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

A. Perelló,[1+] D. Olmo,[2] A. Busquets,[3,4] A. Romero-Munar,[3] B. M. Quetglas[3,5], P. A. Gost,[3,5] M. Berbegal,[6] J. Armengol,[6] C. Cabot,[3] M. Gomila,[3,4] R. Bosch,[3,4] J. Vadell,[3] J. Cifre,[5] and C. Poschenrieder[7]

[1]UCA-FICA, Pontificia Universidad Católica Argentina, Facultad de Ingeniería y Ciencias Agrarias Av. Alicia Moreau de Justo 1300, CABA, Buenos Aires, Argentina.[2]Serveis de Millora Agrària i Pesquera, Govern Balear, 07009 Palma, Spain. [3]Department of Biology, [4]IMEDEA (CSIC-UIB), [5]Department of Engineering, Universitat de les Illes Balears, 07122 Palma, Spain. [6]Instituto Agroforestal Mediterráneo, Universitat Politècnica de València, Camí de Vera S/N, 46022 Valencia, Spain. [7]Department of Plant Physiology, Universitat Autònoma de Barcelona, 08183 Cerdanyola del Vallès, Barcelona, Spain

+email: analiaperello@uca.edu.ar

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In 2021, grapevines (*Vitis vinifera* L.) cv. Callet growing in a commercial vineyard located at Pollença (northeast of the island of Majorca, Spain) showed severe symptoms of shoot blight during spring and early summer, with an incidence of 70%. Symptoms consisted of elongated cankered-like lesions, surrounded by water-soaked darker tissues, that developed at the base or around the middle nodes of the green shoot. Fungal isolation was performed by collecting shoot samples with lesions, surface disinfesting with 2% NaClO for 90s, rinsed twice with deionized water and placed in Petri plates containing potato dextrose agar (PDA). The plates were incubated at 25°C under 12 h light-darkness for 6 days. Isolations consistently yielded one type of fungal colony that produced white mycelium and black spherical to elongated sclerotia of 6.4 (2 to 10) mm (n=22). Morphological characterization was consistent with the description of *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 isolates 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, 1996; 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 with accession numbers MZ604647 and MZ604648 for ITS (524 bp), OK634402 and OK634403 for BTUB (456 bp) and OK634404 and OK634405 for CAL (489 bp). BLASTn search against the genome of the

well characterized *Sclerotinia sclerotiorum* strain 1980 UF-70 (Amselem et al. 2011) revealed that all six amplicons from both UIB strains showed 99.8% identity with their homologous sequences of strain 1980. Pathogenicity tests were conducted using eight one-year 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 made at the base and at the distal part of each shoot with a sterile scalpel with a total of eight inoculation points per plant. Inoculated wounds were sealed with Parafilm tape to avoid rapid dehydration. Inoculated plants and an equal number of wounded but non-inoculated plants (negative controls) were maintained at 25 ± 1°C for 48 h in plastic containers to ensure a high relative humidity (>90%). The experiment was repeated once with similar results. After 5 days, the resulting infection girdled and rotted the green new shoots, whereas the older partially lignified shoots developed a localized long brown lesion that reached 16 cm in length. Due to the 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 remaining attached (Fig 1 A, B). Reisolations from diseased shoots were successfully performed on PDA to fulfill Koch's postulates. *S. slerotiorum* was previously reported on grapevine causing shoot blight in Chile (Latorre and Guerrero, 2001), Korea (Jong-Han et al. 2009), California-USA (Boland and Hall, 1994) and Australia (Hall et al. 2002). Also *S. sclerotiorum* was reported 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 that crop. So, this is the first report of the occurrence of *S. sclerotiorum* as a pathogen of grapevines in Spain causing symptoms of canker and shoot blight. This finding highlights a potential risk of this fungal disease for the wine industry in the Mediterranean region and specially for Spain, the country with the largest acreage devoted to grapevines. Although chemical and biological controls have been developed for this disease management in a perennial system like grapevine production is difficult as sclerotia of *Sclerotinia* can remain viable in the soil for up to eight years (Adams and Ayears, 1979), with limited ability to apply cultural practices to manage the initial inoculum. Epidemiological studies are needed in Spain to understand the impact and important of *S. sclerotiorum* on grapevines and to build tools to better anticipate potential outbreaks of this new pathogen on grapevine in Spain.

Figure 1. Symptoms of *S. sclerotiorum* on grapevine cv Cabernet Sauvignon after 7 days of inoculation. A) rot and blight of shoots, wilting of petioles and break down of shoot. B) detail of necrotic lesion on young shoot.

References:

Adams, P. B.; Ayers, W. A. Ecology of Sclerotinia species. 1979. Phytopathology, St. Paul, 69: 896-899.

Amselem J, Cuomo, C.A., van Kan J.A.L., Viaud ,M., Benito, E.P., et al. 2011. Genomic Analysis of the Necrotrophic Fungal Pathogens *Sclerotinia sclerotiorum* and *Botrytis cinerea*. PLOS Genetics 7(8): e1002230.

Boland, G. J. and Hall, R. 1994. Index of host plants of *Sclerotinia sclerotiorum*. Canadian Journal of Plant Pathology 16, 93–108.

Bolton, M. D., et al. 2006. *Sclerotinia sclerotiorum* (Lib.) de Bary: Biology and molecular traits of a cosmopolitan pathogen. Mol. Pl. Path. 7: 1–16.

Carbone, I., and Kohn, L. M. 1999. A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia, 91(3), 553–556. https://doi.org/10.2307/3761358

Gardes, M., and Bruns, T. 1993. ITS primers with enhanced specificity for Basidiomycetes - application to the identification of mycorrhizae and rusts. Mol. Ecol. 2: 113-118.

Glass, NL and Donaldson GC. 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Applied and Environmental Microbiology 61 (4).

Gonzalez, V. and Tello, M.L. 2011. The endophytic mycota associated with *Vitis vinifera* in central Spain, Fungal Diversity 47:29–42.

Hall, B. H., et al. 2002. First report of *Sclerotinia sclerotiorum* on grape (*Vitis vinifera*) in South Australia. Austr. Pl. Path. 31: 417–418.

Jong-Han, P., et al. 2009. *Sclerotinia* Shoot Rot of Grapevine (Vitis spp.) Caused by *Sclerotinia sclerotiorum* in Korea. Res. Pl. Dis. 15: 259 – 261.

Latorre, B. A. and Guerrero M. J. 2001. First Report of Shoot Blight of Grapevine Caused by *Sclerotinia sclerotiorum* in Chile. Pl. Dis. 85: 1122 DOI 10.1094/PDIS.2001.85.10.1122C.

White, T. J., et al. 1990. Page 315 in: PCR Protocols: a Guide to Methods and Applications. Academic Press, San Diego, CA.

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60x80mm (220 x 220 DPI)

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