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First Report of *Diaporthe amygdali* Associated with Twig Canker and Shoot Blight of Nectarine in Spain

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Nectarine (*Prunus persica* (L.) Batsch var. *nucipersica* (Suckow) C. K. Schneid.) is a fruit crop widely cultivated throughout the Mediterranean basin. In Spain, it is mainly grown in eastern regions of the country. In March 2018, 5-year-old nectarine trees showing twig canker symptoms were observed after a rainy spring period in a 0.5 ha orchard located at Alaior, Menorca island (Spain). Cankers were frequent on affected trees (approximately, 80% of the total trees), thus leading to shoot blight. Ten twig segments of one-year old wood with cankers were cut, washed under running tap water, surface disinfected for 1 min in a 1.5% sodium hypochlorite solution and rinsed twice in sterile distilled water. Small pieces (2 mm) of affected tissues were taken from the margin of the cankers and plated on potato dextrose agar (PDA) supplemented with 0.5 g/L of streptomycin sulphate (PDAS). The plates were then incubated at 25 ºC in the dark for 7 to 10 d. Actively growing colonies were first hyphal-tipped and then transferred to PDA and 2% water agar supplemented with sterile pine needles and incubated at 21-22ºC under a 12h/12h near UV / darkness cycle during 21 d (León et al. 2020). Colonies were white at first, becoming light cream, with visible solitary and aggregate pycnidia at maturity. Alpha conidia were aseptate, fusiform, hyaline, multi-guttulated (mean ± SD = 7.4 ± 0.7

× 2.8 ± 0.4 µm, n = 100). Beta and gamma conidia were not observed. The morphological and cultural characteristics of the isolates were congruent with those of *Diaporthe* spp. (Gomes et al. 2013). The ITS1-5.8S-ITS2 (ITS) region and fragments of β-tubulin (*tub2*), the translation elongation factor 1-alpha (*tef1-α*) gene regions, histone H3 (*his3*) and calmodulin (*cal*) genes of representative isolate DAL-59 were amplified and sequenced (Santos et al. 2017). The BLASTn analysis revealed 100% similarity with sequences of *D. mediterranea* (Synonym *D. amygdali*) (Hilário et al. 2021) isolate DAL-34 from almond (ITS: MT007489, *tub2*: MT006686, tef1-α: MT006989, his3: MT007095 and *cal*: MT006761). Sequences of isolate DAL-59 were deposited in GenBank Database (ITS: MT007491, *tub2*: MT006688, *tef1-α*: MT006991, *his3*: MT007097 and *cal*: MT006763). Pathogenicity tests were conducted using one-year-old potted plants of nectarine cv. Boreal, which were inoculated with isolate DAL-59. In each plant, a 3 mm wound was made in the center of the main branch (about 30 cm length) with a scalpel. Colonized agar plugs with 3 mm diameter, which were obtained from active 10-day-old colonies growing on PDA, were inserted underneath the epidermis and the wounds sealed with Parafilm. Inoculated plants were incubated in a growth chamber at 23 ºC with 12 h of light per day. Controls were inoculated with uncolonized PDA plugs. There were twelve plants per treatment, which were arranged in a completely randomized design. Five days after inoculation necrosis development was observed in the area of inoculation. Wilting and twig blight symptoms over the lesion occurred 3-wk after inoculation and pycnidia were detected, while the controls remained asymptomatic. *Diaporthe amygdali* was re-isolated from symptomatic tissues and identified as described above to satisfy Koch's postulates. To our knowledge, this is the first report of *D. amygdali* causing twig canker and shoot blight disease on nectarine in Spain.

Keywords: fungi, branch, pathogen detection

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