Identification of a Natural Weed Host of Tomato Mottle Geminivirus in Florida

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ABSTRACT

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Surveys were conducted in southwest and west central Florida to identify naturally occurring weed hosts of the whitefly-transmitted tomato mottle geminivirus (TMoV). More than 780 samples representing 42 species in 14 families were collected from 35 field sites over 3 yr. Detection procedures included nucleic acid spot hybridization assays with confirmation by polymerase chain reaction amplification, Southern blot analysis, and virus inclusion visualization. Experimental transmission of TMoV was also attempted to more than 340 plants representing 20 species in seven families by using whiteflies (*Bemisia argentifolii* [*B. tabaci* biotype B]). One exotic weed from the Solanaceae, tropical soda apple (*Solanum viarum*), was found to be an experimental host of TMoV and was also found to be naturally infected in the field at a low incidence.

Additional keywords: *Lycopersicon esculentum* Mill.

Since its recognition in 1989, a disease of tomato (*Lycopersicon esculentum* Mill.), caused by tomato mottle geminivirus (TMoV) (1), has become a major limiting factor for tomato production in southern Florida (D. J. Schuster, *unpublished data*). Symptoms of TMoV in tomato include stunting, interveinal mottling, distortion of shoots and leaves, and reduced yields (14). The virus is transmitted by the silverleaf whitefly (*Bemisia argentifolii* Bellows & Perring [*B. tabaci*, biotype B]) (11) and has a narrow experimental host range limited to *Phaseolus vulgaris* L. and certain nonnative, solanaceous plants, including *L. cheesmanii* Riley, *L. chilense* Dunal, *L. esculentum*, *L. hirsutum* Humb. & Bonpl., *L. pennellii* (Corr.) D'Arcy, *L. peruvianum* (L.) Mill., *L. pimpinellifolium* (L.) Mill., *Nicotiana benthamiana* Domin., *N. edwardsonii* Christie & Hall, *N. tabacum* L., *Physalis alkekengi* L., *P. ixocarpa* Brot. ex DC., and *P. wrightii* A. Gray (14).

Weeds have been reported to be reservoirs of both primary inoculum and vectors for other geminiviruses (3,10). *Cynanchum acutum* L. (Asclepiadaceae) plays a significant role in the epidemiology of tomato yellow leaf curl in the Jordan valley (5). However, the identification and importance of weed hosts have not been determined in most geminivirus epidemics. This research was conducted to gain information about the weed host range of TMoV so that effective management strategies can be developed.

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MATERIALS AND METHODS

Field surveys. Samples of symptomatic and asymptomatic weeds were collected from 35 different sites in eight counties in southwest and west central Florida. Sites were selected on the basis of high incidences of tomato plants exhibiting characteristic symptoms of TMoV. The counties sampled accounted for more than 70% of the state's tomato production during 1990–1991 and 1992–1993 (2). Weed surveys were conducted both during and a few weeks after the spring 1991, 1992, and 1993 and fall 1992 production periods. Foliar samples were collected from weeds that are frequently found in great abundance in or around tomato fields, that are feeding and/or reproductive hosts of *B. argentifolii*, that are hosts of other geminiviruses, or that exhibited viruslike symptoms.

Experimental transmission. Experimental transmission of TMoV was attempted to a select group of weeds commonly found in close proximity to tomato fields for confirmation and extension of field survey results. Transmission studies used the characterized TMoV isolate (1) passaged mechanically from tomato through tobacco (*N. edwardsonii*) and back to tomato. Infected tomato cultivars Sunny and Florida Lanai were used to rear colonies of viruliferous *B. argentifolii*, while nonviruliferous colonies were maintained on the same tomato cultivars free of TMoV. Weed species exposed to nonviruliferous *B. argentifolii*, tomato plants (Sunny or Florida Lanai) exposed to nonviruliferous *B. argentifolii*, and tomato plants exposed to viruliferous *B. argentifolii* were used as controls in each transmission experiment. The fourth treatment consisted of weed plants exposed to viruliferous *B. argentifolii*. Each treatment consisted of four to eight seedlings maintained in screened polystyrene cages in transmission rooms under fluorescent lights at 23–28 C or in a greenhouse at 25–32 C. Approximately 50 whiteflies per plant were introduced into the cages and allowed to feed on test plants for 48–72 hr before they were killed with insecticidal soap.

Transmission of TMoV to tomato plants from certain field-collected weeds expressing symptoms of virus infection, including *Desmodium canadense* (L.) DC. (beggarweed), *Macroptilium lathyroides* (L.) Urban (phasibean), *Sida acuta* J. Burm. (broomweed), and *S. rhombifolia* L. (arrowleaf sida), was also attempted with approximately 250 whiteflies. The whiteflies were introduced into cages containing symptomatic weed plants and allowed to feed for 24 hr. Eight tomato plants were then placed in each cage. About 250 additional whiteflies were introduced on a weekly basis for 3–4 wk. Attempts were also made to mechanically transmit virus to tomato from symptomatic plants of *D. canadense*, *M. lathyroides*, *S. acuta*, and *S. rhombifolia* by using pulverized tissue (1:10, w/v) in a buffer containing 0.1 M KH_2PO_4 and 0.2% mercaptoethanol at pH 7.4 (16). Cotton-tip applicators were used to rub virus inoculum on the leaves of test plants coated with Carborundum (320 mesh). Positive controls were inoculated with tissue macerates from TMoV-infected tomato plants, and negative controls were treated with buffer alone. Mechanically inoculated plants were maintained as described above.

All inoculated plants were monitored for disease expression for at least 4 wk. Test plants were then cut back, and symptom expression was monitored for at least an additional 4 wk. Presence or

Table 1. Field survey of tomato mottle virus (TMoV) in weeds and experimental transmission of TMoV to selected plant species

		Number of samples and detection of TMoV[a]	
	Common name	**Field survey**	**Experimental transmission**
Amaranthaceae			
Amaranthus viridus L.	Slender amaranth	5 (−)	ND[b]
A. spinosus L.	Spiny amaranth	5 (−)	ND
Asteraceae			
Ambrosia artemisiifolia L.	Common ragweed	23 (−)	ND
Bidens bipinnata L.	Spanish needles	24 (−)	12 (−)
Eclipta alba (L.) Hassk.	Eclipta	7 (−)	ND
Erechtites hieracifolia (L.) Raf. ex DC.	Fireweed	9 (−)	ND
Sonchus asper (L.) J. Hill	Spiny sowthistle	6 (−)	ND
Brassicaceae			
Lepidium virginicum L.	Peppergrass	14 (−)	ND
Commelinaceae			
Commelina sp.		8 (−)	ND
Convolvulaceae			
Ipomoea sp.	Morning glory	2 (−)	ND
Cucurbitaceae			
Momordica charantia L.	Wild balsam apple	12 (−)	8 (−)
Euphorbiaceae			
Euphorbia cyathophora J. Murr.	Painted leaf	13 (−)	12 (−)
E. hirta L.	Hairy spurge	10 (−)	8 (−)
E. hypericifolia L.	Spurge	21 (−)	8 (−)
Fabaceae			
Crotalaria rotundifolia (Walt.) Gmel.	Rabbit bells	5 (−)	ND
C. mucronata Desv.	Rattle box	12 (−)	ND
Crotalaria sp.		20 (−)	ND
Desmodium canadense (L.) DC.	Beggarweed	7 (−)	ND
Galactia sp.	Milk pea	2 (−)	ND
Indigofera hirsuta L.	Hairy indigo	18 (−)	ND
Macroptilium lathyroides (L.) Urban	Phasibean	48 (−)	14 (−)
Rhynchosia minima (L.) DC.	Rhynchosia	ND	13 (−)
Sesbania punicea (Cav.) Benth.	Sesban	20 (−)	5 (−)
Malvaceae			
Hibiscus sp.	Wild hibiscus	8 (−)	ND
Sida acuta J. Burm.	Broomweed	40 (−)	18 (−)
S. rhombifolia L.	Arrowleaf sida	35 (−)	ND
Urena lobata L.	Caesarweed	29 (−)	ND
Onagraceae			
Ludwigia bonariensis (Micheli) Hara		37 (−)	28 (−)
L. decurrens Walter		9 (−)	8 (−)
L. erecta (L.) Hara		27 (−)	8 (−)
L. leptocarpa (Nutt.) Hara		3 (−)	ND
L. linifolia Poir. in Lam.		2 (−)	ND
L. octovalvis (Jacq.) Raven		36 (−)	12 (−)
L. peruviana (L.) H. Hara	Primrose-willow	3 (−)	ND
Ludwigia sp.		2 (−)	ND
Rubiaceae			
Diodia teres Walter	Poor Joe	6 (−)	ND
Solanaceae			
Lycopersicon esculentum Mill.	Tomato	ND	80 (−)
Physalis angustifolia Nutt.	Narrow leaf ground cherry	4 (−)	12 (−)
Physalis sp.	Ground cherry	14 (−)	ND
Solanum capsicoides All.		2 (−)	4 (−)
S. nigrum L.	Common nightshade	61 (−)	22 (−)
S. tampicense Dunal		ND	3 (−)
S. torvum Sw.		ND	25 (−)
S. viarum Dunal	Tropical soda apple	168 (+)[c]	46 (+)[d]
Urticaceae			
Boehmeria cylindrica (L.) Sw.	False nettle	1 (−)	ND
Verbenaceae			
Lantana camara L.	Lantana	6 (−)	ND

[a]TMoV detection was based on nucleic acid spot hybridization assay (NASHA), nuclear inclusion visualization, polymerase chain reaction, and Southern blot analysis.

[b]Not done.

[c]Initially, 25 samples of *S. viarum* were collected from two sites adjacent to tomato fields with high incidences of tomato mottle symptoms. Two of 10 samples from one site tested positive for TMoV by NASHA. An additional 143 samples were collected from the same two fields and five additional sites located 0.6–3.1 km from the nearest tomato field. All *S. viarum* samples from the second survey tested negative for TMoV.

[d]The transmission rate of TMoV to *S. viarum* averaged approximately 15%.

absence of TMoV in test plants was determined by the detection procedures outlined below and by back transmission to tomato by *B. argentifolii.*

Detection of TMoV. Field-collected and experimental plant materials were screened for geminivirus by means of a general probe for whitefly-vectored geminiviruses consisting of cloned TMoV component A DNA (A-probe) and a probe specific for the TMoV genome consisting of cloned TMoV component B DNA (B-probe). Cloned genome components A and B were separately labeled with ^{32}P-CTP with a Megaprime DNA labeling system (Amersham Corp., Arlington Heights, IL). These probes were used to detect viral DNA in nucleic spot hybridization assays (NASHA) (14) and Southern blots (17). Positive results for TMoV by NASHA were confirmed through visualization of characteristic geminivirus nuclear inclusions in plant tissue sections (4), by polymerase chain reaction (PCR) amplification of DNA (15), and by Southern blot hybridization. Medium stringency hybridization conditions for both NASHA and Southern blots were used with the A-probe (2× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 65 C), and high stringency conditions were used with the B-probe (0.2× SSC, 65 C).

PCR. Several experimental protocols (6–9) and two commercial protocols (Magic Minipreps DNA purification system, Promega Corp., Madison, WI; and Genereleaser, Bioventure, Inc., Murfreesboro, TN) were tested for the isolation of geminivirus nucleic acid from different plant species. A slight modification (reduced amounts of sample and liquid nitrogen for grinding tissue were used) of the procedure of Doyle and Doyle (8) was selected because reliable results were obtained with a wide range of tissues evaluated. Other procedures did not permit reliable amplification of geminivirus DNA, which was added to the plant samples before test extraction. Leaf disks approximately 1 cm in diameter were chilled with liquid nitrogen and ground to a powder in microcentrifuge tubes with disposable plastic pestles. The tissue was resuspended in 0.5 ml of 1× SSC homogenization buffer, and the remainder of the extraction reagents of the procedure were scaled accordingly. Positive controls included a leaf sample spiked with 10 ng of the plasmid pGEMEX-1, which contained the cloned B component of TMoV (1), and a tomato leaf infected with TMoV extracted as above. Samples from all extraction procedures were resuspended in a final volume of 100 µl of buffer containing 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA. PCR components were as follows: 50 mM KCl; 10 mM Tris base (pH 9.0); 0.1% Triton X-100; 2.5 mM $MgCl_2$; 0.5 mM each of dATP, dCTP, dGTP, and dTTP; 0.15 mM of each primer; 1 unit of *Taq* DNA polymerase; and 3 µl of sample. The total volume of the reaction was 50 µl. Primers were selected to amplify a 960-bp fragment of the TMoV B component (nucleotides 80–1,040). These primers were used to sequence the genome and were suitable for TMoV B component amplification. The sequences of the primers are Bv80, 5′ GGAGTATTAGAGTAA 3′, and Bc1040, 5′ CGTCACCATCAACGT 3′. Each sample was overlaid with 50 µl of mineral oil. A Combi Thermal Reactor TR2 (Hybaid, Ltd., Teddington, Middlesex, UK) was used to implement the following thermal cycle: 5 min at 95 C for strand separation and then 35 cycles at 94 C (1 min, 0.5 sec/C), 35 C (1 min, 0.2 sec/C), and 72 C (3 min, 0.2 sec/C). A positive control of 10 ng of cloned TMoV B component was included. A negative control was provided by testing all of the PCR reaction components minus DNA. Amplification products were separated by electrophoresis in a 1% agarose gel and were detected by staining with ethidium bromide and Southern blotting.

Southern blot hybridization analysis. Nucleic acid extracts of samples and PCR products were electrophoresed in a 1% agarose gel. The DNA was transferred bidirectionally onto nylon membranes (12,17). The B-probe was hybridized to one of the membranes (14). Membranes were exposed to X-ray film

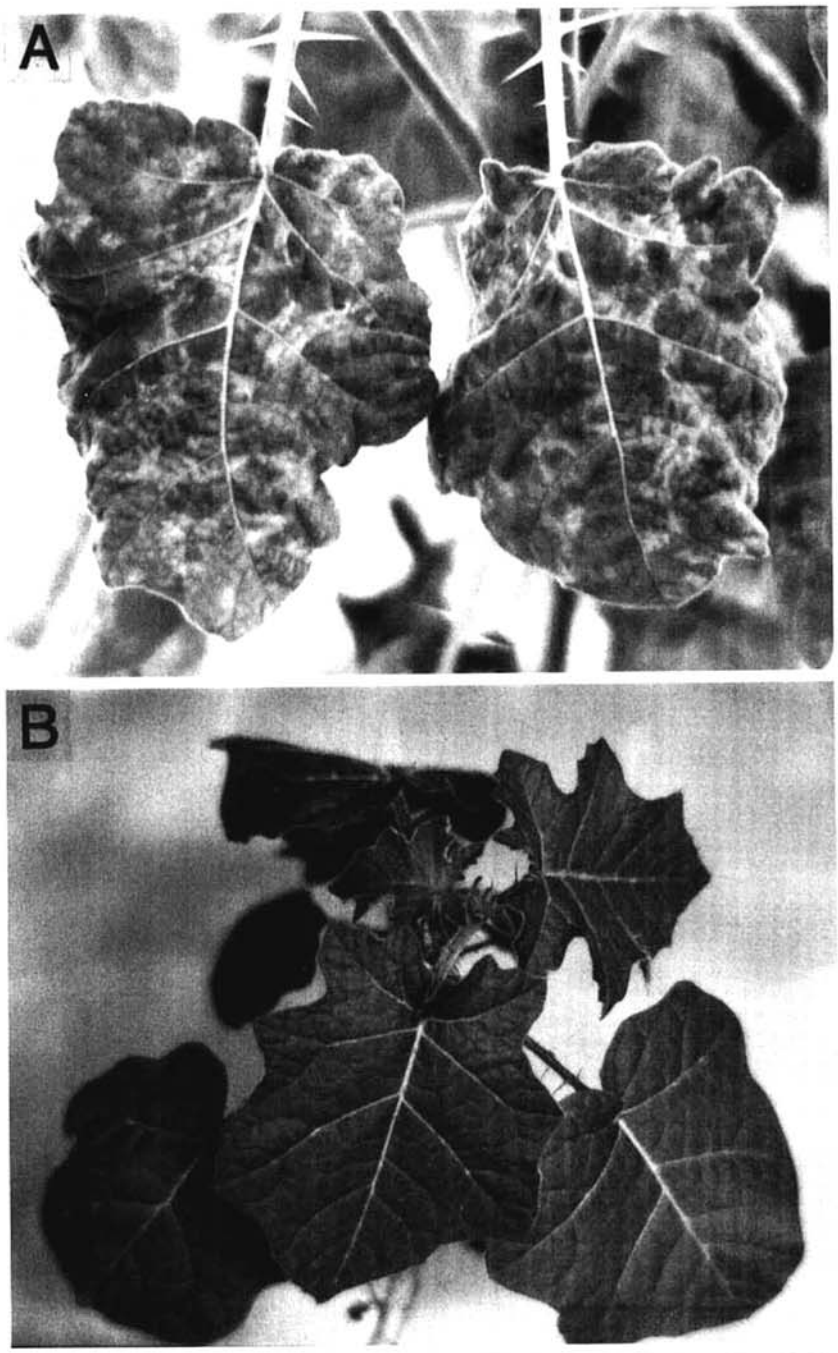

Fig. 1. (A) *Solanum viarum* (tropical soda apple) infected with tomato mottle geminivirus by means of *Bemisia argentifolii* and **(B)** uninfected plant.

for 24 hr at room temperature, and film was developed by standard photographic procedures.

RESULTS AND DISCUSSION

More than 780 field samples representing 42 species in 14 families (Amaranthaceae, Asteraceae, Brassicaceae, Commelinaceae, Convolvulaceae, Cucurbitaceae, Euphorbiaceae, Fabaceae, Malvaceae, Onagraceae, Rubiaceae, Solanaceae, Urticaceae, and Verbenaceae) were tested for TMoV (Table 1). All plants collected from the field were negative for TMoV by NASHA with both A- and B-probes, with the exception of *S. viarum* (tropical soda apple). Initially, 25 samples of *S. viarum* were collected from two sites adjacent to tomato fields in which many plants exhibited characteristic symptoms of tomato mottle. Two of 10 samples from one of the sites tested positive by NASHA. An additional 143 samples were collected from the same two sites and five additional sites located 0.6–3.1 km from the nearest tomato field. All subsequent *S. viarum* samples tested negative for TMoV. Symptoms of TMoV infection in *S. viarum* include stunting, leaf distortion, and chlorotic mottling (Fig. 1).

Three symptomatic, field-collected weed species, *M. lathyroides, S. acuta,* and *S. rhombifolia,* tested positive with the A-probe, were negative with the B-probe, and displayed typical geminivirus nuclear inclusions. These results confirmed previous observations of infection with other whitefly-vectored geminiviruses distinct from TMoV (4). We were unable to transmit any virus to tomato from symptomatic *Sida* spp., *M. lathyroides,* or *D. canadense,* either mechanically or via *B. argentifolii.* Mechanical transmission of TMoV from tomato to tomato was 25–30%, while whitefly transmission was 60–100%.

Occasionally, samples of three nonsymptomatic, ubiquitous weed species, *Ludwigia decurrens* Walter, *L. erecta* (L.) Hara, and *L. octovalvis* (Jacq.) Raven, tested positive by NASHA with either or both probes. Such results conflicted with those of transmission experiments in which these species could not be infected with TMoV by viruliferous whiteflies and the typical nuclear inclusions could not be visualized. Since certain samples of *Ludwigia* spp. often gave surprisingly strong positive results by NASHA, they were selected for more intensive study. Infection by TMoV could not be confirmed in *Ludwigia* spp. by means of Southern blot analysis of either amplified or unamplified nucleic acid extracts. In Southern analysis, the B-probe hybridized with the linear TMoV B component DNA (2,451 bp) (Fig. 2, lane 2) and with DNA extracted from TMoV-infected tomato (lane 3) but did not hybridize with DNA extracted from *Ludwigia* samples that gave either positive (lane 7) or negative (lane 8) reactions in NASHA. Southern analysis of the PCR-amplified products gave similar results. The B-probe hybridized with a 960-bp DNA fragment amplified from TMoV-infected tomato (lane 9) and from cloned TMoV B component DNA (lane 15). No such fragment was detected in amplified products of nucleic acids extracted from *Ludwigia* sp. regardless of NASHA results (lanes 13 and 14). Amplification product was visualized in lanes containing nucleic acid extracts of *Ludwigia* sp. spiked with TMoV DNA before extraction (not shown). False positives in NASHA by *Ludwigia* spp. may have resulted from nonspecific binding of the probes with plant material.

A subset of field-collected weeds, composed of more than 340 plants in 20 species and seven families (Asteraceae, Cucurbitaceae, Euphorbiaceae, Fabaceae, Malvaceae, Onagraceae, and Solanaceae) were inoculated with TMoV by *B. argentifolii.* All inoculated plants tested negative for TMoV by NASHA, with the exception of positive tomato controls and *S. viarum.* The transmission rate of TMoV from tomato to *S. viarum* by *B. argentifolii* averaged approximately 15%. Whitefly transmission of TMoV from tomato to tomato consistently averaged above 75%. Transmission of TMoV from tomato to *S. viarum* was confirmed by the detection of geminivirus inclusions and Southern blot analysis of amplified and unamplified nucleic acid extracts. The B-probe hybridized to DNA extracted from inoculated *S. viarum* (Fig. 2, lane 5) but not to DNA from uninoculated plants (lane 6). A 960-bp fragment was amplified with B component primers from inoculated *S. viarum* but not from uninoculated plants (lanes 11 and 12, respectively). This fragment comigrated with a fragment amplified from TMoV-infected tomato and cloned TMoV B component (lanes 9 and 15, respectively).

Six attempts were made to transmit TMoV by *B. argentifolii* from infected *S. viarum* to at least eight tomato plants per experiment. All attempts were unsuccessful, possibly because of the low feeding preference exhibited by *B. argentifolii* for *S. viarum.* We have observed that *S. viarum* appears to support lower populations of *B. argentifolii* than tomato does.

Tropical soda apple is an extremely thorny, exotic weed, presumably introduced into Florida from the Caribbean Basin or South America within the last three decades (13), and has recently been declared a noxious weed by the Florida Department of Agriculture, Division of Plant Industry (P. L. Hornby, *personal communication*). It functions as a perennial in the southern half of the state and has rapidly become a major problem of range areas, currently infesting an estimated 61,000 ha in south Florida. This number is increasing annually (13). We have also found it to be a natural reservoir for other viruses that infect solanaceous crops (R. J. McGovern, *unpublished*).

Unlike the geminiviruses spread by leafhoppers, it is not unusual for whitefly-vectored geminiviruses to have very narrow host ranges (3). The importance of *S. viarum* in TMoV epidemiology is most likely minimal because of the plant's low incidence near tomato fields,

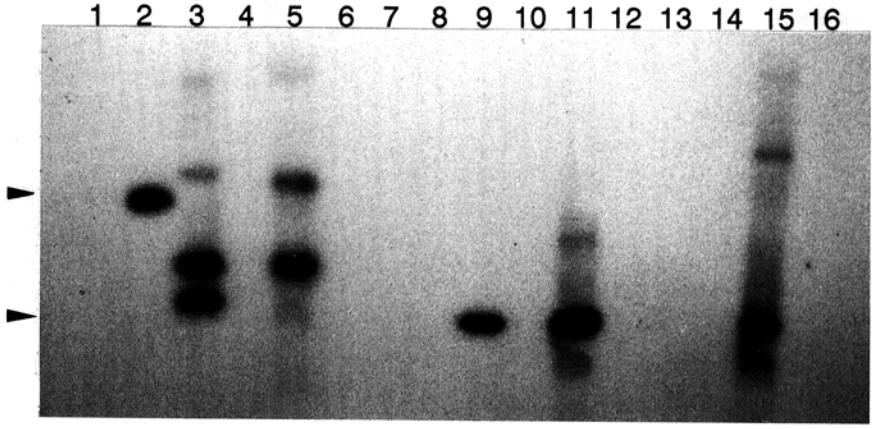

Fig. 2. Southern blot of nucleic acid extracts from samples of *Lycopersicon esculentum* (tomato), *Solanum viarum* (tropical soda apple), and *Ludwigia* sp. (water primrose) either unamplified (lanes 2–8) or amplified (lanes 9–15). Upper arrow indicates the position of the 2,541-bp DNA of the full-length linear tomato mottle geminivirus (TMoV) B component. Lower arrow indicates the position of the 960-bp DNA fragment amplified from nucleotides 80–1,040 of the TMoV B component. The gel was blotted to a nylon membrane and hybridized to cloned ^{32}P-labeled DNA of the TMoV component B (B-probe). The arrangement of samples is as follows: lanes 1 and 16, DNA MW Marker III (Boehringer Mannheim), not visible; lanes 2 and 15, cloned TMoV DNA component B; lanes 3 and 9, DNA of tomato inoculated with TMoV by whiteflies; lanes 4 and 10, DNA of noninoculated tomato; lanes 5 and 11, DNA of tropical soda apple inoculated with TMoV by whiteflies; lanes 6 and 12, noninoculated tropical soda apple; lanes 7 and 13, DNA of *Ludwigia* sp., which gave strong positive results in a nucleic acid spot hybridization assay (NASHA) with the B-probe; and lanes 8 and 14, DNA of *Ludwigia* sp. sample, which gave negative results in NASHA with the B-probe.

the low rate of naturally occurring TMoV infection and transmission from tomato, and the difficulty encountered in transmitting the virus from the weed to tomato. However, as the range and incidence of this weed increase, its role in the epidemiology of TMoV may increase.

The results of this study indicate that old tomato crops and volunteers appear to be the most important sources of TMoV. Thus, growers have been encouraged to improve field sanitation at the end of the season and to voluntarily create a tomato-free period between production seasons during the summer months to help in the management of tomato mottle.

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