Diseases Caused by Fungi and Fungus-Like Organisms

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 1-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/liter of streptomycin sulfate. The plates were then incubated at 25°C in the dark for 7 to 10 days. 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 to 22°C under a 12-h/12-h near-UV/darkness cycle for 21 days (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, and multiguttulate (mean $\pm$ SD = $7.4 \pm 0.7 \times 2.8 \pm 0.4$ μ m, $n = 100$). 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- α (*tefl- α*) gene regions, histone H3 (*his3*), and calmodulin (*cal*) genes of representative isolate DAL-59 were amplified and sequenced (Santos et al. 2017). BLASTn analysis revealed 100% similarity with sequences of *Diaporthe mediterranea* (synonym *D. amygdali*) (Hilário et al. 2021) isolate DAL-34 from almond (ITS, MT007489; *tub2*, MT006686; *tefl- α* , MT006989; *his3*, MT007095; and *cal*, MT006761). Sequences of isolate DAL-59 were deposited in GenBank database (ITS, MT007491; *tub2*, MT006688; *tefl- α* , MT006991; *his3*, MT007097; and *cal*, MT006763). Pathogenicity tests were conducted using 1-year-old potted plants of nectarine cultivar 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°C with 12 h of light per day. Controls were inoculated with uncolonized PDA plugs. There were 12 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 weeks after inoculation, and pycnidia were detected, whereas the controls remained asymptomatic. *D. amygdali* was reisolated 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.

References:

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