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Flavescence dorée phytoplasma affecting grapevine (*Vitis vinifera*) newly reported in Portugal

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In 2007, during an official Flavescence dorée (FD) survey, typical FD symptoms, associated with desiccation of inflorescences, withering of clusters, irregular maturation of the wood, leaf rolling, typical vein banding, leaf yellowing on white grapevine variety Loureiro and leaf reddening on red variety Vinhão, were observed in vineyards from Amares region in northern Portugal. In 2002, similar but less severe symptoms occurring in the same region were associated with 16SrI phytoplasmas ('*Candidatus* Phytoplasma asteris'), subgroup 16SrI-B (De Sousa *et al.*, 2003). Cuttings and leaf samples were collected from plants with and without symptoms. Total DNA was extracted (Angelini *et al.*, 2001) and assayed in a nested polymerase chain reaction (PCR) using primers P1/P7 (Deng & Hiruki, 1991) followed by either R16F2n/R16R2 (F2n/R2) (Gundersen & Lee, 1996) or R16F1(V)/R16V₁₇₃₁₇ (F1/V1) (Martini *et al.*, 1999). Phytoplasma strains from group 16SrV ('*Ca.* Phytoplasma ulmi'), elm yellows (EY1) and rubus stunt (RUS), were used as reference controls. Healthy *C. roseus* and no template DNA were used as negative controls. All plants with symptoms and reference controls showed PCR products (F2n/R2 and F1/V1), absent from samples from symptomless plants. Two representative F2n/R2 amplicons, were cloned, sequenced and deposited in GenBank (FJ611961 and FJ611962). The phytoplasma 16S rRNA sequences were identical and shared 99–100% identity with those of group 16SrV. FD phytoplasmas from Portugal exhibited the characteristic FD-D TaqI RFLP pattern.

This is the first report of FD-D in grapevine in northern Portugal. Although the current FD outbreak is limited to Amares region, the high pressure of the *Scaphoideus titanus* vector populations imposes a threat for FD spread, and a current alert for the Ministry of Agriculture

(DGADR). The implementation of strategies for effective control of *S. titanus* and FD have been suggested and included in the Portuguese legislation (Portaria n° 976/2008, 1 September).

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