Spinach Yellow Dwarf Virus

Claude E. Thomas, R. S. Halliwell, and R. E. Webb

Research Plant Pathologist, Plant Science Research Division, ARS, USDA, Weslaco, Texas 78596; Associate Professor of Virology, Department of Plant Sciences, Texas A&M University, College Station 77843; and Investigations Leader, Vegetable and Ornamentals Research Branch, PSR, ARS, USDA, Beltsville, Maryland 20705.

Mention of a trademark or proprietary produce does not constitute a guarantee or warranty of the product by the USDA, and does not imply its approval to the exclusion of other products that may also be suitable.

ABSTRACT

Spinach yellow dwarf virus (SYDV) is a rigid rod with mean dimensions of 250 nm × 15 nm which aggregates into definite crystals within infected cells. In host range studies, SYDV was confined to spinach alone.

Phytopathology 63:538-539

Additional key words: electron microscopy, partial purification.

Spinach yellow dwarf virus (SYDV) has been described from both California (6) and Texas (2). Neither the virus nor the disease that it incites has been characterized further, even though it is of widespread occurrence in commercial fields (3, 7). It is a limiting factor in spinach (*Spinacea oleracea* L.) production. Our studies included re-examinations of host range and physical properties, as well as partial purification and electron microscopy of SYDV.

SYDV was obtained from the infected spinach cultivar Early Hybrid 424. Leaves were ground in 0.1 M phosphate buffer (pH 7) and filtered through cheesecloth pads. Healthy spinach plants, dusted with No. 500-mesh Carborundum, were mechanically inoculated with the extracted juice. Symptoms typical of SYDV infection developed 14 to 28 days after inoculation in plants maintained at 20 C with 8 hr of illumination/day (Fig. 1). No symptoms developed on control plants rubbed with abrasive and sterile distilled water and otherwise treated the same.

The following plant species were mechanically inoculated as described above: squash (*Cucurbita pepo* L.), cantaloup (*Cucumis melo* L.), cucumber (*Cucumis sativus* L.), watermelon (*Citrullus lanatus* [Thunb.] Mansf.), *Luffa acutangula* (L.) Roxb., pea (*Pisum sativum* L.), sweet pea (*Lathyrus odoratus* L.), cowpea (*Vigna sinensis* [Torner] Savi), lima bean (*Phaseolus lunatus* L.), crimson clover (*Trifolium incarnatum* L.), sweet clover (*Melilotus alba* Desr.), morning glory (*Ipomoea tricolor* Cav.), petunia (*Petunia hybrida* Vilm.), *Nicotiana glutinosa* L., tabasco pepper (*Capsicum frutescens* L. var. *longum* Bailey), bell pepper (*Capsicum frutescens* L. var. *grossum* Bailey), garden beet (*Beta vulgaris* L.), New Zea-

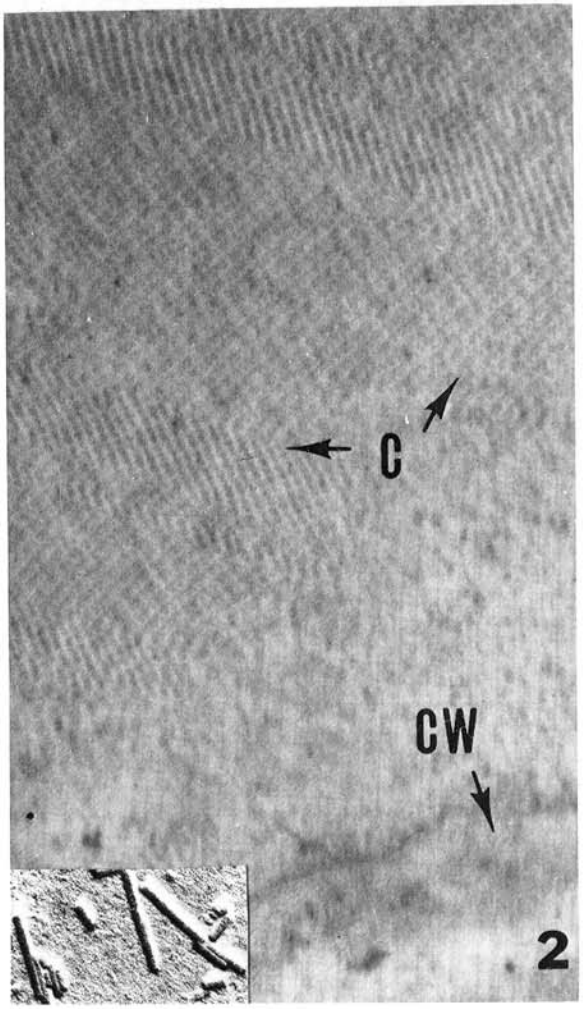

Fig. 1-2. Spinach yellow dwarf virus. **1)** Infected spinach plant showing typical vein-banding, yellow mottle, and leaf distortion. **2)** Electron micrographs of spinach yellow dwarf virus (SYDV) particles (inset) and crystal in a portion of an infected mesophyll cell. C = crystal. CW = cell wall. (× 40,000).

TABLE 1. Flow diagram of purification procedure for spinach yellow dwarf virus

200 g infected frozen tissue

Added 200 ml 0.05 M Sodium EDTA (pH 9.5) and ground to slurry in blender

Filtered slurry through 4 layers of cheesecloth; filtrate should be slightly acid (pH 6-6.5)

Added 5 g activated charcoal (Merck) and 5 g Celite/100 ml and mixed

Filtered mixture through 1.25 cm Celite pad in Büchner funnel

Filtrate — Pad

Resuspended filter pad in 200 ml 0.01 M K_2HPO_4 (pH 8.5)
Readjusted pH to 8.5 with NaOH

Filtered suspension through 0.5 cm thick Celite pad which was prewashed with K_2HPO_4 buffer (pH 8.5) in a Büchner funnel

After filtration, washed pad with 20 ml K_2HPO_4 (pH 8.5)

Pad — Filtrate

Centrifuged at 75,000 *g* for 2 hr

Supernatant — Pellet

Resuspended in distilled H_2O

land spinach (*Tetragonia tetragonioides* [Pallas] O. Ktze.), *Gomphrena globosa* L., *Chenopodium amaranticolor* Coste & Reyn., lambsquarter (*Chenododium album* L.), purslane (*Portulaca oleracea* L.), and wild sunflower (*Helianthus annuus* L.). In addition to the above species, the following spinach cultivars were also inoculated with the extraced juice of SYDV-infected plants: Packer 530H, Viking 269, Winter Bloomsdale, Chesapeake Hybrid, Northland, Packer Hybrid, King of Denmark, Long Standing Bloomsdale, Virginia Blight Resistant, Old Dominion, Viroflay, Hybrid 612, Early Hybrid 424, Dark Green Bloomsdale, Dixie Market, Virginia Savoy, Hybrid 7, Norgreen, Bounty, and America. All spinach cultivars, but none of the other species, were susceptible to SYDV in these tests, even after subindexing to spinach. This agrees with results of previous studies (2, 6), in which spinach was the only suscept.

The dilution end point was 1:10, the thermal inactivation point was 50 C, and longevity in vitro was 24 hr. The first studies reported on SYDV (7) give these physical properties as 1:20, 55 C, and 8 days, respectively.

A partially purified virus suspension, prepared as described in Table 1, was then aspirated onto Formvar grids, shadow-cast with platinum:palladium (80:20),and examined with an Hitachi H_S7_S electron microscope. The virus was a rigid rod (inset Fig. 2). The mean dimensions of the particles were 250 nm in length and 15 nm in diam. The suspension, used to inoculate healthy plants mechanically, was infectious and produced the typical SYDV syndrome in 14 to 28 days on spinach, but was not infectious to cucumber, cowpea, lima bean, *L. acutangula*, bell pepper, lambsquarter, or purslane.

Young leaves of mechanically inoculated, systemically infected spinach plants were fixed in 6% glutaraldehyde (5). All wash solutions prior to dehydration were carried out in 0.1 M phosphate buffer, pH 7. The specimens were postfixed with 2% osmium tetroxide for 4 hr and washed. Specimens were dehydrated in a graded ethyl alcohol series. The specimens were infiltrated with propylene oxide and, subsequently, epoxy resin (8). Tissue sections cut on a Porter-Blum MT-2 ultramicrotome, were mounted on grids and stained with uranyl acetate and lead citrate with each step followed by washing (1, 4). Virus particles were found in large numbers throughout the cytoplasm and were often aggregated into crystals with a distinct "herringbone" pattern in cells of infected plants (Fig. 2). No such structure was found in cells from healthy plants.

The most pertinent question still left unanswered is that of an alternate host for SYDV. The virus may well be confined to spinach alone, surviving from season to season on a few volunteer plants, but this seems unlikely; thus, the search for other hosts of SYDV will continue.

LITERATURE CITED

1. HUXLEY, H. E., & G. ZUBAY. 1961. Preferential staining of nucleic acid containing structures for electron microscopy. J. Biophys. Biochem. Cytol. 11:273-296.
2. MC LEAN, D. M. 1964. Occurrence of an unknown virus infecting spinach in South Texas. J. Rio Grande Valley Hort. Soc. 18:78-81.
3. MC LEAN, D. M., R. E. WEBB, & B. A. PERRY. 1965. Virus disease symptoms on spinach in the lower Rio Grande Valley. Texas A&M Univ. Exp. Sta. Bull. MP-775. 8 p.
4. REYNOLDS, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain for electron microscopy. J. Cell Biol. 17:208-212.
5. SABATINI, D. D., K. BENSCH, & R. J. BARRNETT. 1963. The preservation of cellular ultrastructure and enzymatic activity of aldehyde fixation. J. Cell Biol. 17:19-57.
6. SEVERIN, H. H. P., & D. H. LITTLE. 1947. Spinach yellow dwarf. Hilgardia 17:555-566.
7. THOMAS, C. E., & R. E. WEBB. 1968. Texas spinach viruses. Plant Dis. Reptr. 52:761-763.
8. WINBORN, W. B. 1965. Dow epoxy resin with triallyl cyanurate, and similarly modified Araldite and Maraglas mixtures, as embedding media for electron microscopy. Stain Tech. 40:227-231.