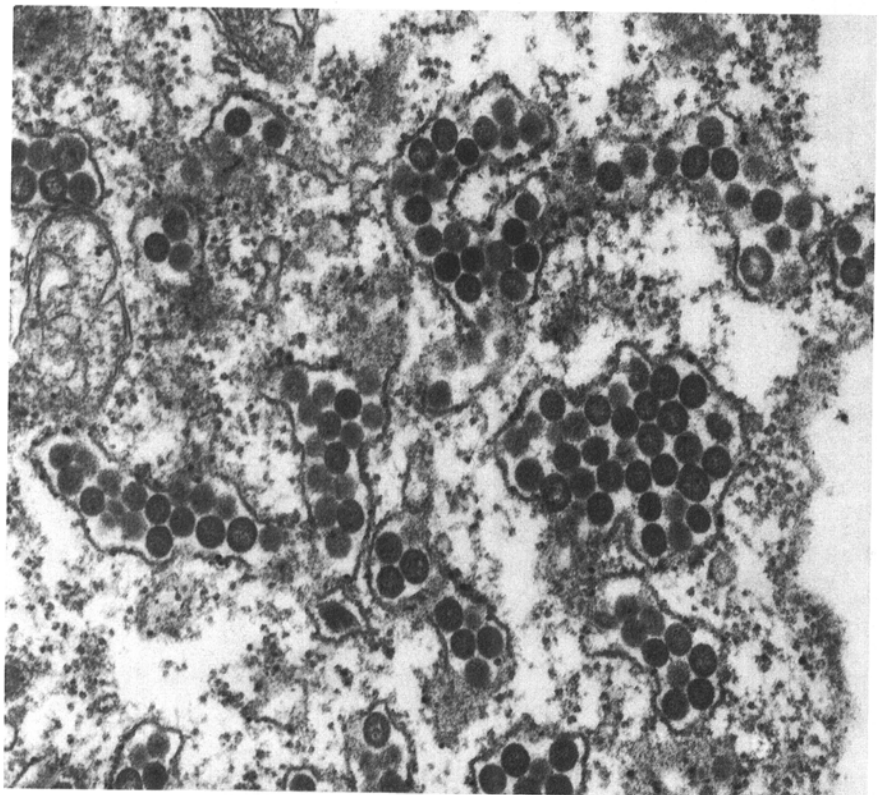

**Fig. 3.** Electron micrograph of an ultrathin section of tobacco leaf inoculated with tomato spotted wilt virus from the hydrangea Imaculata. Virus particles are grouped within membranes. ×50,000.

## DISCUSSION

Properties of the virus isolated from hydrangea, tansy ragwort, puncture vine,

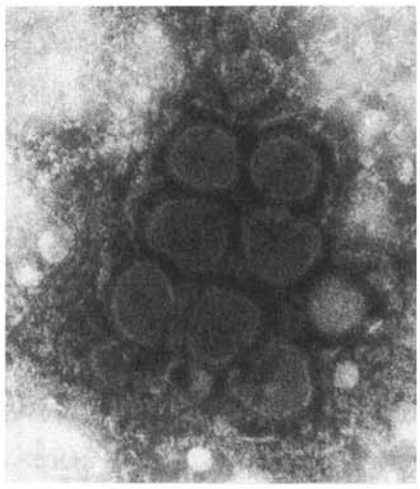

**Fig. 4.** Electron micrograph of tomato spotted wilt virus particles from infected Samsun NN tobacco extracts fixed in 10% formalin, then negatively stained with 2% sodium phosphotungstate, pH 7.0. ×131,000.

purslane, and cutleaf nightshade are characteristic of TSWV. This virus was suspected after symptoms and host range were observed. Evidence of the virus's extreme instability in vitro helped confirm its identity (3). The most convincing evidence, however, was the presence of characteristic virus particles seen within membranes in ultrathin sections of infected Samsun NN leaf cells. Similar particles were observed in formalin-fixed extracts of infected leaves. Serological identification was not attempted.

Francki and Hatta (2) felt that the most reliable method for TSWV identification was electron microscopy of infected plant cells. Particles of TSWV became too distorted in extracts for absolute identification. Sap transmission to selected hosts can provide strong evidence and tests on virus stability may be sufficiently conclusive to identify TSWV. In addition, serology was considered only as a potential future means of TSWV identification.

To find unreported natural hosts of TSWV is not unusual because it has one of the widest host ranges of any virus (1,2). Two factors, however, contribute to the importance of these findings. First, we now know of four additional weeds that can serve as symptomless sources of TSWV. These weeds all are associated with crops in Oregon. Especially interesting is tansy ragwort, the object of an eradication program because it is toxic to cattle. Our findings suggest a second reason to eradicate this weed.

Probably the most important discovery reported in this paper is the isolation of TSWV from hydrangea and its production of local lesions on *G. globosa*. HRSV was the only virus previously isolated from hydrangea that produced local lesions on *G. globosa*. This has resulted in the use of *G. globosa* in some programs as the exclusive diagnostic host for HRSV infection in hydrangea. Consequently, TSWV would not be identified, thrips that transmit the virus would not be the subject of a control program, and spread of the virus would occur unchecked.

**ACKNOWLEDGMENT**

We are grateful to Chris Weiss of the Electron Microscope Facility at Oregon State University for preparation of tobacco leaves for in situ studies.

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