

Diseases Caused by Bacteria and Phytoplasmas

First Report of Flavescence Dorée-Related Phytoplasma in a Productive Vineyard in Germany

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Flavescence dorée (FD) and Bois noir (BN) are the principal grapevine yellows in Europe caused by distinct phytoplasmas: BN by *Candidatus* Phytoplasma solani, and FD by 16SrV-C and -D phytoplasmas (FDp) transmitted by the introduced Nearctic Deltocephalinae *Scaphoideus titanus*. FDp is listed as a quarantine pest in the European Union (Regulation (EU) 2019/2072). Black Alder (*Alnus glutinosa*) is a common asymptomatic host of 16SrV phytoplasmas in Europe and considered the original host of FDp (Malembic-Maher et al. 2020). Palatinate grapevine yellows (PGY) transmitted from alder to grapevine by the Macropsinae *Oncopsis alni* is not transmissible by *S. titanus* (Malembic-Maher et al. 2020). Germany is considered free from FD in grapevine and from its vector. A single case in a nursery in 2014 was eradicated (EPPO 2017), and FD was never before detected in a vineyard. Since *S. titanus* appeared in 2016 in the neighboring French region of Alsace, monitoring of FD was carried out in Germany following a risk based strategy. It was focused on vineyard plots within a distance of 100 m from stands of alder. A geodata-based risk map (Jalke 2020) was used to localize those plots. All symptomatic vines sampled until September 2020 proved to be infected by BN or, occasionally, by PGY. Eight vines with typical symptoms were sampled in vineyards adjacent to alder stands in the winegrowing region of Rheinhessen in September 2020. Symptoms consisted of leaf rolling and discoloration, incomplete lignification, and black pustules arranged in lines along the shoots. Diseased shoots were black and necrotic in December. Leaf midribs were sampled for total nucleic acid extraction. The phytoplasma 16S rRNA gene was amplified by generic primers

R16F2/R2-mod followed by a nested PCR using 16Sr(V) group-specific primers R16(V)F1/R1 and primers R16(I)F1/R1 (Lee et al. 1995) to detect '*Candidatus* Phytoplasma solani' associated with BN. While BN was detected in seven vines, one sample tested positive for 16SrV phytoplasma. This result was confirmed by triplex real-time Taq-Man assay based on rpl14 gene sequences (IPADLAB), by multiplex real-time PCR of map locus, as well as by loop-mediated isothermal amplification (LAMP) according to the EPPO diagnostic standard PM 7/079(2) (EPPO 2016). PCR products of the *map* and *vmpA* genes (Malembic-Maher et al. 2020) were sequenced and compared with reference sequences to distinguish between FD- and non-FD genotypes. The isolate from the diseased vine (GenBank MW 727272) exhibited 100% identity with map-M38 (GenBank LT221933), a genotype of the map-FD2 cluster. The same genotype was detected in *A. glutinosa* and *Allygus* spp. sampled at the infested site. A 234-bp sequence of the first repeat of the *vmpA* gene (GenBank MW727273) showed 100% identity with the homologous part of isolate FD-92 (GenBank LN680870) of the *vmpA*-II cluster. It can be concluded that the symptomatic grapevine was infected by FD and not PGY. This is the first report of FD in a productive vineyard in Germany. The infected vine of cv. Silvaner was 25 years old. While infected planting material is an unlikely source of the infection, a transmission of FDp from alder is highly probable. Finding a single FD infection after several years of testing implies a low risk originating from the wild compartment, but the approach and possible establishment of *S. titanus* expected to be able to colonize the area (EFSA Panel on Plant Health [PLH] 2016) justifies further monitoring activities. The infected vine was eradicated.

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