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DISEASE NOTES

First Report of the Leafhoppers *Ceratagallia nitidula* and *Empoasca abrupta* (Hemiptera: Cicadellidae) as Vectors of 'Candidatus *Phytoplasma trifolii*'

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Citation

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In May 2016, leafhoppers (Cicadellidae) of two species, *Ceratagallia nitidula* Oman and *Empoasca abrupta* DeLong, were collected in the municipality of Calera de V.R., Zacatecas (22°57'55.03" N, 102°41'04.79" W) and identified using diagnostic characters of the male genitalia (Hamilton 1998). To test for their ability to transmit phytoplasmas, the two leafhopper species were allowed to feed on 'Candidatus *Phytoplasma trifolii*'-infected chili pepper plants for 1 week. Inoculations were done in duplicate, allowing groups of 25 leafhoppers of each species to feed on each of 10 healthy chili pepper plants exposed separately for 48 h; five healthy plants were used as negative controls (no insect infestation). After this period, the leafhopper populations were recovered from every cage and tested for 'Ca. *P. trifolii*' presence. Infested chili pepper plants and negative controls were maintained separately at 22°C, and symptoms were evaluated 40 days after leafhopper infestation. No symptoms were observed in negative controls, whereas six out of 10 and seven out of 10 plants inoculated by *C. nitidula* and *E. abrupta*, respectively, showed symptoms including foliar deformation, long internodes, fall of flowers, and yellowing. Symptomatic and asymptomatic plants as well as pools of 20 insects of each species were tested for phytoplasma infection by direct (Smart et al. 1996) and nested (Gundersen and Lee 1996) polymerase chain reaction (PCR) assays. The nested PCR amplicons (1.2 kb)

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obtained from all the positive extracts were cloned separately and directly sequenced. No PCR products were obtained from negative controls and symptomless plants. BLAST analyses of the amplified sequences confirmed the presence of 'Ca. *P. trifolii*' strains in *C. nitidula*, *E. abrupta*, and symptomatic chili pepper. Computer-simulated restriction fragment length polymorphism analysis was performed using iPhyClassifier (<https://plantpathology.ba.ars.usda.gov/cgi-bin/resource/iphyclassifier.cgi>), and the patterns obtained from the aligned 16S rDNA sequence (MG958642) isolated from *C. nitidula*, *E. abrupta*, and chili pepper were most closely related (98.7%) to the reference strain (AY390261) of the group 16SrVI ('Ca. *P. trifolii*'), subgroup A (Hiruki and Wang 2004). Although there are reports about the presence of 'Ca. *P. trifolii*' affecting crops of economic interest in Mexico (Salas-Muñoz et al. 2016), the information on their vectors is limited. To our knowledge, this is the first report of these two leafhopper species as vectors of 'Ca. *P. trifolii*'. Only one other species of *Empoasca*, *E. papayae* Oman from the Caribbean region, has been reported as a phytoplasma vector (Sein and Adsuar 1947). *Austroagallia torrida* (Evans), which belongs to the same leafhopper tribe (Agalliini) as *Ceratagallia*, was reported as a vector of the phytoplasma associated with rugose leaf curl disease of clovers in Australia (Grylls et al. 1974), but no other species of this tribe have been identified as phytoplasma vectors. The identification of these two new vectors of phytoplasmas can help to establish strategies to avoid the spread of diseases caused by 'Ca. *P. trifolii*' in Mexico and other countries.

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