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DISEASE NOTES

First Molecular Evidence of *Tomato chlorotic spot virus* Infecting Tomatoes in Cuba

Y. Martinez-Zubiaur, **L. Chang Sidorchuk**, **H. González Alvarez**, Plant Pathology Department of the National Center for Animal and Plant Health, Higher Education Ministry, Cuba; **N. Barboza Vargas**, Centro de Investigación en Biología Celular y Molecular, Escuela de Tecnología de Alimentos, Universidad de Costa Rica, Costa Rica; and **G. González Arias**, Virology Laboratory of Plant Protection Research Institute, Agriculture Ministry, Cuba.

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Disease symptoms similar to those caused by tospoviruses have been observed in some tomato production areas since November 2013. To identify possible tospoviruses involved, 147 samples were collected at the locations of Güira de Melena, Quivican, Batabanó, and Cotorro from open field production with an incidence of symptomatic plants ranging between 10 and 50%. The typical symptoms observed were: yellowing, bronzing, necrotic spot, wilting, deformed fruits with chlorotic spots, and some plants showed purple leaves. Multiple symptomatic samples were found positive for *Tomato spotted wilt virus* (TSWV) using Agdia immunostrips. However, these same samples were negative by RT-PCR when TSWV-specific primers (Mumford et al. 1996) were used, whereas positive amplification results were obtained when universal primers for tospovirus species detection (Dewey et al. 1997) were used. The RT-PCR fragments of the expected size of 450 bp were amplified with these universal primers. Four samples representing each of the four locations were cloned and sequenced. The four sequences (GenBank Accession Nos. KT218527, KT218528, KT218530, and KT218531) were identical and showed 98 to 99% nucleotide identity with *Tomato chlorotic spot virus* (TCSV) reported in Florida (Londoño et al. 2012) and the Dominican Republic (Batuman et al. 2014). To confirm the presence of TCSV in the four samples, RT-PCR fragments were generated for L, M, and S RNA segments using generic primers (Batuman et al. 2014). The expected fragments of 450, 849, and 871 bp were amplified and sequenced. The sequences of each fragment were identical to a consensus sequence, showing 97, 99, and 99% identity with TCSV-L, M, and S RNA,

respectively (KU19743, KU19744, and KU19745). The nucleocapsid protein gene was amplified with specific primers (Baysal-Gurel et al. 2015); the sequence generated (KU19746) from a 948-bp fragment revealed 99% identity with TCSV from the United States (KP172480). Biological information was obtained by inoculating tobacco (*Nicotiana tabacum* cv Criollo 98), pepper (*Capsicum annuum*), lettuce (*Lactuca sativa*), and tomato (*Solanum lycopersicum*). Necrotic local lesions on tobacco, severe leaf necrosis on pepper and lettuce, and chlorotic spots with central necrotic lesions on tomato were induced. TCSV was detected in all inoculated plants by dot blot hybridization test with TCSV N gene fragment as probe. These results are also consistent with the wide distribution of *Frankliniella schultzei* Tryborn in vegetables at the same localities where symptomatic plants were collected. Although symptoms associated with tospoviruses have been observed in the country, this is the first report of TCSV in Cuba; studies are currently being conducted to confirm its presence in other crops of economic interest. Presence of TCSV has important implications for the complex phytosanitary situation of the tomato crop, as TCSV could be coinfecting this crop together with TYLCV-IL(CU) with a significant impact on production, suggesting the need for developing an appropriate management strategy.

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