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DISEASE NOTES

First Report of Beet Leafhopper Transmitted Virescence Agent Phytoplasma in *Capsicum annum* and *Circulifer tenellus* in Mexico

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ABSTRACT

Chili pepper (*Capsicum annuum* L.) plants in Durango and Zacatecas, Mexico, in September and October 2014, had small, chlorotic, curled leaves, plant stunting, and/or big bud symptoms characteristic of phytoplasma infection (Lee et al. 2004). Symptomatic pepper plants were found in fields ranging from 6 to 10 ha at an incidence of 5 to 15% near Poanas, Durango (33 sampled); Sombrerete, Zacatecas (seven sampled); Villa de Cos, Zacatecas (33 sampled); Laguna Seca, Zacatecas (40 sampled); and Calera, Zacatecas (41 sampled). Total DNA was extracted from foliar tissue with the CTAB extraction method (Munyaneza et al. 2010), and tested using universal phytoplasma nested PCR with primers P1/P7 and FU5/RU3 (Crosslin et al. 2006). Of 156 samples, 104 (67%) were positive for phytoplasma, including three from Poanas, seven from Sombrerete, 27 from Villa de Cos, 31 from Laguna Seca, and 36 from Calera. All positive samples were tested for beet leafhopper transmitted virescence agent (BLTVA) phytoplasma of the clover proliferation group 16SrVI, subgroup A, using nested PCR with primers P1/P7 and BLTVA-specific FU5/BLTVA-int (Crosslin et al. 2006); all were positive for BLTVA. DNA was extracted from BLTVA beet leafhopper vectors, *Circulifer tenellus* (Baker), collected from pepper fields and weeds near pepper fields in Zacatecas, Mexico, in September and October 2014, using the CTAB method (Crosslin et al. 2006) and tested for BLTVA using nested PCR with primers P1/P7 and FU5/BLTVA-int. Of 52 insect samples, 23 (44%) were positive for BLTVA. Sequence analysis of the FU5-BLTVA-int amplicon from six pepper plants (two each) from Poanas, Durango; Sombrerete, Zacatecas; and Laguna Seca, Zacatecas; and three *C. tenellus* samples from Zacatecas, produced a consensus sequence from each host (base pairs 224 to 1,415, GenBank accession KY047614 and KY047615, respectively). BLAST analysis of this region of 16S rRNA gene was 100% identical to Columbia Basin purple top phytoplasma (known as BLTVA) (Crosslin et al. 2006), from the 16SrVI Clover proliferation group (KR072666.1). To verify phytoplasma infection, six plants and three insect samples were subjected to PCR using universal phytoplasma nested primers P1/P7 and R16F2n/R16R2 (Lee et al. 2004), and the amplicons were sequenced. A consensus sequence resulted from both the plant and insect samples (base pairs 1 to 1,250, KY047614 and KY047615, respectively). In silico restriction fragment length polymorphism analysis was done using *iPhyClassifier* analysis tool (Zhao et al. 2009). Both sequences were identified as 16Sr group VI, subgroup A, showing 99.7% similarity with '*Candidatus* Phytoplasma trifolii' (AY390261). '*Ca. Phytoplasma trifolii*' was reported in peppers in Mexico (Mauricio-Castillo et al. 2015), but the specific strain of 16SrVI-A group was not determined. Therefore, this is the first report of the clover proliferation group strain BLTVA phytoplasma in peppers and its insect vector in this region of Mexico. BLTVA is an economically important pathogen in solanaceous crops in the U.S. (Lee et al. 2004), and poses a threat to the pepper-growing region in Mexico, highlighting the need for control of the beet leafhopper vector of BLTVA phytoplasma.

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