## Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Shoot Blight of Grapevine Caused by *Sclerotinia sclerotiorum* in Illes Balears, Mallorca, Spain

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In 2021, grapevines (Vitis vinifera L.) cv. Callet in a commercial vineyard in Pollença (northeast of the island of Majorca, Spain) had severe symptoms of shoot blight during spring and early summer, with an incidence of 70%. Symptoms were elongated canker-like lesions surrounded by water-soaked darker tissues at the base or around the middle nodes of the green shoot. Fungi were isolated by collecting shoot samples with lesions, surface disinfesting in 2% NaClO for 90 s, rinsing twice with deionized water, placing in PDA plates, and incubating at 25°C under 12 h light-dark for 6 days. Isolations consistently yielded one colony type with white mycelium and black spherical to elongated sclerotia of 6.4 (2 to 10) mm (n = 22). Morphology was consistent with Sclerotinia sclerotiorum (Lib.) de Bary (Bolton et al. 2006). Three isolates (UIB 118-1, UIB 118-26, and UIB 129-41) were preserved and deposited in the Culture Collection of Microbiology-Faculty of Sciences, University of Balearic Islands, Spain. Genomic DNA was extracted from UIB 118-26 and UIB 129-41 using the EZNA Miniprep Kit (Omega Bio-Tek, Norcross, GA). The internal transcribed spacer (ITS) region of ribosomal DNA, β-tubulin (BTUB), and calmodulin (CAL) gene regions were amplified using ITS1F-ITS4 (Gardes and Bruns 1993; White et al. 1990), Bt-2a/Bt-2b (Glass and Donaldson 1995), and CAL228F/

CAL737R (Carbone and Kohn 1999) primer sets, respectively. Amplicons were sequenced and deposited in GenBank (ITS: MZ604647, MZ604648 [524 bp]; BTUB: OK634402, OK634403 [456 bp]; CAL: OK634404, OK634405 [489 bp]). BLASTn search against the genome of the wellcharacterized S. sclerotiorum strain 1980 UF-70 (Amselem et al. 2011) revealed that all six amplicons from both UIB strains had 99.8% identity with homologous sequences of strain 1980. Pathogenicity was tested on 81year-old grapevines cv. Cabernet Sauvignon. Old and new green shoots were inoculated by inserting a 6-mm plug of mycelium taken from actively growing cultures on PDA into cuts at the base and at the distal part of each shoot with a sterile scalpel, with eight inoculation points per plant. Wounds were sealed with Parafilm to avoid dehydration. Inoculated plants and an equal number of wounded but noninoculated negative control plants were kept at 25  $\pm$  1°C for 48 h in plastic containers to ensure >90% relative humidity. The experiment was repeated once with similar results. After 5 days, the resulting infection girdled and rotted green new shoots, and older, partially lignified shoots had localized long brown lesions up to 16-cm long. Due to rotting of the basal part of the petiole, leaves turned gray, wilted, and died, easily detaching from the stem. In advanced stages of the disease, 7 days after infection, branches died and fell with the leaves still attached. The fungus was reisolated from diseased shoots on PDA to fulfill Koch's postulates. S. sclerotiorum was reported on grapevine causing shoot blight in Chile, California-U.S.A., Australia, and Korea (Park et al. 2009) and among the endophytic mycobiota associated with Vitis vinifera in the Iberian Peninsula (Gonzalez and Tello 2011), but not as a pathogen causing visible symptoms on the crop. This is the first report of the fungus as a pathogen of grapevines in Spain causing symptoms of canker and shoot blight. This shows the risk of this disease to the wine industry in the Mediterranean, especially Spain, the country with the most area of grapevines. Although chemical and biological controls have been developed for the disease, management in a perennial system like grapevines is difficult as sclerotia of Sclerotinia remain viable in the soil for up to 8 years with limited ability to apply cultural practices to manage the initial inoculum. Epidemiological studies are needed in Spain to understand the impact of S. sclerotiorum on grapevines and to build tools to anticipate outbreaks.

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## e-Xtra

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