Key West Nightshade, a New Experimental Host for Plant Viruses

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ABSTRACT

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Key West nightshade (*Solanum bahamense*) is a perennial solanaceous weed found in the extreme southern portion of Florida. It can be propagated by seed and cuttings and is absent from the noxious weed lists of all U.S. states. Its susceptibility to five viruses common to Florida was evaluated by mechanical inoculation of leaves with *Tomato spotted wilt virus* (TSWV), *Tobacco mosaic virus* (TMV), *Pepper mild mottle virus* (PMMoV), *Cucumber mosaic virus* (CMV), and a putative tobamovirus recently isolated from hibiscus in Florida (HV). TSWV induced chlorotic rings on inoculated leaves and mosaic and malformation of uninoculated leaves. CMV induced necrotic local lesions on inoculated leaves. No symptoms were observed following inoculation with TMV, PMMoV, or HV. TSWV, TMV, and PMMoV systemically infected *S. bahamense* as determined by the use of enzyme-linked immunosorbent assay, reverse transcription-polymerase chain reaction, viral-associated double-stranded RNA analysis, and/or indicator hosts. Active growth of infected plants continued for 7 months following inoculation, making *S. bahamense* suitable for long-term maintenance of viruses in planta. We suggest that *S. bahamense* may be a useful host for virus culture collections and for studies involving large numbers of virus isolates where fresh, infected tissue is continuously required.

Although plant virologists generally focus their research on economically important crops, there are several instances where noncrop plants merit consideration. Such plants, frequently weeds, are important (i) reservoirs for viruses causing economic losses in crop plants, (ii) experimental hosts for detection, identification, maintenance, or easier manipulation of such viruses, and (iii) targets for biocontrol by viruses (8,18). Weeds have long been known to harbor plant viruses and the vectors that transmit them (6,14). Since they can serve as an important source of inoculum for crop plants, numerous weed species have been explored (naturally infected and/or experimentally inoculated) as reservoir hosts. A great many of these are in the Solanaceae (3,4,7,15,20,27), and some weed species, e.g., *Datura stramonium*, have proven to be useful experimental hosts (6). Additional experimental and/or indicator hosts from multiple plant genera are available to virologists. Many of these are also in the Solanaceae (5,19), especially the genus *Nicotiana* (25,26). Although a few of these species are perennials (16,17), the vast majority of

experimental hosts are annuals. A perennial plant species easily manipulated under experimental conditions and susceptible to commonly studied plant viruses may find use in virus culture collections and research with viruses that lose infectivity upon storage.

As part of our research on *Tomato spotted wilt virus* (TSWV) diversity, we have tested many solanaceous plants for their response to infection. American black nightshade, *Solanum americanum*, is regularly employed as an indicator host in our studies. However, *S. americanum*, like all other plants tested to date, has a very short useful life following TSWV infection. Short host life represents a limitation for our research because we have determined that fresh leaf tissue is a better source of inoculum, viral RNA, and viral protein than frozen leaf tissue. We therefore have a need for a TSWV host that will continue active growth following infection.

In this report, we examined the "virus range" of a previously unstudied perennial member of the Solanaceae, Key West nightshade (*Solanum bahamense* L.). The term "virus range," coined by Christie and Crawford (9), is an assessment of the viruses to which a particular host plant is susceptible. Representatives of three different virus genera were assayed. While the virus range reported was not developed by testing all possible viruses that may infect *S. bahamense*, the results with the five viruses assessed here demonstrate the utility of this plant as a new experimental host. We show that *S. bahamense* is a useful long-term host for TSWV, *Tobacco*

mosaic virus (TMV), and *Pepper mild mottle virus* (PMMoV), viruses generally confined to short-lived herbaceous plants, because it continues to grow for many months following inoculation.

MATERIALS AND METHODS

Field collection of *S. bahamense*.

While hiking on Bahia Honda in the Florida Keys, we observed a single shrub-like tree with red-orange fruit (Fig. 1) that were morphologically similar to those of *S. americanum*. As part of our continuing search for a long-term TSWV host suited to our needs, we collected fruit and extracted seeds, which we subsequently planted in the greenhouse. Three seeds germinated and grew into plants identified as Key West nightshade, *S. bahamense*. These three plants were maintained as stock plants for propagation.

Inoculation of *S. bahamense*. In addition to TSWV, four other viruses commonly found in Florida were used to make an initial determination of the "virus range" of *S. bahamense* and to evaluate its suitability as a host for use in virology experiments. Viruses tested were: TMV strain U1 (kindly provided by Dennis Lewandowski), Florida isolates of PMMoV (2), a putative tobamovirus recently detected in hibiscus (HV; 1), *Cucumber mosaic virus* (CMV; kindly provided by Mark Gooch), and TSWV. Inocula were prepared from virus-infected leaf tissue of *D. stramonium* (TSWV), tobacco (*Nicotiana tabacum* cv. Xanthi; TMV, PMMoV, and CMV), and *Chenopodium quinoa* (HV). Inoculum for TSWV was prepared by homogenization of infected leaf tissue in 0.5% (wt/vol) sodium sulfite containing 1% (wt/vol) Celite as an abrasive using a mortar and pestle. Inocula for TMV, PMMoV, HV, and CMV were prepared by homogenization of the infected leaf tissue in 20 mM sodium phosphate buffer (pH 7.0) containing 1% (wt/vol) Celite. Each of five independent groups of *S. bahamense* plants (two or three plants each) was inoculated with one of the five viruses. Cheesecloth was used to apply inocula to several marked leaves per plant. A sixth group of plants was mock-inoculated with phosphate buffer.

Determination of "virus range." Inoculated plants were monitored weekly for symptom development. Following the first appearance of symptoms on TSWV-inoculated (marked) leaves 1 month post-inoculation, uninoculated leaves were collected from all plants and tested for the

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presence of the input virus by at least two of the following techniques: enzyme-linked immunosorbent assay (ELISA), reverse transcription-polymerase chain reaction (RT-PCR), viral-associated double-stranded (ds) RNA analysis, and/or indicator host inoculation. A commercially available ELISA kit (Agdia, Elkhart, IN) routinely employed in our laboratory was used to test for TSWV. RT-PCR was used for detection of TSWV, PMMoV, and TMV according to standard protocols (22,23) with virus-specific primers (Table 1). Briefly, first strand cDNA was synthesized by *Moloney murine leukemia virus* reverse transcriptase (Promega, Madison, WI) at 50°C for 45 min. This was followed by 30 cycles of PCR amplification with *Taq* polymerase at 94°C for 45 s, 55°C for 45 s, and 72°C for 1 min. Products were analyzed by electrophoresis on native 2% agarose gels and detected by ethidium bromide staining. DsRNA analysis was selected for examination of infection by TMV, PMMoV, HV, and CMV, as these four viruses are amenable to detection by this method. DsRNA was extracted

from 7-g samples of uninoculated *S. bahamense* leaf tissue following a protocol previously published (24), although only a single cycle of cellulose chromatography was used. DsRNA was analyzed by electrophoresis on native 5% polyacrylamide gels and detected by silver staining using a commercially available kit (Bio-Rad, Hercules, CA). Upper noninoculated leaf tissue from inoculated *S. bahamense* plants was homogenized and used to inoculate appropriate indicator hosts for TMV, PMMoV, HV, and CMV based on the literature (1,10,28,29).

RESULTS

Culture of *S. bahamense*. Although the three plants started from field-collected seed grew vigorously and flowered profusely (Fig. 1A and B) in our greenhouse, no fruit were produced. This was in striking contrast to *S. americanum*, which produces abundant fruit in our greenhouse. Hand pollination of *S. bahamense* flowers was necessary for fruit production, but even then, fruit set was quite poor (Fig.

1C). Vegetative propagation via cuttings from the original three plants was found to be a much easier and more expedient means of plant production, especially with the use of commercially available auxin (Rootone, Green Light Co., San Antonio, TX). Cuttings rooted and were ready for inoculation in 2 weeks, while 2 to 3 months were required for seed to germinate and produce plants suitable for propagation by cuttings.

“Virus range” of *S. bahamense*. Chlorotic rings and ring patterns developed on *S. bahamense* leaves inoculated with TSWV by 4 weeks postinoculation (Fig. 2A) on two of three inoculated plants. Symptoms of systemic infection, including malformation of leaves (Fig. 2B), a generalized mosaic, and localized necrosis, were readily apparent several weeks later on these plants, an observation confirmed by the use of ELISA and RT-PCR (Table 2). RT-PCR with TSWV-specific primers TSWV723 and TSWV722 (Table 1) amplified the expected 620-bp product from total RNA extracted from the TSWV-infected

Fig. 1. *Solanum bahamense* plants greenhouse-grown from field-collected seed. **A**, Whole plant with meter stick for scale. **B**, Oblong to lanceolate leaves and racemose inflorescence with purple corolla. **C**, Glabrous immature fruit set following hand pollination. Note poor fruit set compared to number of flowers on inflorescence in (**B**).

Table 1. Oligonucleotides used for reverse transcription-polymerase chain reaction amplification

Name	Sequence (5'-3')	Region of viral RNA ^a	Product size (bp)
TSWV723	CACAAGGCAAAGACCTTGAG	2698-2717 ^b	620
TSWV722	GCTGGAGCTAAGTATAGC	2098-2118 ^c	
TMV3'vc	TGGGCCCTACCGGGG	6379-6395 ^b	360
TMV5'vAccI	TAATACGACTCACTATAGGTTATTTTACAACAATTACCAAC ^d	6054-6087 ^c	
PMMoV3'vcA	AATGGGCCCTGGGCCGCTACCGCGGTTTC ^e	6338-6357 ^b	419
tob268CPv	GAYWCHMGDAAYAGRRYHHATHGA	5949-5971 ^c	

^a Indicated in nucleotides on the *Tomato spotted wilt virus* genomic S RNA segment or the *Tobacco mosaic virus* and *Pepper mild mottle virus* genomic RNA.

^b Complementary to the viral genome in this region.

^c Corresponds to the viral genome in this region.

^d Includes a T7 RNA polymerase promoter (underlined).

^e Includes an *Apa*I site (underlined) and three additional 5' nucleotides to optimize restriction.

but not the mock-inoculated plants (Fig. 3, lanes 6 and 7).

Red-brown local lesions developed on *S. bahamense* leaves inoculated with CMV by 2 weeks postinoculation (Fig. 2C) on both of the inoculated plants. No symptoms of systemic infection were apparent, an observation verified by the use of dsRNA analysis and indicator hosts (Table 2). No CMV-associated dsRNA was recovered from uninoculated leaves of *S. bahamense* (Fig. 4, lane 6). However, an endogenous dsRNA was detected in the CMV-inoculated and all other *S. bahamense* plants assayed, including the mock-inoculated plants (compare Fig. 4, lane 2, with lanes 3 to 6). At 9 weeks

postinoculation with CMV, sap from uninoculated *S. bahamense* leaves did not contain CMV as judged by the absence of symptoms on indicator hosts (Table 2).

Although no symptoms were observed through 7 months postinoculation, TMV and PMMoV both systemically infected all inoculated *S. bahamense* plants as determined by the use of RT-PCR, dsRNA analysis, and indicator hosts (Table 2). RT-PCR with the TMV-specific primers TMV3'vc and TMV5'vAccI or PMMoV-specific primer PMMoV3'vcA and degenerate tobamovirus primer tob268CPv (Table 1) amplified the expected 360- and 419-bp products from total RNA extracted from the TMV-infected (Fig. 3, lane 3) and

PMMoV-infected plants (Fig. 3, lane 5), respectively. No products were amplified from total RNA extracted from mock-inoculated plants (Fig. 3, lanes 2 and 4). DsRNA analysis provided additional support for systemic infection of *S. bahamense* by both TMV and PMMoV, as evidenced by the isolation of a dsRNA from both TMV- and PMMoV-inoculated plants that comigrated with the TMV dsRNA marker (Fig. 4, lanes 1, 3, and 4). As late as 7 months postinoculation with TMV or PMMoV, uninoculated *S. bahamense* leaves were an excellent inoculum source as judged by the symptoms on indicator hosts (Table 2).

As with the two recognized tobamoviruses, TMV and PMMoV, no symptoms

Fig. 2. Symptoms of virus infection in inoculated *Solanum bahamense* plants. **A**, Large (1 to 2 cm) chlorotic rings on inoculated leaf following inoculation with *Tomato spotted wilt virus* (TSWV). **B**, Malformation of uninoculated leaves on TSWV-inoculated plant. Note thorns on stem. **C**, Small (2 to 3 mm) red-brown necrotic local lesions following inoculation with *Cucumber mosaic virus*.

Table 2. Determination of “virus range” of *Solanum bahamense* by enzyme-linked immunosorbent assay (ELISA), reverse-transcription polymerase chain reaction (RT-PCR), viral-associated double-stranded RNA analysis (dsRNA), and inoculation of indicator hosts

Virus	ELISA	RT-PCR	dsRNA	Indicator host	
				Symptoms	Species
<i>Tomato spotted wilt virus</i>	+ ^a	+	ND ^b	ND	NS ^c <i>Nicotiana benthamiana</i>
<i>Cucumber mosaic virus</i>	ND	ND	—	—	NS <i>Nicotiana tabacum</i> cv. Xanthi
					NS <i>Nicotiana tabacum</i> cv. Xanthi nc
					NS <i>Nicotiana glutinosa</i>
					NS <i>Datura stramonium</i>
					NS <i>Cucumis sativus</i>
<i>Tobacco mosaic virus</i>	ND	+	+	+	SN <i>Nicotiana benthamiana</i>
					SM <i>Nicotiana tabacum</i> cv. Xanthi
					LL <i>Nicotiana tabacum</i> cv. Xanthi nc
					LL <i>Nicotiana rustica</i>
					LL <i>Datura stramonium</i>
					LL <i>Chenopodium quinoa</i>
<i>Pepper mild mottle virus</i>	ND	+	+	+	LL <i>Nicotiana tabacum</i> cv. Xanthi nc
					LL <i>Nicotiana glutinosa</i>
					LL <i>Nicotiana rustica</i>
					LL <i>Nicotiana sylvestris</i>
					LL <i>Datura stramonium</i>
<i>Hibiscus virus</i>	ND	ND	—	—	NS <i>Chenopodium quinoa</i>

^a Systemic infection of *Solanum bahamense* confirmed (+) or not (–) by technique indicated.

^b ND = not determined.

^c NS = no symptoms, SN = systemic necrosis, SM = systemic mosaic, LL = necrotic local lesions.

were observed following inoculation with the putative tobamovirus HV. However, unlike TMV and PMMoV, HV did not systemically infect any of the three inoculated *S. bahamense* plants as determined by the use of dsRNA analysis and indicator hosts (Table 2). No HV-associated dsRNA was recovered from uninoculated leaves of *S. bahamense* (Fig. 4, lane 5). At 10 weeks postinoculation with HV, sap from uninoculated *S. bahamense* leaves did not contain HV as judged by the absence of symptoms on *C. quinoa* (Table 2).

DISCUSSION

This report demonstrates that *S. bahamense* meets our needs as a long-lived TSWV host. We work with many isolates of TSWV in a single experiment, and *S. bahamense* provides a means of keeping fresh, infected tissue for multiple isolates available simultaneously for months on end. This report also suggests that *S. bahamense* may find more general application as a perennial host for viruses of herbaceous plants, making another experimental tool available to plant virologists. Additional research is required to develop a more complete "virus range" for this species, but the current report demonstrates its usefulness as a long-term host for one tospovirus and two tobamoviruses.

Many other members of the Solanaceae, especially those in the genus *Nicotiana*, have previously been demonstrated to be hosts for a plethora of plant viruses (25,26). One widely used *Nicotiana* species is *N. benthamiana*, which has been known for nearly 30 years to be susceptible to many plant viruses (9,21). Most of these solanaceous species are annual plants with short life cycles, frequently made shorter by virus infection. While this has little impact on the use of these species for detection, identification, and/or propagation of viruses, it is a major limitation for long-term maintenance of viruses such as TSWV in these plants.

Most prior research has focused on developing herbaceous plants to facilitate the study of viruses of woody perennial ornamental and fruit crops (11,12). Notable exceptions to the herbaceous host trend are working research collections of viruses of perennial fruit crops, e.g., citrus, which are maintained in the crop species (13). Several perennial ornamental species in the Solanaceae have also been explored for this purpose (16,17).

This report documents several characteristics that make *S. bahamense* suitable as a perennial host for viruses that generally infect annual crops. Our original three plants have been growing for a year and a half in the greenhouse. Although they were maintained in large containers and allowed to grow quite large (>1 m as seen in Fig. 1A), this was only to maintain sufficient tissue for propagation. Plants used in experiments were routinely maintained in 4-

inch pots (up to 7 months) and regularly trimmed to maintain a height of 30 cm or less. New growth on TSWV-, TMV-, and PMMoV-infected plants was always infected. Vegetative propagation allows for rapid production of *S. bahamense* cuttings once a stock plant is established. Vegetative propagation also eliminates plant-to-plant variation that can sometimes arise in

experimental hosts grown from seed. Inefficient fruit production by *S. bahamense* is a good trait because it makes escape of this plant into the environment unlikely, a fact noted by its absence on noxious weed lists for all U.S. states. On a practical level, *S. bahamense* has numerous thorns on both its stems (Fig. 2C) and leaves. This can lead to sore fingers for the incautious vi-

Fig. 3. Detection of systemic infection in inoculated *Solanum bahamense* plants by reverse transcription-polymerase chain reaction (RT-PCR). Total RNA was extracted from uninoculated leaves of mock- (M; lanes 2, 4, and 6) and virus-inoculated (V; lanes 3, 5, and 7) *S. bahamense* plants, amplified by RT-PCR with virus specific primers, analyzed by native electrophoresis on a 2% agarose gel, and stained with ethidium bromide. TMV = Tobacco mosaic virus, PMMoV = Pepper mild mottle virus, and TSWV = Tomato spotted wilt virus. Lanes 1 and 8 contain markers with sizes in base pairs (bp) indicated to the left of the gel.

Fig. 4. Detection of systemic infection in inoculated *Solanum bahamense* plants by viral-associated double-stranded (ds) RNA analysis. DsRNA was extracted from uninoculated leaves of mock- (lane 2) and virus-inoculated (lanes 3 to 6) *S. bahamense* plants, analyzed by native electrophoresis on a 5% polyacrylamide gel and stained with silver. TMV = Tobacco mosaic virus, PMMoV = Pepper mild mottle virus, HV = hibiscus tobamovirus, and CMV = Cucumber mosaic virus. A mixture of TMV and CMV dsRNAs isolated from *Nicotiana tabacum* cv. Xanthi was used for reference (lanes 1 and 7) with positions of the one TMV dsRNA and the four CMV dsRNAs indicated to the sides of the gel. The position of an endogenous *S. bahamense* dsRNA is indicated with a triangle.

rologist but has no effect on viral RNA or protein isolation! While *S. bahamense* was found to be a systemic albeit symptomless host for TMV and PMMoV, it was not susceptible to infection by HV. This suggests that it is not universally susceptible to tobamoviruses and demonstrates the difficulty of accurately predicting susceptibility to one member of a virus genus from results with other members of that same genus. Finally, *S. bahamense* was observed to contain an endogenous dsRNA (Fig. 4, lane 2), a phenomenon previously documented in other species including several members of the Solanaceae (24).

Many noncrop species in the genus *Solanum* have previously been shown to host viruses naturally (7,14,15,20) and/or experimentally (4,6,19), but to the best of our knowledge, this is the first report of *S. bahamense* being a host for any plant virus. While many excellent experimental hosts are available to plant virologists, the current report demonstrates that useful experimental hosts remain to be discovered, a fact noted by van Dijk and colleagues (26) 15 years ago.

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