Transmission of a Bacilliform Virus of Sowthistle and Bidens pilosa

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ABSTRACT

Sonchus yellow net virus (SYNV) was isolated from sowthistle (*Sonchus oleraceous*) and *Bidens pilosa* in Florida. SYNV was mechanically transmitted to a *Nicotiana* hybrid (*N. clevelandii* × *N. glutinosa*), sowthistle, *B. pilosa*, *Nicotiana glutinosa*, *Nicotiana clevelandii*, *Zinnia elegans*, and lettuce (*Lactuca sativa*). Inoculum prepared by triturating leaf tissue in water lost infectivity rapidly, but was substantially stabilized by 0.5% sodium sulphite. SYNV was transmitted by *Aphis coreopsidis*, but not by *Hyperomyzus lactucae* or *Dactynotus* sp. Nuclear inclusions were observed by light microscopy. Electron microscopy of ultrathin sections of leaf tissue revealed bacilliform particles in the cytoplasm, but more often in the nucleus. Similar particles were found in cells of the aphid vector. The properties of SYNV are discussed in relation to other bacilliform viruses known to infect sowthistle, including sowthistle yellow vein virus, lettuce necrotic yellows virus, and Gomphrena virus. SYNV has been found in widely separated locales in Florida, and can be considered a potential threat to Florida lettuce production.

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In 1954 a serious outbreak of a virus disease infecting lettuce (*Lactuca sativa* L.) in Australia led L. L. Stubbs, later with the help of R. G. Grogan, to initiate a comprehensive study of the disease (22). The causal virus, lettuce necrotic yellows (LNYV), was found to occur naturally in sowthistle (*Sonchus oleraceous* L.) and to be transmitted in a persistent manner to lettuce by the aphid *Hyperomyzus lactucae* (L.). Later work by Harrison and Crowley (13) showed that preparations from diseased tissue contained bacilliform particles, placing LNYV morphologically in a large group of viruses, the rhabdoviruses, that cause a range of diseases extending from the higher plants to man (14).

Sowthistle yellow vein virus (SYVV) was reported in California and Arizona by Duffus (8) and in England by Duffus and Russell (9). SYVV is similar to LNYV in that both are transmitted by *H. lactucae* in a persistent manner, infect sowthistle (8) and lettuce (10, 21), and have a bacilliform morphology (17, 21).

Kitajima and Costa (16) reported on Gomphrena virus (GV) in Brazil. They concluded that GV was LNYV or closely related to it.

The present report describes characteristics of sonchus yellow net virus (SYNV), which was isolated from sowthistle, and *Bidens pilosa* L. in Florida. SYNV is similar in many respects to LNYV, SYVV, and GV; but it also has significant differences which are discussed here.

MATERIALS AND METHODS.—*Mechanical transmission trials.*—Leaf tissue was triturated with a mortar and pestle with 2 ml sterile distilled water or sodium sulphite added/gram tissue. Celite (0.2%, w/v) was added after grinding. Plants were held at least 5 wk

after inoculation for a final reading of symptom expression.

Aphid transmission trials.—A clone of *H. lactucae* known to transmit SYVV was obtained from Jean Richardson, University of California at Berkeley. Individuals of *H. lactucae* were collected locally from sowthistle. Individuals tentatively identified as a *Dactynotus* species also were collected locally from sowthistle. All aphids were reared in cages on sowthistle plants that had been mechanically inoculated with SYNV and which were showing distinct symptoms. *Aphis coreopsidis* (Thomas) were collected locally from *B. pilosa* and reared in cages on SYNV-infected *B. pilosa*. All aphid colonies were reared at least 30 days on infected plants before being used in experiments outlined here. Exposure of test plants to aphids was made either with a small brush, or by placing test plants in cages which contained aphid colonies on virus infected plants. The aphids were killed with a Cygon soil drench. The test plants were then held at least 5 wk for a final reading of symptoms.

Light and electron microscopy.—Epidermal strips were stained for light microscopy with azure B (5) or calcomine orange-"Luxol" brilliant green (4). Leaf material was negatively stained for electron microscopy by slicing small pieces in a few drops of 1% potassium phosphotungstate, pH 6.7, containing 0.05% bovine serum albumin. A droplet of the stain-sap mixture was placed on a grid coated with Formvar and carbon. The liquid was allowed to remain on the grid for 1 min and removed with filter paper. Material was prepared for ultra-thin sectioning as described by Edwardson et al. as the "conventional" method (11).

RESULTS.—*Symptoms on indicator plants.*—The original source of SYNV was a sowthistle plant showing distortion and general yellowing. Leaf material was ground in water with Celite added after grinding. This provided inoculum for a set of test plants consisting of (four each) *Nicotiana* hybrid (*N. clevelandii* × *N. glutinosa*) (6), *Capsicum frutescens* L. 'California Wonder', *Gomphrena globosa* L., *Chenopodium amaranticolor* Coste & Reyn., and *C. quinoa* Willd. Figure 1 depicts the appearance of the healthy *Nicotiana*. Four weeks after inoculation, two of the *Nicotiana* hybrids showed systemic vein-clearing and leaf cupping (Fig. 2). No disease symptoms were observed on the other test plants used. By using the infected *Nicotiana* hybrids as a source of inoculum the virus was transmitted to healthy sowthistle seedlings which developed symptoms as in Fig. 3. Species subsequently infected were: *B. pilosa*, Great Lakes lettuce (*Lactuca sativa* L.), *Nicotiana clevelandii* Gray, *N. glutinosa* L., and *Zinnia elegans* Jacq. Repeated attempts failed to transmit SYNV to Turkish tobacco (*N. tabacum* L.), *Datura stramonium* L., *G. globosa*, *C. quinoa*, and *C. amaranticolor*.

Symptom expression of SYNV in sowthistle is striking. Distinct chlorotic local lesions usually appear on inoculated leaves in 10 to 14 days. The earliest vein-clearing usually appears a few days later (Fig. 3). These systemic symptoms are similar to those of SYVV described and pictured by Campbell (3). Later, small yellow patches appear between the veins and enlarge until the leaf is almost completely yellow. In the advanced stages some of the yellowed areas turn brown.

Systemic symptoms in the *Nicotiana* hybrid are also quite distinct but the initial chlorotic local lesions, which usually are visible in 7 to 10 days are vague. Vein-clearing and strong downward cupping of systemically infected leaves (Fig. 2) follows a day or two after local lesion formation (compare to Fig. 1, a healthy *Nicotiana* hybrid). These symptoms do not appear in later growth but leaves are small and coarse textured and virus is still present, as determined by light microscopy and transmission trials. Occasionally these leaves may show a light chlorotic spotting. SYNV has been maintained in individual *Nicotiana* hybrids for periods of over 6 mo. The time elapsed from inoculation to symptom expression varied greatly in both the *Nicotiana* hybrid (6 to 30 days) and in sowthistle (10 to 30 days).

Great Lakes lettuce shows a light vein-clearing, general leaf yellowing, and very severe stunting of the heart, followed by some recovery. *N. clevelandii* and *N. glutinosa* exhibited symptoms similar to the *Nicotiana* hybrid. *Z. elegans* showed a transient systemic vein-clearing, followed by light systemic mottling. Under our conditions, SYNV-infected *B. pilosa* was symptomless.

Factors affecting mechanical transmission.—Transmission rates were greatly increased upon the addition of sulphite to the inoculum. To test an assumption that sulphite exerts a stabilizing effect on the inoculum, three sets of 30 *Nicotiana* hybrids each were marked to indicate the order of inoculation. Leaf tissue was collected from a SYNV-infected lettuce plant, chopped into small pieces, mixed, and weighed into three equal portions. The inoculum for each set of plants was prepared immediately before use and all of the inoculations were made by the senior author. The grinding medium for the first two sets was water. The third set was inoculated from material ground in sulphite solution. Only the No. 1 and No. 2 plants of the first set and the No. 1 plant of the second set became infected. All of the plants in the third set became infected except No. 29.

Sowthistle plants were inoculated from three SYNV-infected sources (sulphite added). The results were (successful transmissions/no. of plants tested): lettuce 8/30, sowthistle 18/30, and *Nicotiana* hybrid 14/14. The greatest number of lesions produced on any one leaf in these trials was eight from lettuce, 16 from sowthistle, and 150 from the *Nicotiana* hybrid.

Two experiments showed the *Nicotiana* hybrid to be a more sensitive test plant for SYNV than sowthistle. In the first experiment, 30 sowthistles were inoculated first and followed by five *Nicotiana* hybrids. The plants were marked to indicate the order of inoculation. Sowthistle was used as the source of inoculum (sulphite added). Eighteen of the first 20 sowthistles became infected, but none of the last 10 developed symptoms. All five of the *Nicotiana* hybrids inoculated subsequently became infected. In the second experiment, in which 30 sowthistles were inoculated from SYNV-infected lettuce, only eight became infected. Twenty-nine out of 30 *Nicotiana* hybrids inoculated from the same source plant became infected. Nevertheless, sowthistle was a useful test plant in that it produced the most discrete local lesions of any plant tested.

In an attempt to determine if SYNV is identical to SYVV, which it closely resembles in its symptoms on sowthistle, the following test was carried out: Five *Nicotiana* hybrids followed by 20 sowthistles again followed by five *Nicotiana* hybrids were inoculated (sulphite added) from SYVV-infected sowthistle leaves. The material used for the inoculum arrived from California in good condition and showed distinctive symptoms. It was felt that if SYVV and SYNV were indeed identical, transmission should be achieved, considering the success we had in transmitting SYNV from sowthistle to sowthistle and the *Nicotiana* hybrid. No transmissions of SYVV were affected.

Aphid transmission trials.—Aphids were allowed to colonize diseased plants at least 1 mo before being used in the trials. In all of the trials in which test plants were caged with aphids, vigorous colonies became established and numerous winged forms developed.

A variety of transmission trials were attempted with *H. lactucae*. Numerous mature aphids were transferred to each of 25 healthy sowthistles. After several days they were killed with a Cygon soil drench. Numerous aphids of

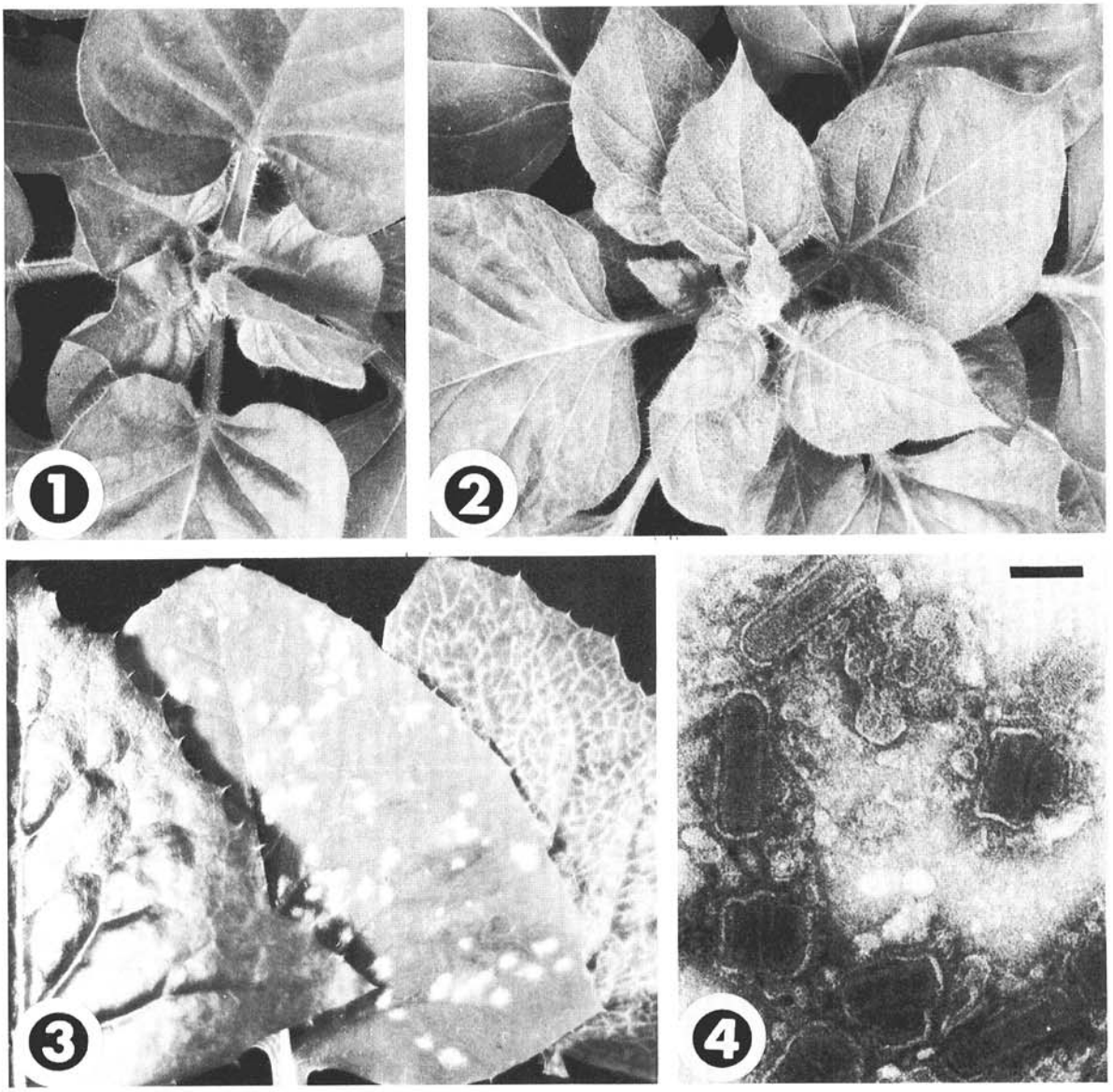

Fig. 1-4. Sonchus yellow net virus (SYNV) infection of *Nicotiana* hybrids and sowthistle. **1-2)** Apical leaves of *Nicotiana* hybrids; **1)** Healthy, and **2)** systemically infected with SYNV showing vein-clearing and cupping of leaves. **3)** Symptoms of SYNV on sowthistle. Left to right: Healthy leaf, local chlorotic lesions on an inoculated leaf, and systemic chlorotic vein-clearing. **4)** Electron micrograph of a leaf dip of SYNV infected *Nicotiana* hybrid negatively stained with potassium phosphotungstate. Note bullet-shaped particles. Scale bar = 100 nm.

various instars were transferred to seven healthy sowthistles and killed after 4 wk. In each of three separate trials, two diseased sowthistles with established colonies of *H. lactucae* were placed in insect-proof cages with 10 healthy sowthistles for several months. In another trial, nonviruliferous *H. lactucae* of a clone known to be vectors of SYVV were reared for 1 mo on four SYNV-infected sowthistles in an insect-proof cage. At the end of this period, 16 healthy sowthistle seedlings were placed in the cage. After 1 wk, three plants were removed and the aphids on them killed. Six more sowthistles and other susceptible plants were placed in the cage for 1 wk before

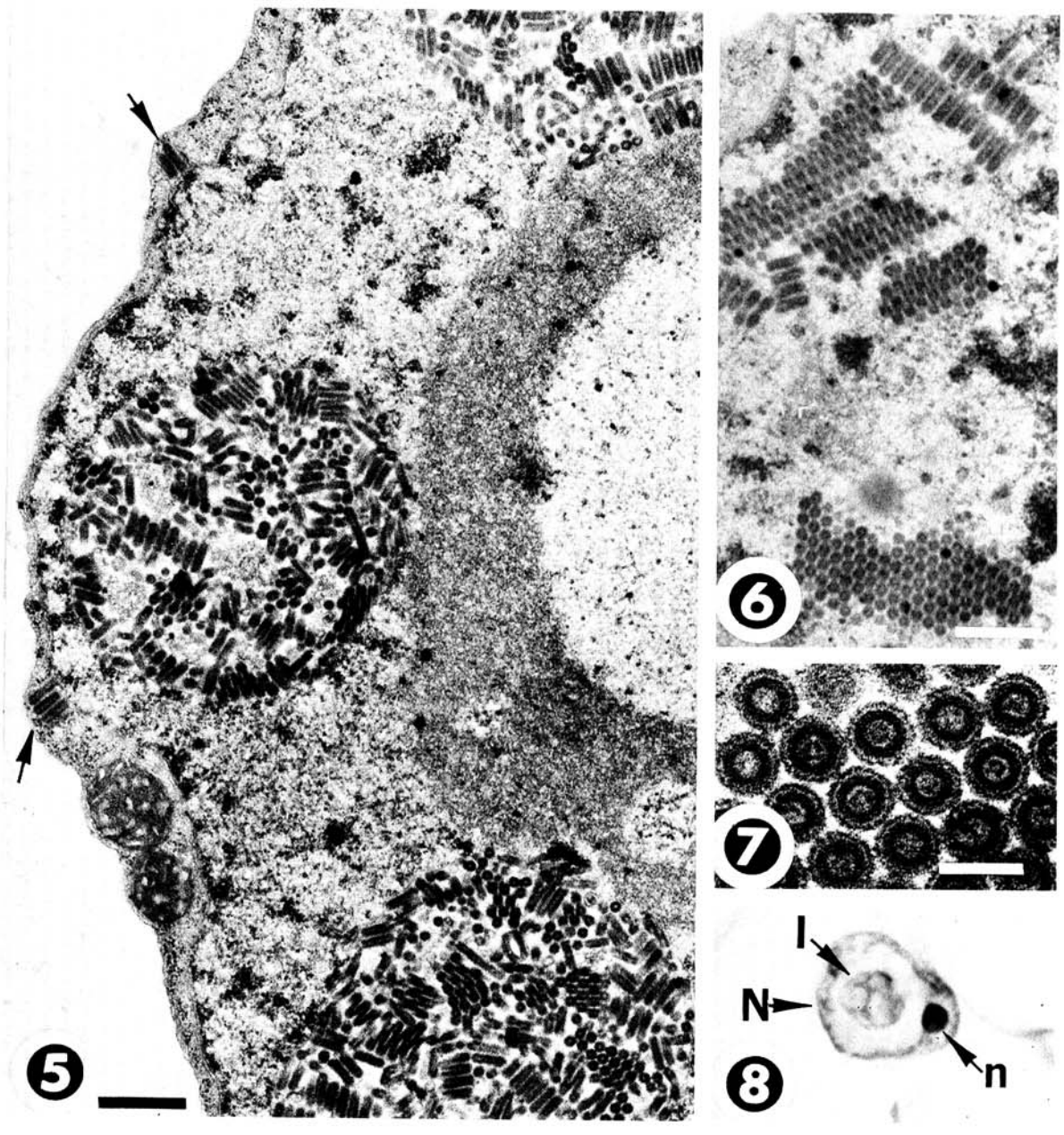

Fig. 5-8. Electron and light micrographs of SYNV-infected sowthistle and *Nicotiana* hybrid. **5)** Electron micrograph of ultrathin section. Globular aggregates of virus particles in nucleus. Note particles in cytoplasm (arrows). Scale bar = 500 nm. **6)** Electron micrograph of ultrathin section. Crystal arrays of virus particles in nucleus. Scale bar = 500 nm. **7)** Electron micrograph of ultrathin section. Detail of virus particles in cross-section. Scale bar = 100 nm. **8)** Light micrograph of an epidermal strip from an SYNV infected *Nicotiana* hybrid stained with azure B. N = nucleus, n = nucleolus, I = inclusion. Note nonstaining area surrounding inclusion. ×1,500.

all of the test plants were removed and the aphids killed. In all of the trials, test plants were held 5 wk after exposure for final reading of symptoms. Epidermal strips from some of the leaves were checked for the presence of nuclear inclusions in the light microscope. No transmission of SYNV was effected in any of these trials.

Twelve sowthistles and other test plants were caged several months with four diseased sowthistles supporting colonies of a *Dactynotus* species. None of the test plants developed SYNV symptoms.

Four sowthistles and four *Nicotiana* hybrids were caged with a *B. pilosa* plant infected with SYNV and supporting a colony of *A. coreopsidis*. All four sowthistles and two of the *Nicotiana* hybrids became infected. In another trial, aphids of various instars were transferred from an infected *B. pilosa* to 10 sowthistles. Five aphids were placed on each test plant. Three of the 10 plants became infected with SYNV.

Electron and light microscopy.—In the electron microscope, "bullet-shaped" particles were observed in leaf dips made from SYNV-infected material (Fig. 4). Electron microscopy of ultra-thin sections of SYNV-infected sowthistle revealed nuclear inclusions composed of bacilliform particles in globular aggregates (Fig. 5) and crystalline arrays (Fig. 6). Particles were seen both in cross-section and longitudinal section (Fig. 5, 6, 7). Although most particles were found in the nucleus, they occasionally were seen in the cytoplasm (Fig. 5). Bacilliform particles were also seen in sections of cells of viruliferous aphids.

Stained epidermal strips from SYNV-infected leaves exhibited from one large to many smaller inclusions in nuclei of cells associated with areas of vein-clearing and local lesions. When stained with azure B, the nucleus is blue, the nucleolus is violet, and the nuclear inclusions are pink. With the orange-green stain the nucleus is orange, the nucleolus green, and the nuclear inclusions are deep brown. In older SYNV-infected leaves, nuclei have large nonstaining areas, with a smaller inclusion or none at all (Fig. 8). These observations applied to the tissue of all the SYNV-infected plants studied. Such nuclear inclusions were not observed in healthy tissue.

DISCUSSION.—In only a few cases have aphids been implicated as vectors of bacilliform viruses of plants. *H. lactucae* (8) and *Macrosiphum euphorbiae* (1) are vectors of SYVV; *H. lactucae* (22) and *H. carduellinus* (19) of LNYV. *Anphorophora rubi* Kalt. is the vector of raspberry mosaic virus (2) which is belived to be a member of the Rhabdovirus group (18). *Chaetosiphon jacobi* H.R.L. is the vector of strawberry crinkle virus (12) shown to be associated with bacilliform particles (20). Kennedy et al. (15) place *H. lactucae*, *H. carduellinus* and *C. jacobi* in the sub-family *Myzinae*, *M. euphorbiae* and *A. rubi* in the Dactynotinae and *A. coreopsidis* in the *Aphidinae*. This report seems to be the first of the occurrence of a rhabdovirus transmitted by the sub-family *Aphidinae*.

SYVV infects lettuce (10, 21) and sowthistle (8). It has no other known hosts and has not been mechanically transmitted (21). LNYV (22), GV (16), and SYNV also infect lettuce and sowthistle but LNYV (22) and GV (16) apparently have wider host ranges than SYVV or SYNV. We have been unable to infect some of the reported hosts of LNYV and GV with SYNV (e.g., *D. stramonium* or *G. globosa*). SYVV and LNYV differ from SYNV in reported vectors. There are no reports on insect vectors of GV. Based on presently known information SYNV is considered to be either a distinct strain of SYVV or LNYV or is a previously undescribed virus of the rhabdovirus group.

During the course of these investigations, SYNV has been found naturally occurring in *B. pilosa*, a common weed in Florida. Leaf samples from southern, central, and northern Florida were ascertained to be infected with SYNV on the basis of nuclear inclusions, the presence of bacilliform particles, and symptoms on test plants. It can therefore be assumed that SYNV is widespread in *B. pilosa* in Florida. In every case in which SYNV was found in *B. pilosa*, it was found in combination with bidens mottle virus (BMV) (7). This is not surprising as SYNV alone is apparently symptomless in *B. pilosa* while in combination with BMV a necrotic etching is usually superimposed over the chlorotic mottle induced by BMV alone is apparently symptomless in *B. pilosa*; while in combination with BMV, a necrotic etching is usually vector of SYNV, makes SYNV a potential problem in Florida lettuce production, and the difficulty of detecting it in *B. pilosa* could further compound the problem.

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