

## Tomato chlorotic spot virus Identified in *Marsdenia floribunda* in Florida

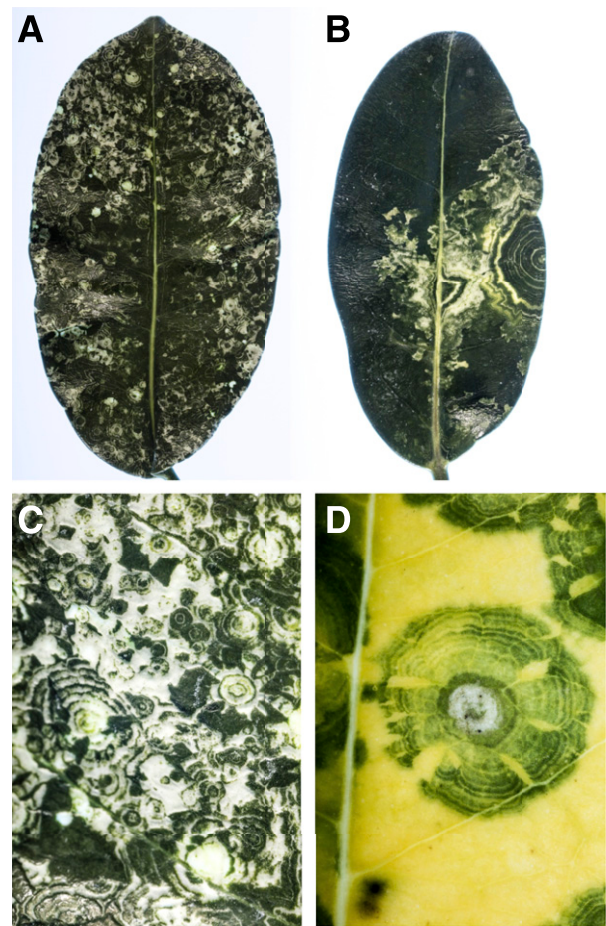
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*Marsdenia floribunda* (Brongn.), also known as Madagascar jasmine, waxflower, and Hawaiian wedding flower (and previously known as *Stephanotis floribunda*), is a flowering plant in the milkweed family, Apocynaceae, and is native to Madagascar. It is grown as an ornamental crop in subtropical and tropical regions. In September 2016, *M. floribunda* plants with typical tospovirus symptoms of chlorotic or bleached ringspots, ring patterns, and line patterns (Fig. 1) were found in a Miami-Dade County nursery by a horticultural inspector. Representative leaf tissue from 10 plants tested positive in serological testing with commercially available *Tomato spotted wilt virus* (TSWV) lateral flow immunoassay reagents (Agdia, Elkhart, IN) and/or broad-spectrum tospovirus enzyme-linked immunosorbent assay reagents (Bioreba, Reinach, Switzerland). Positive results with these tests indicate the presence of one or more tospoviruses because these reagents are known to react with *Tomato chlorotic spot virus* (TCSV) and *Groundnut ringspot virus* (GRSV), in addition to TSWV (Adkins et al. 2015).

Thus, four samples of symptomatic *M. floribunda* were further analyzed by reverse transcription-polymerase chain reaction (RT-PCR) to identify the tospovirus present. Total RNA was extracted from representative samples using an RNeasy Plant Mini Kit (Qiagen, Valencia, CA) and used as template in RT-PCR with primers specific for the nucleocapsid (N) gene of TCSV (Batuman et al. 2014), TSWV (Koehler et al. 2016), or GRSV (Webster et al. 2011). Amplicons of the expected size were produced using the TCSV N gene primers. No amplicons were produced using the TSWV or GRSV N gene primers. Additional TCSV primers specific for the glycoprotein ( $G_N G_C$ ) gene (GLY4-vc and GLY5-v; Webster et al. 2011) were used for further confirmation of TCSV and produced amplicons of the expected size. Amplicons of N and  $G_N G_C$  genes from a representative sample were directly sequenced and deposited in GenBank (Accession Nos. MF109728 and MF109728). The 695-nt N gene sequence had 98 to 99% nucleotide identity with the corresponding region of previously described Florida TCSV isolates, and 96 to 99% nucleotide identity with all TCSV isolates in GenBank. The 112-nt  $G_N G_C$  gene sequence had 98 to 100% nucleotide identity with all TCSV isolates in GenBank.

This is the first report of TCSV infection of *M. floribunda* in Florida or any other location, although TSWV was previously reported from this plant species in Oregon (Green et al. 1988). The recent identification of TCSV in annual vinca (Warfield



**FIGURE 1**

Symptoms of *Tomato chlorotic spot virus* infection of *Marsdenia floribunda* in Florida: (A and B) individual leaves with chlorotic or bleached ringspots, ring patterns, and line patterns; and (C and D) closer views of concentric ring patterns.

et al. 2015), *Hoya wayetii*, and *Schlumbergera truncata* (Baker and Adkins 2015), taken together with our current report, demonstrates that the TCSV host range has expanded into ornamental crops.

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