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# plant disease

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## Disease Notes

### First Report of '*Candidatus* Phytoplasma solani' and '*Ca. P. convolvuli*' Associated with Grapevine Bois Noir and Bindweed Yellows, Respectively, in Georgia

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A survey carried out in Georgian vineyards, located in the Khaketi region, in September 2013, showed the presence of vines of the cultivar Chardonnay with typical grapevine yellows (GY) symptoms including leaf discoloration and curling, berry shriveling, and irregular maturation of wood. In the same vineyards, bindweed (*Convolvulus arvensis* L.) plants showing shoot proliferation and leaf yellowing were found, suggesting the involvement of phytoplasmas in the disease etiology. Total DNA was extracted by a CTAB method from leaf veins of 18 symptomatic and two asymptomatic grapevines, and from four symptomatic and two asymptomatic bindweeds, and analyzed by PCR assays. Moreover, DNA extracted from '*Candidatus* Phytoplasma asteris' strain SAY (group 16SrI), '*Ca. P. solani*' strain STOL (group 16SrXII), and '*Ca. P. ulmi*' strain EY1 (group 16SrV) were used as positive controls. DNA extracted from healthy periwinkle and a reaction mixture without template were employed as negative controls. Nested PCRs targeting the 16S rDNA, carried out using the primer pairs P1/P7 followed by R16F2n/R16R2 (1), produced a band of the expected size (1,250 nt) in all the symptomatic grapevine and bindweed plants, and in the positive controls. No amplification was observed with DNA from asymptomatic plants nor the negative controls. PCR products were sequenced by a commercial sequencing service (Primm, Milan, Italy). The 16S rDNA nucleotide sequences of phytoplasmas identified in all grapevines and in two bindweed samples shared >99.5% sequence identity with '*Ca. P. solani*' reference strain STOL (GenBank Accession No. AF248959), and carried identical STOL-unique signature sequence and distinguishing sequence blocks (3). Moreover, nucleotide sequences of phytoplasmas identified in the other two bindweed samples shared >99.6% sequence identity with '*Ca. P. convolvuli*' reference strain BY-S57/11 (JN833705) (2).

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RFLP and phylogenetic analyses confirmed the affiliation of the phytoplasma strains identified in grapevine and bindweed plants in Georgia to the species '*Ca. P. solani*' (subgroup 16SrXII-A) and '*Ca. P. convolvuli*' (subgroup 16SrXII-H). Representative 16S rDNA nucleotide sequences were deposited in NCBI GenBank website with accession nos. KF996535 and KF996536 ('*Ca. P. solani*' from grapevine and bindweed, respectively), and KF996537 ('*Ca. P. convolvuli*'). Future studies will focus on investigating the spread and impact of '*Ca. P. solani*'-associated bois noir (BN) in Georgia. In particular, the identification of '*Ca. P. solani*' in bindweeds suggested the presence of the insect *Hyalesthes obsoletus*, a polyphagous cixiidae responsible for BN phytoplasma transmission in vineyards in Europe. Accurate surveys and molecular analyses will be performed for identifying the insect vector(s) of the BN associated phytoplasma strains in Georgia. Additional studies will be performed to study the spread and impact of '*Ca. P. convolvuli*,' identified only in Italy, Germany, Serbia, and Bosnia and Herzegovina (2), throughout the Caucasian countries.

*References:* (1) I.-M. Lee et al. *Int. J. Syst. Bacteriol.* 48:1153, 1998. (2) M. Martini et al. *Int. J. Syst. Evol. Microbiol.* 62:2910, 2013. (3) F. Quaglino et al. *Int. J. Syst. Evol. Microbiol.* 63:2879, 2013.