Silver Mottle Disease of Watermelon Caused by Tomato Spotted Wilt Virus

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ABSTRACT

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The causal virus of the silver mottle disease that occurred in watermelon in Okinawa, Japan, was identified as tomato spotted wilt virus (TSWV) on the basis of particle morphology, host reactions, instability in crude sap, effects of sodium sulfite and L-cysteine on stability, and thrips transmissibility. This is the first report of TSWV naturally infecting Cucurbitaceae.

In 1982, watermelon plants (*Citrullus vulgaris* Schrad.) showing silver mottle on leaves and chlorotic mottle on fruits were found in Okinawa Prefecture, Japan. Yield of infected watermelon plants was considerably decreased, as was quality, because infected plants produced malformed fruits.

Watermelon mosaic virus (8), cucumber green mottle mosaic virus (9), and cucumber mosaic virus (9) have been reported on watermelon in Japan. These viruses cause large yield losses; however, they cause mosaic or mottle symptoms on leaves and fruit deterioration of watermelon that are quite distinct from symptoms caused by the virus described in this report.

From our investigations, the causal virus of silver mottle disease was identified as a new strain of tomato spotted wilt virus (TSWV). This paper describes the host range, symptoms, transmission, stability in crude sap, and electron microscopy of the virus.

MATERIALS AND METHODS

A virus was isolated from leaves of naturally infected watermelon showing silver mottle symptoms. Leaves of naturally infected watermelon were macerated in a mortar with 0.05 M sodium and potassium phosphate buffer (PB), pH 7.0, containing 0.5% sodium sulfite and 1 mM L-cysteine and rubbed onto 600-mesh Carborundum-dusted leaves of test plants grown in a glasshouse. Preliminary tests indicated that mechanically inoculated *Nicotiana glutinosa* plants produced obvious symptoms and were a suitable propagative host. In host range studies, inoculations were made using infected *N. glutinosa* leaves as the source of inoculum. Virus stability in crude sap was tested using the extract of diseased *N. glutinosa* prepared in 0.05 M PB either with or without 0.2% sodium sulfite and 1 mM L-cysteine.

Leaf-dip preparations for electron microscopy were prepared by grinding small pieces of diseased leaves previously fixed in 4% glutaraldehyde for 1–2 hr in 2% potassium phosphotungstic acid, pH 7.0.

For ultrathin sectioning, small pieces of diseased leaves were fixed with 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.2, for 1 hr, then postfixed with 1% osmium tetroxide in the same buffer for 4 hr. Fixed samples were dehydrated with an alcohol series and embedded in Spurr's resin (12). Ultrathin sections were cut with glass knives mounted on an LKB III ultratome. Sections were stained with uranyl acetate and lead citrate and examined in a Hitachi Model H-500 electron microscope.

Aphid transmission tests were carried out using *Aphis gossypii* Glover and *Myzus persicae* Sulzer reared on eggplant and turnip plants, respectively. In nonpersistent type transmission tests, aphids were starved in a glass beaker for about 2 hr and given access to diseased plants for 10–30 min, then five to 20 insects were transferred to each test plant for 24 hr of inoculation access. In persistent type transmission tests, aphids were allowed to feed on diseased plants for acquisition access periods of 4–24 hr, then five to 20 insects were transferred to each test plant for 24 hr of inoculation access.

Whitefly transmission tests were carried out using the virus-free greenhouse whitefly (*Trialoirodes vaporariorum* Westwood) reared on bean plants.

In thrips transmission tests, thrips (*Thrips* sp.) were reared on diseased watermelon, then five to eight adult insects were transferred to each healthy watermelon, cucumber, or tomato. After 3 days of inoculation access, the insects were removed by spraying an insecticide.

Seed transmissibility of the virus was investigated using commercial seeds of watermelon in Okinawa, seeds collected from infected watermelon fruits in fields, and seeds from infected petunia, *Physalis floridana*, and *Tetragonia expansa* plants in a glasshouse.

RESULTS

Symptoms on watermelon. In the fields, diseased watermelon plants showed dwarfing with shortened internodes, and their young leaves showed rugosity with veinal mottle. Later, younger leaves showed silver mottle. Diseased leaves were malformed with narrow or curled leaf blades and appeared "moldy" because of the presence of many hairs (Fig. 1A). Diseased fruits showed chlorotic mottle symptoms (Fig. 1B), and their surfaces became uneven.

In a glasshouse, watermelon plants inoculated with the virus showed necrotic spots and rings on first uninoculated leaves, followed by systemic veinal mottle and silver mottle. Leaf blades became narrow and curled. Later, infected plants became dwarfed with shortened internodes.

Host range. The virus infected 23 plant species representing six families among 37 species representing nine families tested. The virus systemically infected many species of Cucurbitaceae and Solanaceae.

N. glutinosa and *N. clevelandii* were susceptible to the virus and showed local chlorotic spots or chlorotic rings on inoculated leaves followed by veinclearing and rolling of young leaves.

N. tabacum cv. Bright Yellow frequently showed chlorotic spots or chlorotic rings on inoculated leaves and chlorotic spots, chlorotic rings, and necrotic rings on upper leaves. As the plants matured, the upper leaves showed a mild rugosity.

Lycopersicon esculentum were dwarfed and showed chlorosis, narrowing, and curling on younger leaves, with prominent purpling of veins on the undersides of leaves.

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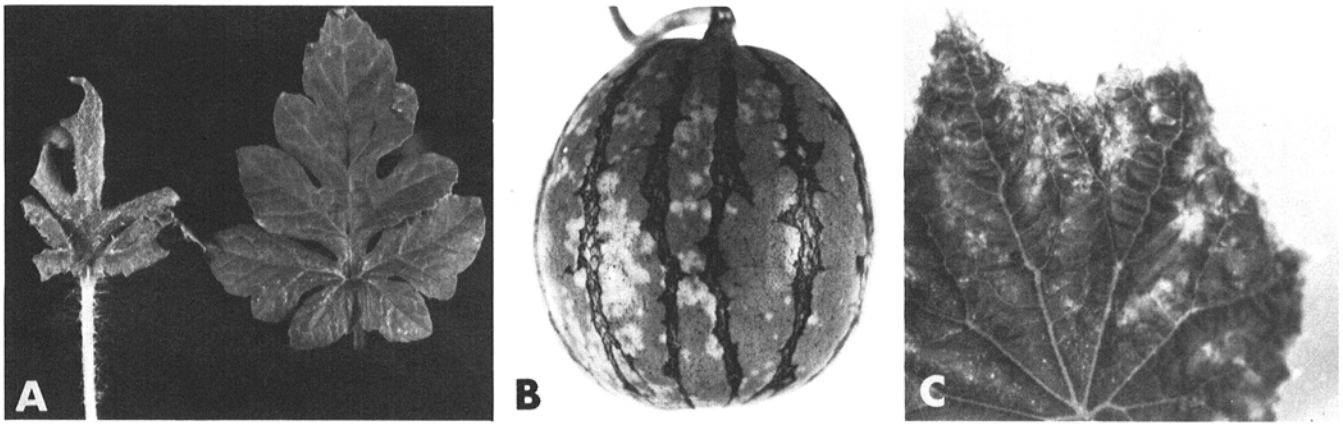

Fig. 1. (A) (Left) Silver mottle symptom on watermelon leaf; (right) healthy leaf. **(B)** Chlorotic spots on watermelon fruit. **(C)** Interveinal chlorotic spots on cucumber leaf.

Cucumis sativus showed small chlorotic spots on upper leaves; thereafter, the margins of younger leaves became chlorotic and cupped upward or downward (Fig. 1C).

T. expansa, Gomphrena globosa, Vigna sesquipedalis, Capsicum annuum, C. annuum var. *anglosum, P. floridana, Datura stramonium, Petunia hybrida, Cucurbita pepo, C. maxima, C. maxima* × *C. moschata, Cucumis melo,* and *C. melo* var. *conomon* were infected systemically with the virus and showed symptoms.

Chenopodium amaranticolor, C. quinoa, and *V. unguiculata* cv. Blackeye showed chlorotic or necrotic lesions in inoculated leaves. *Phaseolus vulgaris* cv. Kintoki was infected with the virus in inoculated leaves without symptoms. These plants were not infected systemically.

The following plants were not infected with the virus: *Arachis hypogaea, Beta vulgaris, Brassica rapa, Cucurbita moschata, Glycine max, Lactuca sativa, Lagenaria siceraria, P. vulgaris* cv. Top Crop and Kisaya Kurosando, *Pisum sativum, Sesanum indicum, Solanum melongena, Spinacia oleracea, Vicia faba, Vigna radiata,* and *Zinnia elegans.*

Stability in crude sap. When PB was used for macerating the diseased leaves, thermal inactivation point, dilution end point, and longevity in vitro were 40–45 C for 10 min, 2×10^{-2}–10^{-2}, and 2–4 hr at 20 C, respectively. When PB containing 0.2% sodium sulfite and 1 mM L-cysteine was used for macerating the diseased leaves, thermal inactivation point, dilution end point, and longevity in vitro were 45–50 C for 10 min, 10^{-2}–2×10^{-3}, and 2–4 hr at 20 C, respectively.

Electron microscopy. Dip preparations made from fruits and leaves of diseased watermelon collected in the fields contained enveloped, roughly spherical particles 80–100 nm in diameter (Fig. 2A). These characteristic particles were also detected in dip preparations from leaves of mechanically inoculated cucumber, watermelon, *N. glutinosa,* tobacco, tomato, *Chenopodium quinoa, G. globosa,* and *T. expansa* in a glasshouse (Fig. 2B). The diameters of the particles in negative stain were greater than in ultrathin sections and more variable, presumably because of flattening. These particles were always surrounded by membranes and could not be observed in healthy plants.

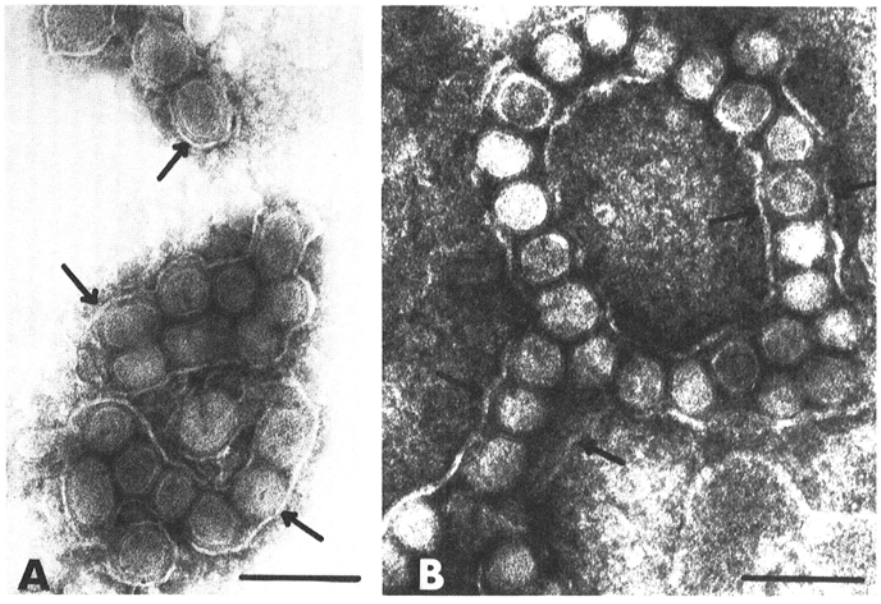

Fig. 2. Negatively stained virus particles in dip preparations from leaves of infected **(A)** watermelon and **(B)** cucumber. Enveloped, roughly spherical particles encircled by membranes of host plant (arrows). Bars = 0.2 μm.

Ultrathin sections of the same plants used for dip preparations contained the spherical particles 80–90 nm in diameter that were present within the cisternae of the endoplasmic reticulum in the cytoplasm (Fig. 3A). Viroplasm densely staining amorphous masses was observed in the cytoplasm and associated with the endoplasmic reticulum that sometimes contained spherical particles (Fig. 3B). These particles were observed in cells of leaf epidermis and mesophyll as well as in sieve tubes and phloem parenchyma of leaf veins.

Transmission. In aphid transmission tests, *M. persicae* failed to transmit the virus from tobacco or cucumber to tobacco in a nonpersistent manner (0/18). *A. gossypii* also failed to transmit the virus from tobacco or watermelon to tobacco, cucumber, or *Cucumis melo* var. *conomon* in a nonpersistent (0/24) and persistent manner (0/24).

The greenhouse whitefly (*Trialoirodes vaporariorum*) failed to transmit the virus from tobacco to tobacco (0/19) in trials of 2 days each of acquisition and inoculation access periods.

Thrips sp. transmitted the virus from watermelon to watermelon (6/8), cucumber (10/10), and tomato (3/6). Ten to 20 days after inoculation, these plants showed symptoms identical to those produced by sap inoculation of the virus. These infected plants contained spherical particles 80–100 nm in diameter.

Seedlings produced from seeds of infected plants, such as watermelon (0/236), petunia (0/672), *Tetragonia expansa* (0/80), and *P. floridana* (0/300), showed no symptoms. These results indicate that the virus is not seed-transmitted.

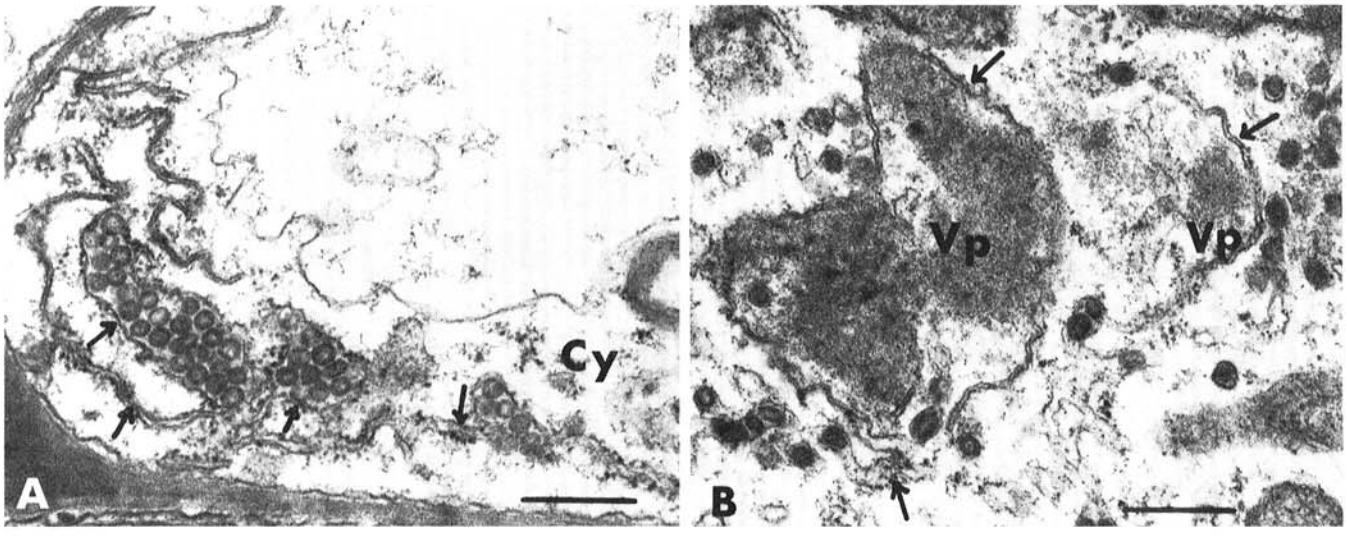

Fig. 3. Ultrathin sections of infected plants. **(A)** Leaf cell of infected watermelon. Spherical particles appear within the cisternae of the endoplasmic reticulum (arrows) in the cytoplasm (Cy). Bar = 500 nm. **(B)** Fruit cell of naturally infected watermelon. Viroplasm (Vp) associated with the endoplasmic reticulum (arrows) in the cytoplasm. Spherical particles surrounded by membranes of the endoplasmic reticulum. Bar = 500 nm.

DISCUSSION

The properties of the virus isolated from watermelon showing the characteristic silver mottling indicate that the causal agent of this disease is TSWV. This virus is distributed worldwide and has a wide host range, infecting 157 dicotyledonous and six monocotyledonous species in 29 and five families, respectively (1). However, TSWV strains reported so far produced only chlorotic local lesions on inoculated leaves of Cucurbitaceae plants but did not invade them systemically. Frequently, cucumber has been used as a diagnostic host of TSWV because of its characteristic local lesions (3).

In Japan, TSWV induced severe disease in dahlia (5), pimiento (11), tomato (7), and *Belamcanda chinensis* (14) in the field. These isolates of TSWV produced only local lesions in inoculated leaves of Cucurbitaceae plants. Recently, TSWV was isolated from tobacco in the Ryukyu islands (13) and our preliminary tests indicated that this isolate infected cucumber systemically. In our study, watermelon was newly included as a natural host of TSWV. The host range of the watermelon isolate was similar to those of other TSWV isolates, with the exception of its systemic invasion of Cucurbitaceae. Symptoms of the watermelon isolate were usually milder in many plant species than those of other isolates.

For detection of TSWV particles in negatively stained dip preparations, samples must be fixed (2,10). Fixation prevents much of the distortion of virus particles, and consequently, the envelope around the core particle can be observed (Fig. 2). Ultrathin sections of infected plants showed that TSWV particles were always present within the cisternae of the endoplasmic reticulum. These results are similar to those reported for other TSWV (2,4,6).

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