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Craig G. Webster, Galen Frantz, Stuart R. Reitz, Joseph E. Funderburk, H. Charles Mellinger,
Eugene McAvoy, William W. Turechek, Spencer H. Marshall, Yaowapa Tantiwanich,
Margaret T. McGrath, Margery L. Daughtrey, and Scott Adkins

First, seventh, eighth, and twelfth authors: U.S. Department of Agriculture, Agricultural Research Service, U.S. Horticultural Research
Laboratory, Fort Pierce, FL 34945; second and fifth authors: Glades Crop Care, Inc., Jupiter, FL 33458; third author: Oregon State
University, Department of Crop and Soil Science, Ontario, OR 97914; fourth author: University of Florida, Department of Entomology
and Nematology, North Florida Research and Education Center, Quincy, FL 32351; sixth author: University of Florida/IFAS, Hendry
County Extension, LaBelle, FL 33935; ninth author: Plant Pathology Research Group, Plant Protection Research and Development Office,
Department of Agriculture, Ministry of Agriculture and Cooperatives, Chatuchak, Bangkok, 10900, Thailand; and tenth and eleventh
authors: Cornell University, Department of Plant Pathology and Plant-Microbe Biology, Long Island Horticultural Research and Extension
Center, Riverhead, NY 11901.
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ABSTRACT

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Groundnut ringspot virus (GRSV) and *Tomato chlorotic spot virus*
(TCSV) are two emerging tospoviruses in Florida. In a survey of the
southeastern United States, GRSV and TCSV were frequently detected in
solanaceous crops and weeds with tospovirus-like symptoms in south
Florida, and occurred sympatrically with *Tomato spotted wilt virus* (TSWV)
in tomato and pepper in south Florida. TSWV was the only tospovirus
detected in other survey locations, with the exceptions of GRSV from to-
mato (*Solanum lycopersicum*) in South Carolina and New York, both of
which are first reports. Impatiens (*Impatiens walleriana*) and lettuce
(*Lactuca sativa*) were the only non-solanaceous GRSV and/or TCSV
hosts identified in experimental host range studies. Little genetic diversity
was observed in GRSV and TCSV sequences, likely due to the recent
introductions of both viruses. All GRSV isolates characterized were re-
assortants with the TCSV M RNA. In laboratory transmission studies,
Frankliniella schultzei was a more efficient vector of GRSV than *F. occi-
dentalis*. TCSV was acquired more efficiently than GRSV by *F. occiden-
talis* but upon acquisition, transmission frequencies were similar. Further
spread of GRSV and TCSV in the United States is possible and detection
of mixed infections highlights the opportunity for additional reassortment
of tospovirus genomic RNAs.

Tomato spotted wilt virus (TSWV), the type member of the
genus *Tospovirus* in the family *Bunyaviridae*, is one of the most
economically important plant viruses worldwide, infecting many
vegetable and ornamental plant species (24,28). TSWV has long
been the major tospovirus problem of vegetable, peanut, and
tobacco producers in the southeastern United States (6,7,12). Two
other tospoviruses, *Impatiens necrotic spot virus* and *Iris yellow
spot virus*, occur in the southeastern United States (27). Recently,
two additional tospoviruses previously not known to occur in the
United States have emerged in vegetable production areas of
south Florida.

Groundnut ringspot virus (GRSV) was first detected in tomato
(*Solanum lycopersicum*) in late 2009 and subsequently in pepper
(*Capsicum annuum*), tomatillo (*Physalis philadelphica*), and
eggplant (*Solanum melongena*) in south Florida (33,35). *Tomato
chlorotic spot virus* (TCSV) was subsequently detected in tomato
in south Florida in 2012 (20), and in tomato, pepper, jimsonweed
(*Datura stramonium*), and lettuce (*Lactuca sativa*) in Puerto Rico
(13,32) and tomato in the Dominican Republic (3). TSWV re-
mains present in all of these areas (3,22,35). Diagnosis is

complicated due to visually indistinguishable symptoms: ring-
spots and necrotic and chlorotic areas are induced in tomato and
pepper by GRSV, TCSV, and TSWV (14,20,33,39). Additional
hosts reported for GRSV and/or TCSV in other parts of the world
include peanut (*Arachis hypogaea*; 9,11), coriander (*Coriandrum
sativum*; 14,19), and lettuce (14), as well as several weeds (4,14).

The genomes of GRSV and TCSV each consist of three RNA
segments designated small (S), medium (M), and large (L), analo-
gous to TSWV and other tospoviruses (reviewed in 36). The S
RNA is ambisense and encodes a nucleocapsid (N) protein and a
nonstructural silencing suppressor protein (NSs). The M RNA is
also ambisense and encodes a precursor for two glycoproteins (G_N
and G_C) and a nonstructural movement protein (NSm). The L
RNA is negative sense and encodes a multifunction protein (L)
including an RNA-dependent RNA polymerase domain.

The tripartite genomes of tospoviruses, and members of the
family *Bunyaviridae* in general, allow for reassortment (reviewed
in 5). Such reassortment may occur between isolates of a single
virus species as observed for TSWV (25,31), or between virus
species as observed for GRSV and TCSV in Florida (34). In fact,
all isolates of GRSV characterized to date from Florida have an
$L_G M_T S_G$ genotype with GRSV S and L RNAs and a TCSV M
RNA. In contrast, the S, M, and L RNAs of TCSV isolates re-
cently reported from Florida, Puerto Rico, and the Dominican
Republic all share high levels of nucleotide identity with the cor-
responding RNAs of TCSV isolates from South America (3,13,
20,32), and thus have the standard TCSV genotype (i.e., are not

Corresponding author: S. Adkins, E-mail address: scott.adkins@ars.usda.gov

interspecies reassortants). Similar to research with other members of the family *Bunyaviridae* (reviewed in 5), studies of reassortment between isolates of TSWV suggest novel biological properties can arise (e.g., TSWV N-gene-derived resistance breaking; 26) from this process. Biological properties of interspecies tospovirus reassortants, like Florida GRSV isolates, have not been characterized.

Virus species must occur sympatrically for reassortment to be possible (5). We define sympatry as the occurrence of different virus species infecting the same host species in a given geographic area at the same time. The presence of a common host species, plant or thrips, makes it possible for multiple virus species to infect a single individual of that species, which enables reassortment if co-infection of a plant host or insect vector occurs. For much of this study, GRSV, TSWV, and TCSV occurred sympatrically in south Florida. GRSV and TSWV were observed to occur in the same tomato fields and occasionally co-infect the same tomato plant in south Florida at the start of the study (33, 34). Beginning in 2012, TCSV was reported to occur in tomato in one of the same counties (Miami-Dade County) from which GRSV and TSWV were also reported in south Florida (20,35).

Tospovirus transmission occurs exclusively by thrips (order Thysanoptera) but relatively few thrips species are known to acquire and transmit tospoviruses (27,36). Feeding by recently hatched larvae on tospovirus-infected host plants is required for the successful acquisition (including infection of thrips cells by virus) and transmission (subsequent migration of virus to the salivary glands and delivery to noninfected plants), as previously reviewed (36). Viruliferous winged adult thrips are able to migrate and disseminate the virus. Transmission efficiencies vary by virus source, virus isolate, and thrips population (10,15,16,37), and therefore local knowledge of virus isolates and thrips populations is necessary to identify which thrips species are most likely to transmit tospoviruses in a given geographic area.

The western flowers thrips (*Frankliniella occidentalis*) is a major vector of several tospoviruses, including TSWV, GRSV, and TCSV, and is widespread in the United States and much of the world (27,34,37). The common blossom thrips (*F. schultzei*) is also an efficient vector of TSWV, GRSV, and TCSV in South America but this species is not widespread in the United States (23,27,37). However, *F. schultzei* has recently been reported in vegetable production areas where GRSV and/or TCSV have been detected in the continental United States and Puerto Rico (13, 17,18,32). The potential of other thrips species to transmit GRSV or TCSV isolates in Florida has not been determined. This includes the Florida flower thrips (*F. bispinosa*) and tobacco thrips (*F. fusca*) that are known to transmit tospoviruses (27) and are commonly found in the southeastern United States.

Vegetable production in the southeastern United States shifts north from Florida to other states during spring and summer and returns south to Florida during the fall and winter to follow suitable growing conditions. A wide variety of vegetable and other crops, many of which may be GRSV and/or TCSV hosts, are grown throughout the eastern United States. Therefore, we determined experimental host ranges of Florida GRSV and TCSV isolates with particular emphasis on important crop and weed species. Although TSWV is well known in most major U.S. vegetable production areas, the extent of spread of GRSV and TCSV beyond south Florida is not known. We used surveys of GRSV, TCSV, and TSWV across major tomato growing regions of Florida and the southeastern United States to investigate the establishment of GRSV and TCSV and determine the current geographic range and potential for further spread and/or reassortment of these viruses. We also examined the genetic diversity of all GRSV and TCSV isolates collected from new hosts and new locations during this study. Finally, we evaluated the ability of several important thrips species to transmit GRSV and TCSV. Collectively, the results of this research provide a current assess-

ment of these emerging tospoviruses in the southeastern United States and the foundation for improved management strategies.

MATERIALS AND METHODS

Isolation and maintenance of tospoviruses. Two single local lesion-passaged GRSV isolates and one single local lesion-passaged TSWV isolate from tomatoes in Florida were maintained as described previously (34). A TCSV isolate collected from tomato in southwest Florida was similarly maintained. Local lesions of TCSV and selected newly collected GRSV isolates were generated by mechanical inoculation of *Nicotiana glutinosa* using 0.5% (wt/vol) sodium sulfite. A single local lesion was excised and used to mechanically inoculate systemic tospovirus hosts including American black nightshade (*Solanum americanum*), jimsonweed (*Datura stramonium*), tomato, and pepper. These local lesion-passaged GRSV and TCSV isolates were used for host range and transmission experiments.

Plants sources and inoculations. For the majority of plants in host range experiments, commercially available seeds were used (Johnny's Selected Seeds, Winslow, ME; Burpee Seeds and Plants, Warminster, PA; Asgrow, St. Louis, MO). Weed and indicator host species were grown from seeds produced from virus-free source plants in the USHRL greenhouse. Brugmansia (*Brugmansia × candida*), Key West nightshade (*S. bahamense*), and strawberry (*Fragaria × ananassa*) were propagated from virus-free stock plants. Plants were kept in an air conditioned greenhouse under natural lighting with a daytime maximum temperature of 30°C.

For host range experiments, 10 plants of each species or cultivar were mechanically inoculated as described above at the two to three true leaf stage. Two plants of each species or cultivar were mock inoculated with sodium sulfite solution. Inoculated and upper, noninoculated leaves were examined weekly for symptoms. The presence of virus in inoculated (locally infected) or upper, noninoculated (systemically infected) leaves was determined at 2 to 3 weeks postinoculation by using virus-specific tests as described below. For a few species or cultivars (e.g., impatiens and peanut with GRSV), several sets of plants were inoculated. To examine potential differences between mechanical and thrips inoculation, one set of eggplants was mechanically inoculated and a second set was exposed to an *F. occidentalis* population reproducing on TCSV-infected plants.

Geographic distribution of GRSV, TCSV, and TSWV. As a first step to analyze the geographic distribution of GRSV (and later TCSV), plants with typical tospovirus symptoms were collected by trained observers in the major vegetable production areas of Florida, Georgia, South Carolina, North Carolina, and the Eastern Shore of Virginia. These were primarily tomatoes but also included peppers, peanuts, and a variety of weeds. Symptomatic tomato transplants were also collected from transplant production facilities in Florida and symptomatic vegetable plant samples were collected in Indiana and Long Island, New York.

At the start of this study, collected samples were tested only for GRSV and TSWV because TCSV was not known to occur in the United States at that time, and no evidence of TCSV was found in 2009 and 2010 when GRSV was first detected in Florida (33–35). With the detection of TCSV in Florida in 2012 (20), all subsequent survey samples were tested for GRSV, TCSV, and TSWV using virus-specific assays as described below.

Detection of tospoviruses. Enzyme-linked immunosorbent assay (ELISA) and/or reverse transcription-polymerase chain reaction (RT-PCR) were used for tospovirus detection in all plant samples evaluated in this study as previously described (33,34). Commercially available ELISA reagents for GRSV/TCSV or TSWV N protein detection were used according to the manufacturer's instructions (Agdia, Elkhart, IN). RNA was extracted (RNeasy Plant Mini Kit, Qiagen, Germantown, MD) and first

strand cDNA synthesized using random hexamers and *Moloney murine leukemia virus* reverse transcriptase (Promega Corp., Madison, WI). Primers specific for the N gene of GRSV (33), TCSV (32), or TSWV (1) that produce PCR amplicons of 594, 519, or 620 bp, respectively, were initially used.

Further sequence characterization of GRSV and TCSV isolates made use of additional primers to amplify a portion of the NSm (29) and the L genes (32,33). $G_N G_C$ primers Glyco4vc (5′-AGC ATTCTGTTGTTCAGGGCTAC-3′) and Glyco5v (5′-CAGAG TGTGCTTTGAAGTTCCC-3′) were designed from the original Florida GRSV isolate sequence (34) and used for the GRSV isolates from South Carolina and New York. Isolates chosen for further sequencing represented all new hosts and new locations from which either GRSV or TCSV was detected for the first time during the geographic survey. Amplicons were excised from agarose gels, purified (QiaQuick gel purification kit, Qiagen), and ligated to pGEM-T (Promega). Six clones of each amplicon were sequenced in both directions on an ABI 3730XL automated sequencer at the USHRL DNA Sequencing Support Laboratory. Primer and plasmid sequences were removed and a single consensus sequence was obtained from the six clones of each amplicon using Vector NTI Advance software, Version 11 (Invitrogen, Carlsbad, CA). Consensus sequences were deposited in GenBank (accessions KM006992 to KM007037) and aligned using MEGA (Version 4.1; 30) with Clustal W and default parameters. Pairwise comparisons of nucleotides were made using the pairwise deletion option and the number of common sites and compared with sequences obtained from GenBank.

Thrips transmission of GRSV and TCSV. Thrips species known to transmit tospoviruses were collected from vegetables, weeds, and ornamentals in the Florida landscape and identified to the species level with a dissecting microscope. A colony of *F. occidentalis* was maintained on green bean (*Phaseolus vulgaris*) pods that were washed in 0.5% (vol/vol) sodium hypochlorite and Veggie Wash (active ingredient: 1% citric acid, Beaumont Products, Inc., Kennesaw, GA). Typically, *F. schultzei* was collected from watermelon (*Citrullus lanatus*) or cucumber (*Cucumis sativus*) flowers, *F. bispinosa* was collected from crape myrtle (*Lagerstroemia indica*) flowers, and *F. fusca* was collected from perennial peanut (*Arachis glabrata*) flowers. However, *F. bispinosa* was found in flowers of all species.

Adults of each species were maintained in separate containers and allowed to oviposit onto bean pods for 2 to 3 days before being removed. First instar larvae were collected from bean pods within 24 h of emergence and placed on symptomatic leaves of GRSV- or TCSV-infected tomato 'Florida 47' or pepper 'Aristotle' source plants for acquisition and transmission experiments as previously described (34,38). Source plants were of consistent plant age and mechanically inoculated with one of two local lesion-passaged GRSV isolates or one local lesion-passaged TCSV isolate. Prior to use in transmission experiments at 1 to 2 weeks postinoculation, infection of source plants with the expected virus was confirmed by RT-PCR testing for the N gene as described above. Initially, a single adult thrips that was fed as a larva on infected plants and had potentially acquired virus was moved to a single leaf disc (tomato 'Florida 47' or pepper 'Aristotle') placed on agar in 2.5 cm petri dishes and supplemented with Miracle Grow (The Scotts Company, Marysville, OH). Although dishes were sealed with Parafilm (Pechiney Plastic Packaging Company, Chicago, IL), large numbers of thrips, especially *F. bispinosa* and *F. fusca*, escaped. In later experiments, a single thrips was instead placed on a single leaf disc in a 1.5-ml microcentrifuge tube to prevent adult escape. Adult thrips were removed after 2 days on leaf discs. Identifications of thrips were confirmed and thrips were frozen (–20°C) for later testing. Leaf discs were maintained for another 3 to 5 days after thrips removal during which time sterile water was added as needed to prevent dehydration of the discs. Ultimately,

leaf discs were removed and frozen (–20°C) for later testing. Thrips and leaf discs were tested for GRSV or TCSV by either RT-PCR (≈10% of samples) or ELISA (remaining ≈90% of samples) as described previously (34) because single thrips and single leaf discs were found to contain too little virus to permit testing by both methods. An individual thrips and its corresponding leaf disc were always tested by the same method.

Acquisition and transmission of GRSV by four thrips species were compared through contingency table analyses. Where a significant difference among the species was detected, post-hoc multiple comparison tests were conducted to determine which pairs of species differed from one another (40). We further examined the frequency of successful transmission of GRSV by viruliferous thrips of different species using Fisher's exact test because of the small sample sizes for viruliferous thrips. Comparisons of GRSV and TCSV acquisition and transmission by *F. occidentalis* were also made using contingency table analyses, including the use of Fisher's exact test for comparison of transmission of GRSV and TCSV by viruliferous *F. occidentalis*. All analyses were conducted using SAS v. 9.2 (SAS Inc., Cary, NC).

RESULTS

Experimental host ranges of the emerging tospoviruses. Experimental host range tests of 45 species for GRSV and 18 species for TCSV included a range of important vegetable, ornamental, and weed species (Table 1). A more extensive host range was examined for GRSV due to it being a reassortant. All mock-inoculated plants remained virus-free.

GRSV and TCSV severely affected solanaceous species including vegetable, ornamental, and weed species. Symptoms induced by the two viruses were similar in commercial tomato and pepper cultivars and included necrosis, often of the growing point, ringspots, chlorotic or necrotic lesions, and mottle. In general, TCSV-infected plants expressed more severe symptoms than those infected with GRSV. Local and systemic movement of viruses was confirmed by ELISA and/or RT-PCR and typically correlated with the presence of symptoms, although in some cases (e.g., tomato 'Florida 47' infected with GRSV, and 'Tygress' infected with GRSV or TCSV) the virus was detected in inoculated leaves despite a lack of symptoms in these leaves. In most susceptible species, greater than 50% of the inoculated plants developed symptoms, indicating a high susceptibility to infection by mechanical inoculation. However, results of inoculations were inconsistent in brugmansia, eggplant, and tomato. For instance, local infections developed in seven brugmansia inoculated with GRSV and five eggplants inoculated with GRSV or TCSV, whereas systemic symptoms (with virus presence confirmed by RT-PCR) only developed in a single brugmansia and a single eggplant inoculated with GRSV. Although thrips inoculation of eggplant with TCSV essentially doubled the number of locally infected plants observed with mechanical inoculation from 50 to 92%, no systemic infections were detected from either inoculation method. Tomato cultivars 'Black Cherry', 'Black Prince', 'Matt's Wild Cherry', 'Moskvich', 'Purple Calabash', and 'Quincy' developed no local or systemic symptoms following inoculation with GRSV, although four of these tomato cultivars ('Black Prince', 'Matt's Wild Cherry', 'Moskvich', and 'Purple Calabash') displayed systemic symptoms when inoculated with TCSV. In some species, symptoms progressed to plant death within 3 weeks of inoculation. This occurred most often with TCSV although it varied by species and, in some cases, by cultivar. For instance, 12 of 28 tomatillo 'Burpee Green' plants became systemically infected with GRSV, whereas 6 of 10 died following inoculation with TCSV and the remaining four plants were systemically infected. Only 1 of 10 petunia 'Blue Supercascade' plants was systemically infected with GRSV, whereas 8 of 10 were systemically infected with TCSV. In contrast, 6 of 10 petunia 'Red Super-

cascade' plants died following GRSV inoculation with two of four remaining plants systemically infected, whereas only 1 of 10 died following TCSV inoculation with 8 of 9 remaining plants systemically infected. Flower break was observed in all petunia cultivars inoculated with GRSV but not with TCSV.

Although the majority of host plants identified for both viruses were solanaceous, impatiens inoculated with GRSV or TCSV and lettuce inoculated with TCSV were also infected (Fig. 1). Impatiens were locally and systemically infected with GRSV and TCSV but only plants infected with TCSV developed concentric necrotic rings (Fig. 1A) on upper, noninoculated leaves. Neither lettuce cultivar tested was infected with GRSV. TCSV only infected lettuce 'Parris Island', inducing small chlorotic spots on inoculated and upper, noninoculated leaves that turned necrotic and developed into larger necrotic areas spreading along the midrib of the leaf (Fig. 1B). In some cases, this led to collapse of inoculated leaves. Other important crop species, including water-melon, soybean and peanut, and common virus indicator hosts, including globe amaranth and lambsquarters, were not infected with either virus.

Geographic distribution of GRSV, TCSV, and TSWV. In 2013, we detected GRSV outside of Florida for the first time in tomato samples from South Carolina and New York (Fig. 2A). The South Carolina GRSV isolate was detected in a mixed infection with TSWV, and was identified following separation by local lesion-passage in *N. glutinosa* and RT-PCR amplification of portions of all three genomic RNA segments for each virus. The New York GRSV isolate was detected in a single infection although TSWV was common in other samples from this and nearby fields.

GRSV and TCSV were commonly identified in seven, and GRSV was detected in two more, of the ten counties surveyed in south Florida (Florida peninsula south of Orlando; Fig. 2B) although the percentage of south Florida tomato samples infected
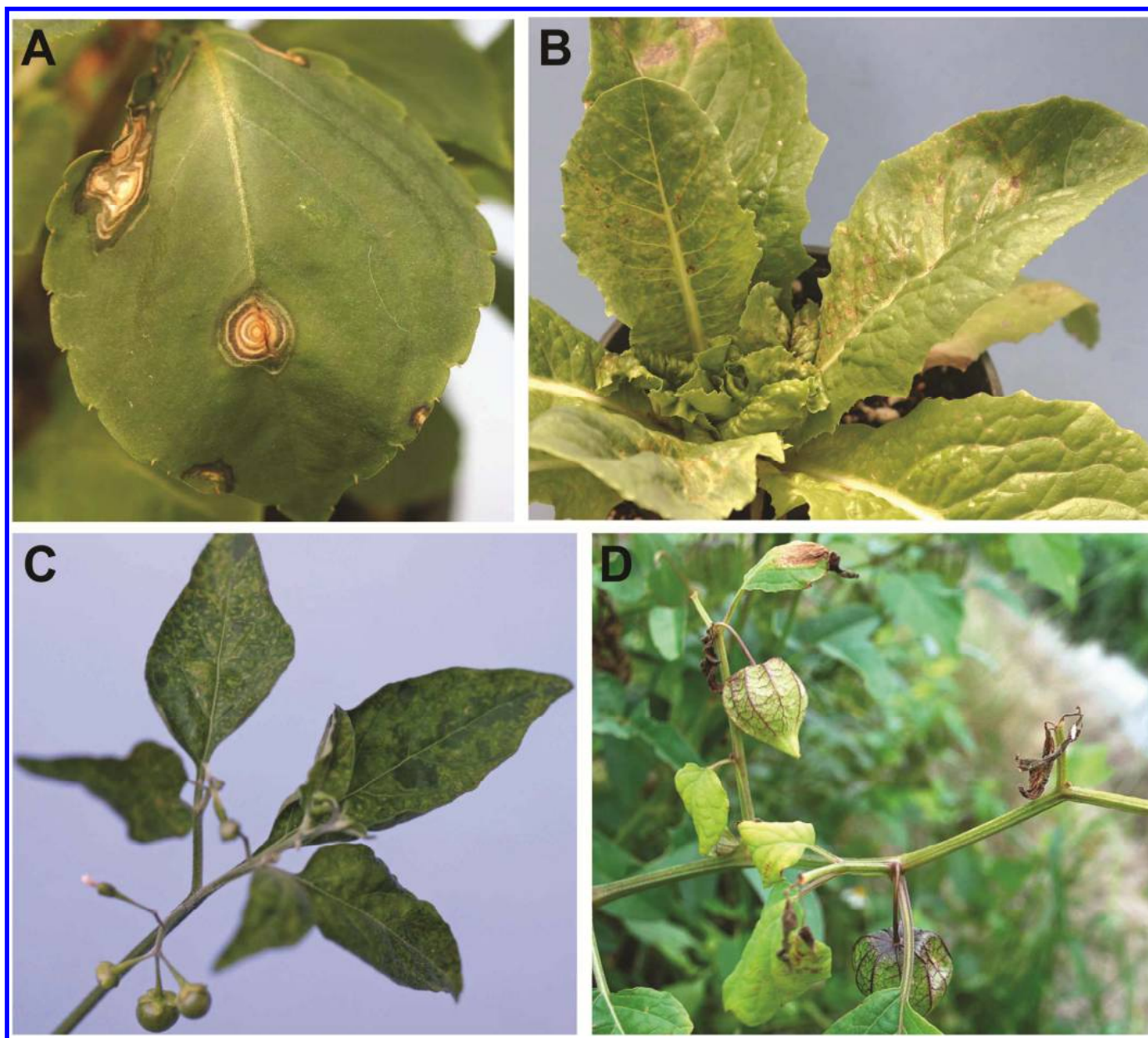

Fig. 1. Symptoms of *Tomato chlorotic spot virus* (TCSV) and *Groundnut ringspot virus* (GRSV) infection of mechanically and naturally infected hosts. **A,** Impatiens (*Impatiens walleriana*) mechanically inoculated with TCSV with concentric necrotic rings on noninoculated (systemically infected) leaf. **B,** Lettuce (*Lactuca sativa*) mechanically inoculated with TCSV with chlorotic and necrotic lesions on noninoculated (systemically infected) leaves. **C,** American black nightshade (*Solanum americanum*) found naturally infected with GRSV with chlorotic ringspots and ring patterns collected in 2011 in Collier County. **D,** Cutleaf groundcherry (*Physalis angulata*) found naturally infected with GRSV with necrotic growing points collected in 2011 in St. Lucie County.

with GRSV decreased from 64% in 2011 to 9% in 2013 (Table 2). TSWV was widespread on a range of solanaceous hosts throughout Florida and in the southeastern United States from 2011 through 2013 (Fig. 2A and B) and also in New York and Indiana. Although two or more viruses were often found in the same field, mixed infections of two tospovirus species in a single plant were not common. Only seven mixed infections were detected among the 431 infected samples detected in this study (Table 2). GRSV was not prevalent outside of south Florida, and TCSV was not found beyond south Florida. However, all three viruses occurred sympatrically in tomato and pepper in seven counties in south Florida (Collier, Hendry, Lee, Manatee, Miami-Dade, Palm Beach, and St. Lucie) during much of our study, and GRSV and TSWV occurred sympatrically in tomato in South Carolina and New York at least sporadically in 2013, fulfilling a major requirement for genome reassortment.

In south Florida, GRSV and TCSV were identified in tomato and/or pepper in several new counties: GRSV was detected in Charlotte, Lee, and St. Lucie Counties; TCSV was detected in Collier, Hendry, Manatee, Palm Beach, and St. Lucie Counties. Only TSWV was detected in north Florida (Fig. 2B). At the start of this research in 2011, only GRSV and TSWV were known to occur in the United States and it was not until after the detection and report of TCSV in 2012 (20) that TCSV was included in testing protocols. GRSV and TCSV were also identified several times each in tomato transplants from transplant production facilities in Florida. Broad spectrum tests for tospovirus were also conducted on selected samples to examine for additional tospoviruses and in all cases were negative (data not shown).

During the geographic survey, several new natural weed hosts for GRSV were identified including American black nightshade (Fig. 1C) and cutleaf groundcherry (Fig. 1D). Symptomatic weeds were collected adjacent to tomato or pepper plants infected with GRSV. Symptoms included ringspots and chlorosis on nightshade, and leaf distortion and necrosis of growing points on groundcherry, and were similar to those induced on mechanically inoculated plants in the greenhouse (Table 1). Neither GRSV nor TCSV was detected in any samples of other crop or weed species. TSWV was detected in peanut in both south and north Florida (Table 2).

The geographic distribution studies in this work considered only plants with typical tospovirus symptoms. Thus, our results do not necessarily reflect the true incidence of GRSV, TCSV, and TSWV in the hosts analyzed but rather document expansion of the known geographic distribution of GRSV and TCSV. Symptomatic samples were infrequent in all 3 years and typically made up less than 1% of the total planting, although higher incidences

TABLE 1. Plants used to establish experimental host range and symptoms of *Groundnut ringspot virus* (GRSV) and *Tomato chlorotic spot virus* (TCSV)

Family, species, and cultivar[u]	Common name	GRSV symptoms[v]	GRSV presence[w]	TCSV symptoms[v]	TCSV presence[w]
Amaranthaceae					
Gomphrena globosa 'Strawberry Fields'	Globe amaranth	–/–	0 of 10/0 of 10	nt	nt
Apiaceae					
Coriandrum sativum	Coriander	–/–	0 of 10/0 of 10	nt	nt
Asteraceae					
Helianthus annuus 'Sunrich Orange Summer'	Sunflower	–/–	0 of 10/0 of 10	nt	nt
Lactuca sativa 'Parris Island'	Lettuce	–/–	0 of 10/0 of 10	CL,N/CL,NL	8 of 10/10 of 10
L. sativa 'Cherokee'	Lettuce	–/–	0 of 10/0 of 10	–/–	0 of 10/0 of 10
Zinnia elegans 'Giant Dahlia'	Elegant zinnia	–/–	0 of 10/0 of 10	nt	nt
Rudbeckia hirta 'Prairie Sun'	Black-eyed Susan	–/–	0 of 10/0 of 10	nt	nt
Balsaminaceae					
Impatiens walleriana 'Athena Mix'	Impatiens	NL,RS/–	4 of 20/1 of 20	N,RS/N,RS	6 of 10/5 of 10
Brassicaceae					
Brassica oleracea 'Champion'	Collards	–/–	0 of 10/0 of 10	nt	nt
B. oleracea 'Farao'	Cabbage	–/–	0 of 10/0 of 10	nt	nt
B. oleracea 'Winterbor'	Kale	–/–	0 of 10/0 of 10	–/–	0 of 10/0 of 10
Caryophyllaceae					
Dianthus barbatus 'Hollandia Mix'	Sweet William	–/–	0 of 10/0 of 10	nt	nt
Chenopodiaceae					
Chenopodium amaranticolor	Lambsquarters	–/–	0 of 10/0 of 10	nt	nt
C. quinoa	Lambsquarters	–/–	0 of 20/0 of 20	–/–	0 of 10/0 of 10
Convolvulaceae					
Ipomoea violaceae 'Giant White'	Moonflower	–/–	0 of 10/0 of 10	nt	nt
Cucurbitaceae					
Citrullus lanatus 'Black Diamond'	Watermelon	–/–	0 of 10/0 of 10	nt	nt
C. lanatus 'Charleston Grey'	Watermelon	–/–	0 of 10/0 of 10	nt	nt
C. lanatus 'Crimson Sweet'	Watermelon	–/–	0 of 10/0 of 10	nt	nt
Cucurbita pepo 'Prelude II'	Squash	–/–	0 of 10/0 of 10	nt	nt
C. pepo 'Zucchini hybrid'	Squash	–/–	0 of 10/0 of 10	nt	nt
Fabaceae					
Arachis hypogaea 'Georgia Green'	Peanut	–/–	0 of 30/0 of 30	nt	nt
Crotalaria juncea	Sunn hemp	–/–	0 of 10/0 of 10	nt	nt
Glycine max '93Y92'	Soybean	–/–	0 of 10/0 of 10	–/–	0 of 10/0 of 10
G. max '95M50'	Soybean	–/–	0 of 10/0 of 10	–/–	0 of 10/0 of 10
G. max '95Y20'	Soybean	–/–	0 of 10/0 of 10	–/–	0 of 10/0 of 10
G. max '97M50'	Soybean	–/–	0 of 10/0 of 10	–/–	0 of 10/0 of 10
					(*Continued on next page*)

[u] Family and species name are according to USDA PLANTS Database (http://plants.usda.gov, accessed October 9, 2012). Cultivar shown where applicable.

[v] Symptoms on inoculated and upper, noninoculated leaves indicated to left and right of slash, respectively; – = no symptoms, C = chlorosis, CL = chlorotic lesion, FB = flower break, M = mottling, N = necrosis, NL = necrotic lesion, NLL = necrotic local lesion, PD = plant death, RS = ringspot, VC = vein clearing, and nt = not tested.

[w] Localized infection and systemic movement of the virus in inoculated and upper, noninoculated leaves in number of plants specified (usually 10 or multiple thereof) indicated to left and right of slash, respectively, with infection confirmed by enzyme-linked immunosorbent assay or reverse transcription-polymerase chain reaction.

[x] Indicates number of plants alive (of original 10) at time of symptom evaluation and virus testing.

[y] *S. lycopersicum* (tomato) denoted with *Sw5* has the *Sw5* gene that confers resistance to *Tomato spotted wilt virus*.

[z] Inoculated by mass exposure to viruliferous *Frankliniella occidentalis*.

were occasionally seen (data not shown). The relative incidence of the three tospoviruses in infected samples collected in south Florida was interesting. No peanuts with GRSV or TCSV infections were detected. Only TSWV was detected in 57% of the peanuts collected in south Florida (35 of 61 samples, Table 2) despite the frequent occurrence of GRSV (20%) and TCSV (16%) in other hosts in this region during the surveys (Table 2). Only two peppers were found infected with TCSV, whereas 41% (19 of 46 samples) of peppers from the same region were infected with GRSV and 28% (13 of 46 samples) were infected with TSWV (Table 2).

Low genetic diversity of GRSV and TCSV isolates. Sequences of portions of the S, M and L genomic RNA segments of GRSV and TCSV isolates from all new hosts and new locations (GenBank accessions KM006992 to KM007037) were compared with previously characterized isolates (GenBank accessions HQ644140, HQ644141, HQ644142, S54325, AF213674, and HQ700667). High levels of nucleotide sequence identity (94 to 100%) of all isolates of both viruses to the respective characterized isolates of GRSV or TCSV confirmed initial identifications. The isolates characterized in this study all were highly similar, with fewer than 1×10^{-2} substitutions/nucleotide (Table

3). The reassorted nature ($L_G M_T S_G$ genotype) of GRSV isolates from all hosts and locations, including South Carolina and New York, was confirmed with greater than 99% nucleotide identity to previously characterized GRSV isolates from south Florida (data not shown).

Acquisition and transmission of GRSV and TCSV isolates by local thrips populations. Thrips species known to transmit tospoviruses and prevalent in the southeastern United States were tested for their ability to acquire and transmit GRSV (Table 4). *F. occidentalis* was included due to previous results showing that this species transmitted Florida GRSV isolates (34). There were significant differences in the ability of the four thrips species to acquire GRSV ($\chi^2 = 53.65$, df = 3, $P < 0.0001$): 30.3% of *F. schultzei* acquired GRSV, which was a significantly higher rate than for the other three species (Table 4). No *F. fusca* successfully acquired GRSV. The overall frequency of transmission of GRSV also differed among the species ($\chi^2 = 60.79$, df = 3, $P < 0.0001$). *F. schultzei* transmitted GRSV at a significantly higher rate than the other three species, and *F. occidentalis* transmitted GRSV at a significantly higher rate than the other two species (Table 4). Although 3.2% of *F. bispinosa* successfully acquired GRSV, no *F. bispinosa* transmitted the virus (Table 4). *F. fusca* was not ob-

TABLE 1. (*Continued from previous page*)

Family, species, and cultivar[u]	Common name	GRSV symptoms[v]	GRSV presence[w]	TCSV symptoms[v]	TCSV presence[w]
Fabaceae					
Phaseolus vulgaris	Kidney bean	–/–	0 of 10/0 of 10	nt	nt
P. vulgaris 'Foremost'	Green bean	–/–	0 of 10/0 of 10	nt	nt
Vigna unguiculata 'California Blackeye #5'	Cowpea	–/–	0 of 10/0 of 10	nt	nt
Malvaceae					
Abelmoschus esculentus 'Clemson Spineless'	Okra	–/–	0 of 10/0 of 10	nt	nt
Gossypium hirsutum 'DES 607'	Cotton	–/–	0 of 10/0 of 10	nt	nt
Lamiaceae					
Ocimum basilicum	Sweet basil	–/–	0 of 10/0 of 10	nt	nt
Rosaceae					
Fragaria × ananassa 'Festival'	Strawberry	–/–	0 of 10/0 of 10	–/–	0 of 10/0 of 10
Rutaceae					
Citrus sinensis 'Ridge Pineapple'	Sweet orange	–/–	0 of 10/0 of 10	nt	nt
Solanaceae					
Brugmansia × candida 'Creamsicle'	Angel's trumpet	RS/VC	7 of 11/1 of 11	nt	nt
Capsicum annuum 'Aristotle'	Bell pepper	CL/M,N,RS	10 of 10/9 of 10	CL,RS/M,N,RS	10 of 10/10 of 10
Datura metel	Downy thorn-apple	CL/NL	6 of 10/6 of 10	nt	nt
D. stramonium	Jimsonweed	RS/CL,N,RS	7 of 10/7 of 10	RS/M,N	10 of 10/10 of 10
Nicotiana benthamiana		N,M,RS/N,M,RS	8 of 10/9 of 10	CL,RS/C,N	10 of 10/10 of 10
N. debneyi		RS/–	9 of 10/0 of 10	nt	nt
N. glutinosa		NLL/–	8 of 10/0 of 10	NLL/N	10 of 10/9 of 10
N. megalosiphon		RS/RS	9 of 10/10 of 10	nt	nt
N. miersii		NL,N/NL	10 of 10/10 of 10	nt	nt
N. occidentalis		RS/RS	10 of 10/6 of 10	nt	nt
N. rustica		NL/N,M	7 of 10/7 of 10	N,RS/–,PD	9 of 9/3 of 9[x]
N. sylvestris		NL/N,M	5 of 10/1 of 10	NL,RS/–	10 of 10/1 of 10
N. tabacum 'Xanthi'	Tobacco	NL/M	8 of 10/6 of 10	RS/–,PD	7 of 8/4 of 8[x]
N. tabacum 'Xanthi nc'	Tobacco	NL/–	8 of 10/3 of 10	RS/–	9 of 10/2 of 10
Petunia × hybrida 'Blue Supercascade'	Petunia	–/NL,RS,FB	0 of 10/1 of 10	N,RS/N,RS,VC	10 of 10/8 of 10
P. × hybrida 'Red Supercascade'	Petunia	NL/N,RS,FB,PD	1 of 4/2 of 4[x]	N,RS/N,RS,PD	9 of 9/8 of 9[x]
P. × hybrida 'Rose Wave Hybrid'	Petunia	NL/N,RS,FB,PD	0 of 9/4 of 9[x]	nt	nt
Physalis philadelphica 'Burpee Green'	Tomatillo	RS,N,M/RS,N,M	11 of 28/12 of 28	C,N/C,N,PD	4 of 4/4 of 4[x]
P. pruinosa 'Goldie'	Husk Cherry	RS,N,M/RS,N,M	3 of 9/7 of 9	nt	nt
Solanum americanum	American black nightshade	RS/RS	8 of 10/7 of 10	–/M,N	8 of 10/8 of 10
S. bahamense	Key West nightshade	–/N,C	2 of 9/8 of 9	nt	nt
S. lycopersicum 'Black Cherry'	Tomato	–/–	1 of 10/0 of 10	nt	nt
S. lycopersicum 'Black Prince'	Tomato	–/–	1 of 10/6 of 10	–/N,NL	8 of 10/9 of 10
S. lycopersicum 'Florida 47'	Tomato	–/M,N,RS	14 of 20/18 of 20	CL/M,N,RS	11 of 20/16 of 20
S. lycopersicum 'Matt's Wild Cherry'	Tomato	–/–	7 of 10/5 of 10	–/N,NL	6 of 10/9 of 10
S. lycopersicum 'Moskvich'	Tomato	–/–	5 of 10/4 of 10	–/N,NL	7 of 10/8 of 10
S. lycopersicum 'Purple Calabash'	Tomato	–/–	0 of 10/5 of 10	–/M,N,RS	7 of 10/9 of 10
S. lycopersicum 'Quincy' (*Sw5*)[y]	Tomato	–/–	0 of 10/0 of 10	nt	nt
S. lycopersicum 'Tygress'	Tomato	–/M,N	2 of 20/8 of 20	–/M,N,RS	5 of 10/8 of 10
S. melongena 'Nightshadow'	Eggplant	RS/RS,M	5 of 10/1 of 10	N,RS/–	5 of 10/0 of 10
S. melongena 'Nightshadow'[z]	Eggplant	nt	nt	N,RS/–	11 of 12/0 of 12
Tropaeolaceae					
Tropaeolum majus 'Double Gleam Mix'	Nasturtium	–/–	0 of 10/0 of 10	nt	nt

served to transmit GRSV. When considering only the rate of transmission by thrips that tested positive for the virus (adjusted transmission rate for viruliferous thrips), the rate of viruliferous *F. schultzei* transmitting GRSV was 64.7%, but this was only significantly higher than the rate for *F. bispinosa* (0.0%).

Both acquisition and transmission frequency were significantly higher for TCSV than GRSV by *F. occidentalis* ($\chi^2 = 98.58$, df = 1, $P < 0.0001$ acquisition; $\chi^2 = 6.09$, df = 1, $P = 0.014$ transmission). Acquisition by *F. occidentalis* of TCSV was 36.8% compared with 5.9% acquisition for GRSV (Table 4). Likewise, the overall transmission rate was 11.8% for TCSV versus 6.4% for GRSV. However, the adjusted rates of transmission for viruliferous thrips were not significantly different between the virus species (21.5% for TCSV versus 28.6% for GRSV; $\chi^2 = 0.38$, df = 1, $P = 0.54$), suggesting that, once acquired, both viruses were transmitted with equal efficiency by *F. occidentalis*.

DISCUSSION

The ability of an insect-transmitted pathogen to become established and impact crops when introduced to a new geographic area depends on several factors. In annual crop production, these would include the presence of suitable reservoir hosts to enable survival when the crop is not in production, and an insect vector that reproduces and feeds on both the crop and reservoir hosts to enable movement. Although TSWV has severely impacted agriculture across the southeastern United States over many years (28), much less information is available regarding potential impacts of GRSV and TCSV. Our geographic distribution survey and host range studies show the widespread presence of both GRSV and TCSV in vegetable production regions of south Florida and that numerous suitable reservoirs are present to facilitate the persistence of these invasive viruses. Our virus acquisition and transmission tests indicate that thrips species common in Florida and elsewhere in the southeastern United States are competent vectors of these viruses. Together this information documents the establishment of both virus species in south Florida vegetable and vegetable transplant production areas, and the potential threat to other geographic areas.

All three viruses occurred in sympatry in the majority of the ten counties sampled in south Florida. It was common to find two of the three viruses in the same field but much less common to find them infecting a single plant (7 of 431 tospovirus-infected samples; Table 2) similar to previous observations (33,34). However, the percentage of samples infected with GRSV or TCSV fluctuated over the course of the study, such that only TCSV (but no GRSV) was detected in south Florida in the last half of 2013. The initial sympatry of the three species in tomato and pepper in south Florida may be due to a common route and near concurrent introduction of GRSV and TCSV to the state. Results from south Florida parallel results from Argentina where GRSV, TCSV, and TSWV are also known to exist. Gracia et al. (14) found all three viruses present in the tomato production areas of Mendoza and Buenos Aires Provinces in Argentina. However, in a similar study conducted in the same region 1 to 2 years later, a distinct geographic segregation of the viruses was found although the reasons for these changes were not fully explored (39).

Across south Florida, multiple solanaceous vegetables and weeds were found to be hosts, in some cases new hosts, of GRSV and/or TCSV in field surveys (Table 2). These findings corresponded with those from experimental host range inoculations (Table 1). Neither GRSV nor TCSV was found in other reported hosts (e.g., peanut and soybean) in field surveys, which also corresponded with our inability to infect a variety of leguminous and other plants by mechanical inoculation in our experimental host range studies (Table 1). For the one plant species (eggplant) in which we compared thrips-mediated with mechanical inoculation, no differences in symptoms (necrosis and ringspots) or distribution of TCSV (local but no systemic infections) were observed, although the number of locally infected plants essen-
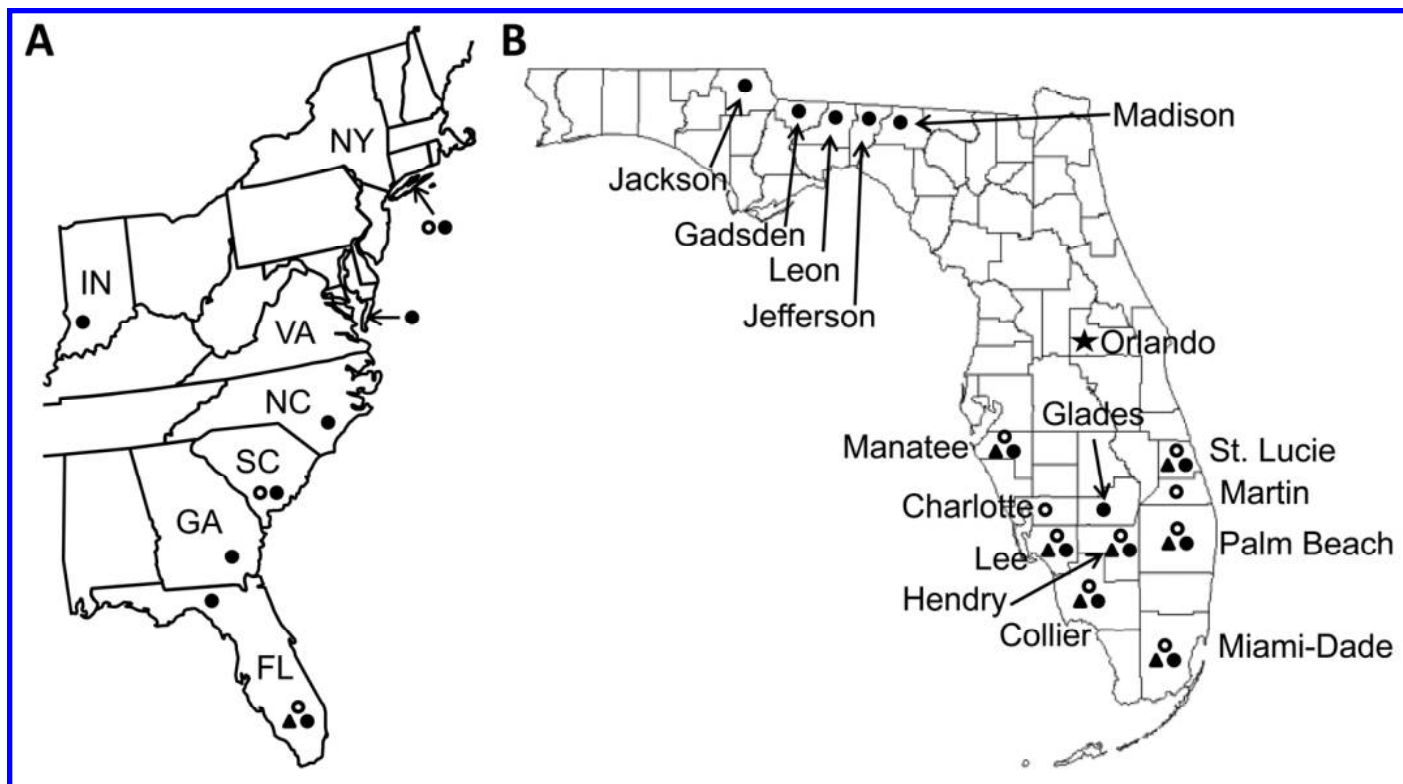

Fig. 2. Geographic distribution of *Groundnut ringspot virus* (GRSV; open circles), *Tomato chlorotic spot virus* (filled triangles) and *Tomato spotted wilt virus* (filled circles) observations. **A,** Samples were collected from Florida (FL), Georgia (GA), South Carolina (SC), North Carolina (NC), Virginia (VA; Eastern Shore), New York (NY; Long Island), and Indiana (IN). **B,** Florida map (county level) with counties surveyed named. The city of Orlando is indicated with a star for reference. Samples of cultivated hosts and/or weed hosts from each state or county were collected and tested for the presence of the three tospoviruses.

tially doubled with thrips-mediated inoculation (Table 1). This suggests that thrips-mediated inoculation was more efficient than mechanical inoculation and raises the possibility that results of our experimental host range studies may have been influenced by the inoculation method. However, correspondence between TCSV symptoms and distribution in lettuce during a recent epidemic in Puerto Rico (13) with results of our experimental host range studies (Table 1) demonstrates the continued usefulness of such studies. All GRSV and TCSV isolates selected for additional sequence characterization from new hosts and new locations displayed low genetic diversity (Table 3). All GRSV isolates characterized in this study, including those from South Carolina and New York, had the same reassorted ($L_G M_T S_G$) genotype that was originally reported for Florida isolates (34). This low genetic diversity for both GRSV and TCSV isolates analyzed in this study is likely due to the recent introductions of both viruses to the United States.

Experimental transmission assays demonstrated that thrips vectors of Florida GRSV isolates include *F. schultzei*, in addition to the previously reported *F. occidentalis* (34). Significantly higher acquisition and transmission rates of GRSV were observed for *F. schultzei* (30.3 and 24.1%, respectively; Table 4) than for *F. occidentalis* (5.9 and 6.4%, respectively; Table 4). This suggests that *F. schultzei* is a contributor to outbreaks of GRSV in Florida even though this species was present in relatively low numbers in tomato and pepper blooms analyzed in this study (data not shown). However, *F. schultzei* has recently been observed breeding on cucurbitaceous and solanaceous crops in Miami-Dade County (17,18). At present it does not seem likely that cucurbit crops are a significant host of GRSV in Florida because impatiens was the only non-solanaceous host that became infected when mechanically inoculated with GRSV. We specifically tested watermelon and other cucurbit species in the GRSV experimental host range because cucurbit flowers were the major source of *F.*

TABLE 2. Geographic and temporal distribution of tospoviruses in crop and weed species in surveyed areas

Year	Region[t]	Plant species	Samples	Number infected[u]			
				GRSV	TCSV	TSWV	Mixed
2011	South Florida	*Solanum lycopersicum*	33	21	nt	4	2[v]
		Capsicum annuum	13	10	nt	2	0
		Physalis angulata	8	1	nt	0	0
		Solanum americanum	4	2	nt	0	0
		Other species[w]	6	0	nt	0	0
	North Florida	*S. lycopersicum*	1	0	nt	1	0
	Southeastern U.S.	*S. lycopersicum*	2	0	nt	2	0
2012	South Florida	*S. lycopersicum*	173	62	43	18	0
		C. annuum	14	3	1	4	0
		Arachis hypogaea	61	0	0	35	0
		S. melongena	1	1	0	0	0
		Other species[x]	36	0	0	0	0
	North Florida	*S. lycopersicum*	36	0	0	30	0
		C. annuum	12	0	0	11	0
		A. hypogaea	15	0	0	3	0
	Southeastern U.S.	*S. lycopersicum*	76	0	0	40	0
		C. annuum	1	0	0	1	0
	Indiana	*S. lycopersicum*	1	0	0	1	0
		C. annuum	3	0	0	3	0
2013	South Florida	*S. lycopersicum*	268	24	58	20	3[y]
		C. annuum	19	6	1	7	1[y]
		S. melongena	1	0	0	0	0
		Other species[z]	12	0	0	0	0
	Southeastern U.S.	*S. lycopersicum*	12	1	0	6	1[y]
	New York	*S. lycopersicum*	20	1	0	7	0
		C. annuum	3	0	0	3	0
Total		All	830	132	102	197	7

[t] Geographic regions from which samples were collected: south Florida (Charlotte, Collier, Glades, Hendry, Lee, Manatee, Martin, Miami-Dade, Palm Beach, and St. Lucie Counties), north Florida (Gadsden, Jackson, Jefferson, Leon, and Madison Counties), southeastern United States (Georgia, South Carolina, North Carolina and Virginia), Indiana (Knox County), and New York (Suffolk County).

[u] Number of samples infected with *Groundnut ringspot virus* (GRSV), *Tomato chlorotic spot virus* (TCSV), *Tomato spotted wilt virus* (TSWV), or combinations thereof (Mixed). All samples tested by enzyme-linked immunosorbent assay with selected samples confirmed by reverse transcription-polymerase chain reaction (RT-PCR) prior to detection of TCSV in Florida in 2012 (nt = not tested for TCSV), after which all samples were tested by RT-PCR.

[v] Two *S. lycopersicum* samples with mixed GRSV and TSWV infections were found in south Florida in 2011.

[w] Other species tested in 2011: *Glycine max*, *Macroptilium lathyroides*, and unknown legume.

[x] Other species tested in 2012: *Ambrosia artemisiifolia*, *Bidens pilosa*, *Citrullus lanatus*, *Eclipta prostrata*, *Gamochaeta pensylvatica*, *Ipomoea batatas*, *Lactuca canadensis*, *Melothria pendula*, *Origanum vulgare*, *Parthenium hysterophorus*, *Phaseolus vulgaris*, *Physalis angulata*, *S. americanum*, *S. tuberosum*, unknown legume, and *Wedelia trilobata*.

[y] Two *S. lycopersicum* samples with mixed GRSV and TCSV infections, one *S. lycopersicum* sample with a mixed TCSV and TSWV infection, and one *C. annuum* sample with a mixed TCSV and TSWV infection were found in south Florida in 2013. One *S. lycopersicum* sample with a mixed GRSV and TSWV infection was found in South Carolina in 2013.

[z] Other species tested in 2013: *Bidens pilosa*, *Lactuca sativa*, *Physalis philadelphica*, *S. americanum*, and *S. tuberosum*.

schultzei for our transmission experiments. Larvae of *F. schultzei* and other thrips species must acquire tospoviruses by feeding on infected plants to subsequently transmit as adults. Thus, *F. schultzei* or any other thrips species developing on noninfected cucurbits would likely not contribute to the primary spread of GRSV or TCSV.

A common thrips species present in south Florida flowers is *F. bispinosa*, which is known to be a TSWV vector (2). In our transmission studies, a small percentage of *F. bispinosa* acquired GRSV (3.2%, Table 4). However, no transmission by *F. bispinosa* was observed, suggesting that this species is not readily able to transmit Florida GRSV isolates. Similar acquisition of tospovirus but vector incompetence is known to occur in other thrips species, and is presumably due to a lack of movement of the virus to the salivary glands, as reported previously (8), although further investigation is required. Our characterization of the ability of common thrips species in the southeastern United States to acquire and transmit GRSV provides a sound basis for future research to determine the role each vector and plant species plays in the epidemiology of GRSV in southeastern tomato and pepper production. Because known vector determinants are present on tospovirus M RNA genome segments, and because GRSV isolates analyzed from Florida, South Carolina, and New York are all reassortants containing the TCSV M RNA, it is likely that our acquisition and transmission results will be broadly applicable to TCSV.

We specifically compared the ability of *F. occidentalis* to acquire and transmit TCSV and GRSV. TCSV was both acquired and transmitted by *F. occidentalis* at a significantly higher rate than GRSV (Table 4). However, once thrips acquired the virus, similar transmission rates by viruliferous *F. occidentalis* were observed for TCSV (21.5%) and GRSV (28.6%), which parallels previous results comparing GRSV and TSWV transmission (34). This suggests that acquisition efficiency is important in determining population level tospovirus transmission rates, with GRSV, TCSV, and TSWV having quite similar rates of transmission by *F. occidentalis* once thrips acquired the virus. Given the non-reassorted genotype of Florida TCSV isolates, it is also expected that *F. schultzei* and other known TCSV vectors (23,37) will play a role in Florida, although plant host, virus isolate and thrips biotype may affect the transmission rates (10,37). While there are relatively low populations of *F. schultzei* in Florida, higher populations are found in Puerto Rico where TCSV has recently become a significant limitation to vegetable production (13,32). Thus, management of *F. schultzei* should be included in integrated pest management strategies for these viruses.

Our inability to infect peanut plants with GRSV and the absence of GRSV in peanut plants with tospovirus symptoms in this study is of interest because peanut is the originally reported host of GRSV (9). No symptoms were observed and no virus was detected in peanut despite repeated mechanical inoculation of peanut plants with GRSV, even using methods shown to be effective for mechanical inoculation of peanut with TSWV (21). It is likely that previous reports of peanut inoculations with GRSV (9,11) made use of non-reassorted isolates. Perhaps the reassorted nature of GRSV isolates present in Florida, South Carolina, and New York renders these isolates incapable of infecting peanut. The presence of the TCSV M RNA, which encodes the NSm movement protein and the G_N and G_C glycoproteins, may be sufficient to alter the host range and/or the vector range of the reassorted GRSV isolates. Such alterations may also explain our observations of no GRSV infection in peanuts with tospovirus

TABLE 3. Genetic diversity of *Groundnut ringspot virus* (GRSV) and *Tomato chlorotic spot virus* (TCSV) from the United States

Virus	RNA[w]	Isolates	GenBank accession numbers	π[x]	SE[y]
GRSV	S	9	KM007024 to KM007032	4.57×10^{-3}	2.30×10^{-3}
	M	10	KM007011 to KM007020	3.76×10^{-3}	1.63×10^{-3}
	L-5′[z]	7	KM006992 to KM006998	7.00×10^{-3}	2.10×10^{-3}
	L-3′[z]	9	KM007002 to KM007010	3.62×10^{-3}	1.28×10^{-3}
TCSV	S	5	KM007033 to KM007037	4.23×10^{-3}	2.25×10^{-3}
	M	3	KM007021 to KM007023	6.43×10^{-3}	2.74×10^{-3}
	L	3	KM006999 to KM007001	8.45×10^{-3}	2.56×10^{-3}

[w] Portions of the GRSV S (542 nt; N), M (520 nt; NSm), or L (692 nt; L-5′; or 708 nt; L-3′) RNAs and TCSV S (478 nt; N), M (520 nt; NSm), or L (899 nt; L) RNAs from virus isolates collected from all new hosts and new locations reported.

[x] Mean nucleotide diversity (π) of the pairwise comparisons of each sequence was calculated within MEGA 4.1 using pairwise deletion and a maximum composite likelihood substitution model.

[y] Standard error (SE) of the mean computed using bootstrapping with 1,000 replications.

[z] 5′ and 3′ portions of the L gene on the L RNA were sequenced for different isolates of GRSV.

TABLE 4. Acquisition and transmission of *Groundnut ringspot virus* (GRSV) by locally important thrips species (top portion of the table) and acquisition and transmission of *Tomato chlorotic spot virus* (TCSV) and GRSV by *Franklinella occidentalis* (lower portion of the table)

	Species	Virus acquisition[x]	Virus transmission[y]	Adjusted transmission[z]
Thrips	*F. schultzei*	30.3% (17/56) a	24.1% (14/58) a	64.7% (11/17) a
	F. occidentalis	5.9% (22/373) b	6.4% (25/389) b	28.6% (6/21) ab
	F. bispinosa	3.2% (5/156) b	0.0% (0/228) c	0.0% (0/5) b
	F. fusca	0.0% (0/39) b	0.0% (0/81) c	–
Virus	TCSV	36.8% (103/280) *a*	11.8% (34/287) *a*	21.5% (23/103) *a*
	GRSV	5.9% (22/373) *b*	6.4% (25/389) *b*	28.6% (6/21) *a*

[x] Virus acquisition determined by enzyme-linked immunosorbent assay (ELISA) or reverse transcription-polymerase chain reaction (RT-PCR) and presented as the number of positive thrips/total number tested. Thrips or virus species sharing the same letter (regular type for thrips and italicized for viruses) are not significantly different according to Tukey-style multiple comparisons of proportions ($\alpha = 0.05$).

[y] Virus transmission to noninfected leaf discs determined by ELISA or RT-PCR and presented as the number of positive discs/total number tested. Thrips or virus species sharing the same letter (regular type for thrips and italicized for viruses) are not significantly different according to Tukey-style multiple comparisons of proportions ($\alpha = 0.05$).

[z] Transmission efficiency using results from viruliferous thrips only presented as the percentage of virus-positive leaf discs from the number of leaf discs fed upon by viruliferous thrips. Numbers in parentheses indicate the total number of virus-positive leaf discs/number of viruliferous thrips. Thrips or virus species sharing the same letter (regular type for thrips and italicized for viruses) are not significantly different according to Tukey-style multiple comparisons of proportions ($\alpha = 0.05$).

symptoms sampled from areas containing GRSV in south Florida (Table 2) and the lack of acquisition or transmission of GRSV by *F. fusca* (Table 4).

GRSV was found in single tomato samples from South Carolina and from New York, but it is not yet clear if these findings reflect establishment of GRSV in these locations or detections of chance infections that may or may not be maintained in the ecosystem. Suitable plant hosts (including weed species) and vectors (*F. occidentalis*) are present in both South Carolina and New York, and across the wider United States. Both states lack significant populations of *F. schultzei*, the most efficient vector yet determined, and have considerably colder winters than Florida. Such factors are likely important for establishment of GRSV and TCSV in new geographic areas.

GRSV and TCSV were identified primarily in south Florida and only GRSV was found outside of Florida in the areas we surveyed. Both viruses appear mostly limited to infections of tomato and other solanaceous vegetables, ornamentals, and weeds, although two non-solanaceous hosts were infected by mechanical inoculation. This suggests that these GRSV and TCSV isolates will remain primarily of concern to tomato, pepper, and other solanaceous crop producers. However, TCSV may become an issue for lettuce producers as recently observed in Puerto Rico (13). Both viruses infected impatiens in our study, and likely infect a wider range of ornamentals than we have tested. The *Sw5* gene for TSWV resistance in tomato also conferred resistance to GRSV ('Quincy' in Table 1) as observed previously (34). However, the between cultivar variability in symptoms and susceptibility to GRSV and/or TCSV observed within species including lettuce, tomato, and petunia makes generalizations across these species difficult.

While *F. schultzei* is an efficient vector of GRSV, TCSV, and TSWV, it currently has a relatively limited distribution within the continental United States and thus is not likely to become a widely important tospovirus vector outside of Florida in the near future. Additionally, the preference of *F. schultzei* for cucurbit flowers coupled with the inability of GRSV to infect cucurbits, suggests a minor role for *F. schultzei* in field transmission of GRSV and TCSV even in Florida. However, *F. occidentalis* is widespread within the continental United States and able to transmit GRSV and TCSV, and likely plays a major role in field spread of GRSV and TCSV in Florida. The presence of *F. occidentalis* in South Carolina and New York, states from which detection of GRSV is first reported in this work, gives the potential for GRSV transmission in these states.

The potential for further spread of these emerging tospovirus species exists, especially given detection of GRSV and TCSV in tomato transplants, and ongoing vigilance is needed to observe and test suspect samples. The rapid change in tospovirus species in Florida with the initial appearance of GRSV and the subsequent appearance of TCSV serves as a warning. Nucleic acid-based tests are required for accurate identification because of the highly similar symptoms induced by GRSV, TCSV, and TSWV in tomato and pepper, and inability of currently used serological tests to unequivocally distinguish these species. It is possible that GRSV and/or TCSV are already present elsewhere in the United States but have been misdiagnosed as TSWV. Although not common, infections of a single plant with GRSV and TSWV, GRSV and TCSV, or TCSV and TSWV reported here and previously (33,34) suggest that tospovirus diagnostics are likely to become more complex through additional reassortment of tospovirus genomic RNAs. Clearly, GRSV and TCSV were both present in a single plant or thrips and able to exchange M RNA segments to create the GRSV reassortant first found in Florida. Our detection of this same GRSV reassortant in South Carolina and New York, where TSWV is already established, provides early warning of new locations where further reassortment may occur.

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