

## Natural Occurrence of Sonchus Yellow Net Virus in Florida Lettuce

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### ABSTRACT

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The causal agent of a newly recognized disease of lettuce (*Lactuca sativa*) in Florida was investigated. A virus isolated from naturally infected lettuce was mechanically transmissible to *Nicotiana* × *edwardsonii*, *N. clevelandii*, *N. debneyi*, *Sonchus oleraceus*, and lettuce. Electron microscopic examination of extracts from infected *N. × edwardsonii* showed rhabdoviruslike particles present in infected plants. When virions were purified and examined by SDS-PAGE and serological analysis of "Western blots," four major virion proteins that reacted with antisera to the type isolate of sonchus yellow net virus (SYNV) were identified. On the basis of host range and electron microscopic and serologic evidence, the causal agent of this Florida lettuce disease is identified as SYNV. This is the first report of natural infection of an economically important crop by SYNV.

Florida's winter lettuce (*Lactuca sativa* L.) crop has remained relatively virus-free since seed indexing to control lettuce mosaic virus (LMV) was implemented in the early 1970s. Besides LMV, bidens mottle virus (BiMV), beet western yellows virus (BWYV), and lettuce big vein (LBV) occur naturally in Florida lettuce (5,6,12), but none have proven to be serious annual problems.

During the 1983-1984 growing season, a previously unrecognized disease was occasionally observed in crisphead and cos lettuce types. Characteristic symptoms were a bright yellow interveinal spotting of older leaves. The incidence of plants showing these symptoms was rare (only five plants in 1984); however, the same symptoms were again seen sporadically during the 1984-1985 growing season. Laboratory studies have since shown that sonchus yellow net virus (SYNV), a plant rhabdovirus (2,10), is the causal agent of this disease.

### MATERIALS AND METHODS

**Virus source, transmission, and host range studies.** Lettuce plants showing a bright yellow spotting of the older leaves were collected from commercial lettuce

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fields near Belle Glade, FL, in the winter and fall of 1984. Inoculations were made from the field plants to indicator species by grinding tissues with a mortar and pestle in 0.03 M potassium phosphate buffer, pH 7.0, containing 0.1% 2-mercaptoethanol. Leaves of indicator plants were dusted with Celite and rubbed with inoculum with a cotton swab. Test plants were kept in an air-conditioned greenhouse (25 C) and observed for symptoms for 6 wk. Because *Nicotiana* × *edwardsonii* showed distinct virus symptoms 7-10 days postinoculation, the virus was maintained in this host by periodic mechanical transfer.

**Identification.** For electron microscopy, tissue pieces from the indicator plants were minced in a small amount of 0.01 M potassium phosphate buffer, pH 7.4. Droplets of the resulting extracts were transferred to carbon-backed, Formvar-coated electron microscope grids. After 1 min, the grids were rinsed successively with buffer and water, then stained with either 2% uranyl acetate or 2% phosphotungstic acid (PTA). They were then examined for virions in the electron microscope.

Virions were purified from infected *N. × edwardsonii* and *N. clevelandii* by the method described for maize mosaic virus (MMV) (8). Purified preparations were further analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described for MMV (8), except the gels were run for 80 min at 200V.

SDS-PAGE-analyzed proteins were also tested by "Western blotting" and serological analysis of the blots (1). After SDS-PAGE, proteins were electrophoretically transferred to nitrocellulose

sheets (BA83, Schleicher and Schuell, Keene, NH) for 2 hr at 10V/cm. The nitrocellulose sheet (blot) was washed for 15 min in PBST (0.02 M phosphate, 0.15 M NaCl, pH 7.4, containing 0.3% polyoxyethylene sorbitan monolaurate) containing 3% fraction V bovine serum albumin. Antisera to MMV (8), to the type isolate of sonchus yellow net virus (SYNV) (from A. O. Jackson), and to the SYDV-NY isolate of potato yellow dwarf virus (9) (from Hei-ti Hsu) were used to probe the blots. MMV and SYNV antisera were diluted 1/500 in PBST and SYDV antiserum to 1/1,000, and the blots were incubated in the antisera solutions with shaking for 1.5 hr. Blots were then washed twice for 10 min each in PBST. Protein A-conjugated horseradish peroxidase (Boehringer-Mannheim, Indianapolis, IN) diluted 1/2,000 in PBST was added to the blots, which were incubated for 1 hr. Blots were washed as before, then placed in 5 ml of orthophenylenediamine substrate. After incubation in the dark for 30 min, blots were briefly washed in PBST, dried, exposed to transmitted visible light, and photographed.

Immunodiffusion tests were done by the SDS method (12) or by grinding infected plant tissue in PBS (0.02 M phosphate, 0.15 M NaCl, pH 7.4) containing 2% Triton X-100. In the latter case, samples were placed in wells of 0.8% Noble agar (in PBS) plates with homologous or heterologous antisera in center wells.

Purified virion preparations were used for antiserum production. Three intramuscular injections, 1 wk apart, using 1 ml purified virions ( $A_{260nm} = 2.0$ ) were given to a single New Zealand white rabbit. Virions were emulsified in 1 ml of complete adjuvant (Difco) for the first injection and in 1 ml each of incomplete adjuvant (Difco) for the second and third injections. Blood was collected starting 20 days after the first injection, and antiserum was processed as described (11). Antiserum was used for indirect ELISA tests as described (7) and for serologically probing "Western blots."

### RESULTS AND DISCUSSION

**Virus source, transmission, and host range studies.** Field incidence of lettuce plants showing the described symptoms was low. Two crisphead lettuce plants

showing bright yellow spotting symptoms were found in January 1984, and two crisphead and one cos lettuce plant were found in November 1984. Serological tests (indirect ELISA and SDS-immunodiffusion) showed that none of these five plants were infected with LMV or BiMV. A virus was mechanically transmitted from all five field-collected lettuce plants to *N. × edwardsonii* and to *Chenopodium quinoa* Willd. Symptoms on *N. × edwardsonii* included necrotic local lesion development on inoculated leaves 6–7 days postinoculation. Systemic symptoms developed 10–14 days postinoculation on upper uninoculated leaves. These included severe leaf curling, yellowing of leaf veins, and interveinal mottling. *C. quinoa* plants developed

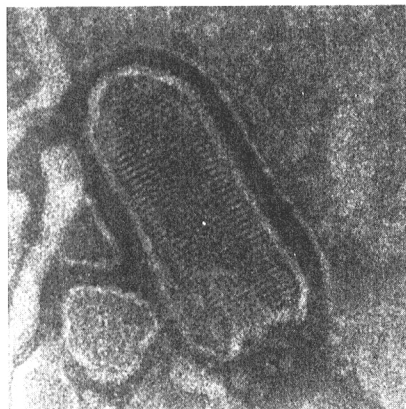


Fig. 1. Electron micrograph ( $\times 304,000$ ) of a phosphotungstic acid-stained leaf-dip preparation from *Nicotiana × edwardsonii* infected with the Florida isolate of sonchus yellow net virus showing a typical "bullet-shaped" virion.

necrotic local lesions on inoculated leaves 10–14 days postinoculation.

Host range studies using infected *N. × edwardsonii* as the inoculum source showed that *N. clevelandii* Gray, *N. debneyi* Domin, and *Sonchus oleraceus* L. also were virus hosts, whereas *Vigna unguiculata* Walp. and *Solanum nigrum* L. were not infected. When plants of lettuce cultivars Minetto and Gallega were inoculated using infected *N. × edwardsonii* as the inoculum source, a systemic, yellow, interveinal chlorosis developed 3–4 wk postinoculation, demonstrating that the virus isolated from symptomatic field plants was the causal agent of the previously observed lettuce disease.

**Identification.** Electron microscopic examination of symptomatic *N. × edwardsonii* plants revealed particles characteristic of the plant rhabdovirus group. Particles were not abundant in leaf dips but were easily differentiated from other material in the preparations. The average dimensions for seven particles were  $226 \pm 12 \times 91 \pm 6$  nm. Typical "bullet-shaped" virion morphology with partial virion detail enhancement was seen when samples were stained with PTA (Fig. 1), whereas intact bacilliform particles that appeared mostly to be superficially stained were seen when samples were stained with uranyl acetate.

Virions were purified from infected *N. × edwardsonii* and *N. clevelandii*. Virion protein analysis by SDS-PAGE showed four major virion proteins (Fig. 2A), which were distinct from those of two other plant rhabdoviruses, MMV and

SYDV. When the SDS-PAGE-analyzed proteins were serologically tested using antisera to three plant rhabdoviruses, positive serological reactions for the Florida lettuce rhabdovirus proteins occurred only with antiserum to the type isolate of SYNV and not with antisera to MMV or SYDV (Fig. 2B–D). Similarly, SYDV antisera reacted only with SYDV proteins and MMV antisera reacted only with MMV proteins. Antisera produced to the Florida lettuce virus also reacted specifically with its SDS-PAGE-separated virion proteins and not those of MMV or SYDV. Similar results showing the serological distinctness of these three rhabdoviruses were achieved by immunodiffusion tests. More distinct reactions were obtained when tests were done in PBS plates than in SDS plates, and each antiserum reacted only with homologous antigens. On the basis of virion morphologies, host range, virion protein content, and serological properties, this virus is designated a lettuce isolate of sonchus yellow net virus.

The Florida SYNV antiserum generally gave weak reactions with sap from infected *N. × edwardsonii* by immunodiffusion tests, and this method was determined unsatisfactory for diagnosing field lettuce samples. However, the Florida SYNV antiserum reacted sufficiently strongly by indirect ELISA with SYNV-infected *N. edwardsonii* ( $A_{405nm} = 0.4$ ) and thus was an acceptable means for diagnosing field lettuce samples for SYNV infection. In November 1984, three field lettuce plants reacted positively for SYNV by indirect ELISA (average  $A_{405nm} = 0.12$ ), and these were confirmed to be SYNV-infected by mechanical inoculation tests to *N. × edwardsonii*. Healthy lettuce ( $A_{405nm} = 0.01$ ) or lettuce plants infected with LMV or BiMV did not react with Florida SYNV antiserum by indirect ELISA.

SYNV was originally discovered in Florida (2). At least two other rhabdoviruses occur naturally in lettuce, sowthistle yellow vein virus (SYVV) and lettuce necrotic yellows virus (LNYV) (4,13). However, SYNV can be distinguished from both SYVV and LNYV by comparing several characters including host range, aphid vector species, and serological and biochemical properties (2–4,10,13). Neither SYVV nor LNYV can be mistaken here for the Florida SYNV because SYVV is not mechanically transmissible as is SYNV, and LNYV has only three major virion proteins while SYNV has four (2–4,10). Lettuce was previously found to be an experimental host for SYNV, and it was pointed out then that SYNV might sometime pose a threat to Florida lettuce (2). On the basis of the low natural incidence of SYNV over the last 2 yr, it does not appear to be an immediate threat to Florida lettuce. However, because SYNV is one of the most thoroughly characterized plant

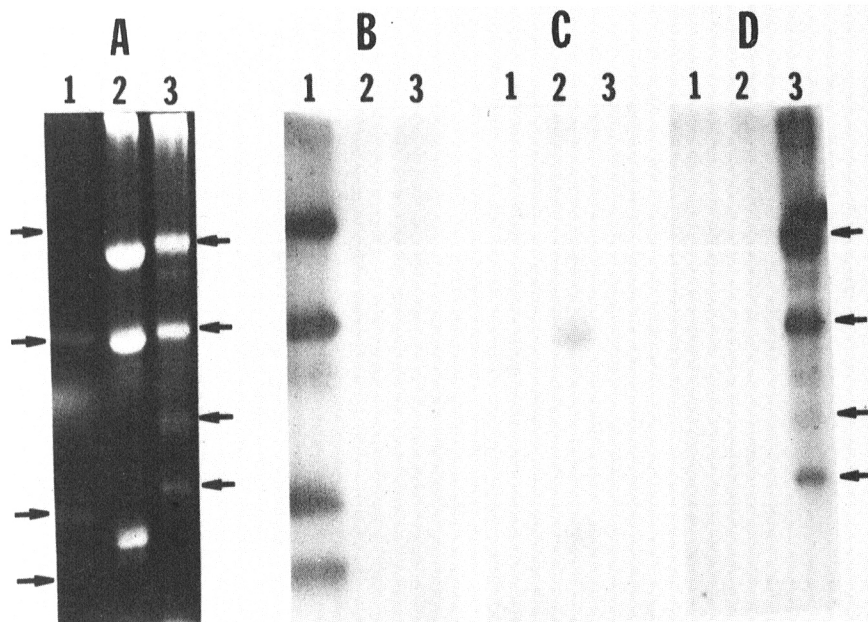


Fig. 2. (A) Sodium dodecyl sulfate-polyacrylamide slab gel showing a comparison of the major virion proteins of potato yellow dwarf virus (SYDV) (lane 1), maize mosaic virus (lane 2), and the Florida isolate of sonchus yellow net virus (SYNV) (lane 3). Arrows on left show locations of the four virion proteins of SYDV and arrows on right show locations of the four virion proteins of the SYNV. (B–D) "Western blots" of the proteins from A after probing with antisera to (B) SYDV, (C) maize mosaic virus, and (D) SYNV. Arrows show reactions of the four virion proteins.

rhabdoviruses (10), information on its natural incidence in an economically important host is significant.

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