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First Report of *Tomato chlorotic spot virus* (TCSV) in Tomato, Pepper, and Jimsonweed in Puerto Rico

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Tomatoes in south and southwestern coastal areas of Puerto Rico were observed with symptoms typical of tospovirus infection in a 2006-2007 tomato survey, and again between December 2012 and February 2013 at the University of Puerto Rico's Juana Diaz Experiment Station and on commercial farms in Santa Isabel. Tomato symptoms during this most recent outbreak included: necrotic spots on leaves; general necrosis of leaves, petioles, and stems; death of growing points of plants; and wilting (Fig. 1A). In 2012 and 2013, bell pepper and jimsonweed (*Datura stramonium*) with ringspots, irregular chlorotic areas, and deformation of leaves (Figs. 1B and 1C) were also observed on commercial farms in this region. Serological testing during both 2006-2007 and 2012-2013 using a variety of commercially available enzyme-linked immunorbent assay (ELISA) and lateral flow immunoassay diagnostic reagents from different manufacturers (Agdia Inc., Elkhart, IN; Envirologix Inc., Portland, ME) suggested the presence of one or more tospoviruses.

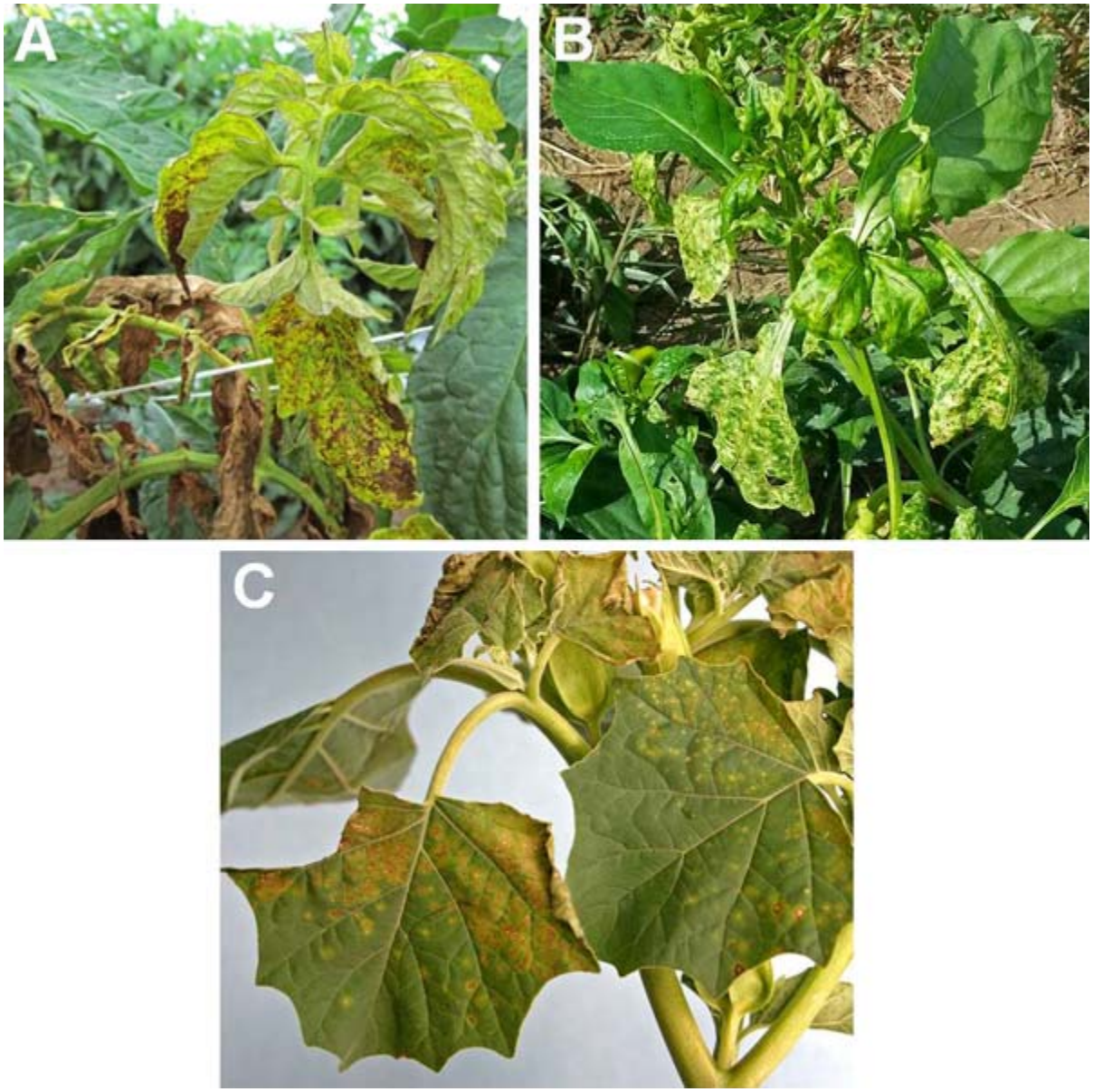

Fig. 1. Symptoms of *Tomato chlorotic spot virus* infection in Puerto Rico include: (**A**) necrotic spots on, and wilting and death of, tomato leaves; (**B**) chlorotic and necrotic spots on, and deformation of, bell pepper leaves; and (**C**) chlorotic and necrotic rings on older jimsonweed leaves (often first appearing near the petiole, and subsequently extending down the leaf blade), and chlorosis and distortion of younger leaves.

Twenty-one tomato, three pepper, and two jimsonweed samples were collected from commercial farms in Santa Isabel in 2012-2013 and tested for *Tomato chlorotic spot virus* (TCSV), *Groundnut ringspot virus* (GRSV), and *Tomato spotted wilt virus* (TSWV) by reverse transcription-polymerase chain reaction (RT-PCR). We tested for these three tospoviruses due to the similarity of reported symptoms that makes visual identification of the virus impossible, the recent detection of TCSV (3) and a GRSV/TCSV reassortant (6) in Florida, and the continuing economic losses in tomato and pepper production worldwide due to TSWV. Total RNA was extracted (RNeasy Plant Mini Kit, Qiagen, Valencia, CA) and tested by RT-PCR with TCSV specific primers designed to amplify a 519 bp portion of the nucleocapsid (N) gene (TCSV-Nv2 5'-CCTAAA-GCTTCTTTAGTGTTATACTTCG-3'; TCSV-Nvc2 5'-CTAACTCAGGCTGGAGAAATC-GAG-3') or a 1011 bp portion of the RNA-dependent RNA polymerase (L) gene (TCSV-3'Lv 5'-AAAGGCAATGCATAAATAGCATCAC-3'; TCSV-3'Lvc 5'-GGAGACC-AAGTCAAGAAAAATTCGTT-3'). Previously described primers (5) were used to amplify a 670 bp portion of the movement protein (NSm) gene. Amplicons of the

expected sizes were produced with all three TCSV primer sets from fifteen tomato, two pepper, and two jimsonweed samples. Primers specific for the N gene of TSWV (1) or GRSV (6) did not amplify products from any samples.

Three TCSV amplicons (N, L, and NSm) from 11 samples (seven tomato, two pepper, and two jimsonweed) were gel-purified and cloned (pGEM-T, Promega, Madison, WI). Six clones of each amplicon were sequenced in both directions, Vector NTI Advance software (Version 11, Invitrogen, Carlsbad, CA) was used for consensus construction and sequences were submitted to GenBank (KC969442 to KC969471). All three genes showed greater than 98% nucleotide identity with TCSV isolates recently described from tomato in Florida (3), and 96-100% nucleotide identity with TCSV isolates from South America and South Africa.

Four thrips species, *Frankliniella schultzei*, *F. kelliae*, *F. bruneri*, and *Thrips palmi*, were identified from tomato, pepper, and weed flowers in the vicinity of symptomatic plants in December 2012 and January 2013. Of these species, *F. schultzei* is known to be an efficient vector of TCSV (4) and has been previously reported as abundant in vegetables in Puerto Rico (2).

To the best of our knowledge this is the first report of TCSV in Puerto Rico and the first report of TCSV infection of jimsonweed from any location. The identification of this solanaceous weed as a TCSV host has implications for its management in Puerto Rico, Caribbean, and Florida vegetable production.

Literature Cited

1. Adkins, S. and Rosskopf, E.N. 2002. Key West nightshade, a new experimental host for plant viruses. Plant Dis. 86:1310-1314.
2. Feliciano, M., Cabrera-Asencio, I. and Rivera-Vargas, L.I. 2008. *Frankliniella occidentalis*, *F. schultzei* and *F. fusca* (Thysanoptera:Thripidae) in Puerto Rico. J. Agric. Univ. P.R. 92:107-110.
3. Londoño, A., Capobianco, H., Zhang, S. and Polston, J.E. 2012. First record of *Tomato chlorotic spot virus* in the USA. Trop. Plant Pathol. 37:333-338.
4. Nagata, T., Almeida, A.C.L., Resende, R.O. and de Ávila, A.C. 2004. The competence of four thrips species to transmit and replicate four tospoviruses. Plant Pathol. 53:136-140.
5. Silva, M.S., Martins, C.R.F., Bezerra, I.C., Nagata, T., de Ávila, A.C. and Resende, R.O. 2001. Sequence diversity of NSm movement proteins of tospoviruses. Arch. Virol. 146:1267-1281.
6. Webster, C.G., Reitz, S.R., Perry, K.L. and Adkins, S. 2011. A natural M RNA reassortant arising from two species of plant- and insect-infecting bunyaviruses and comparison of its sequence and biological properties to parental species. Virology 413:216-225.