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DISEASE NOTES

First Report of 'Candidatus Phytoplasma trifolii'–Related Strain Associated with a New Disease on Garlic in Zacatecas, Mexico

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The state of Zacatecas is considered the largest producer of garlic (*Allium sativum* L.) in Mexico. In February 2016, symptoms of plant stunting, leaf yellowing, leaf malformation, and bright and “waxy” appearance of the leaves were observed in 40% of garlic plants in a commercial field in the municipality of Fresnillo in Zacatecas, Mexico (N: 23° 22.676, W: 102° 59.535 2017 masl). Total DNA was extracted from 15 symptomatic and five asymptomatic garlic plants (collected in the same commercial field) by a modified version of the Dellaporta method (Dellaporta et al. 1983). Direct and nested polymerase chain reaction (PCR) assays targeting the 16S rDNA gene were used to confirm the association of phytoplasma with the disease. The oligonucleotide primers used were P1 (5'-AAGAGTTTGATCCTGGCTCAGGATT-3') and Tint (5'-TCAGGCGTGTGCTCTAACCAGC-3') for direct PCR (Smart et al. 1996) and R16F2n (5'-GAAACGACTGCTAAGACTGG-3') and R16R2 (5'-TGACGGGCGGTGTGTACAAACCCCG-3') for nested PCR (Gundersen and Lee 1996). No PCR products were obtained from the five asymptomatic plants. The nested PCR amplicon (1.2 kb) amplified from each one of the 15 symptomatic plants was cloned separately into pGEM-T Easy Vector (Promega, Madison, WI) and directly sequenced. BLAST analysis of the 16S rDNA sequences revealed that they shared 100% sequence identity to each other and 99.0% sequence identity to 'Candidatus Phytoplasma trifolii' (Cpt) isolate Tomato-Zac (KX092011). Computer-simulated restriction fragment length polymorphism (RFLP) analysis of the 16S rDNA sequence from the Zacatecas garlic phytoplasma sequence

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(GenBank accession no. MH259307) using iPhyClassifier (<https://plantpathology.ba.ars.usda.gov/cgi-bin/resource/iphyclassifier.cgi>) and RFLP profiles was compared with each 16S phytoplasma group and subgroup. The garlic phytoplasma 16S rDNA sequence shared 98.8% identity to the reference strain, Cpt subgroup A (accession no. AY390261). To our knowledge, this is the first report of Cpt associated with a new disease of garlic in Mexico. Also, adults of the beet leafhopper (*Circulifer tenellus* Baker) were collected from the sampled garlic field, and total DNA was extracted from a pool of 20 beet leafhoppers. Amplification of phytoplasma DNA using the same primers as mentioned previously was carried out, and further sequencing of the PCR products confirmed the presence of Cpt DNA with a nucleotide identity of 100% to the phytoplasma sequence detected in symptomatic garlic plants. Cpt has been associated to the big bud disease of pepper (Mauricio-Castillo et al. 2015) and dwarfing and yellowing of tomato plants in Zacatecas (Salas-Muñoz et al. 2016). Infection of members of different botanical families, Solanaceae (pepper and tomato) and Alliaceae (garlic), by Cpt in this area of Mexico may reflect the polyphagous habit of *C. tenellus*, and consequently a wider host range may be expected.

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Section:

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