Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Diaporthe foeniculina* Associated with Grapevine Trunk Diseases on *Vitis vinifera* in Cyprus

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In June 2017, three vineyards were surveyed in the regions of Droushia (30-year-old cv. Mavro), Ineia (50-year-old cv. Xynisteri), and Lemona (15-year-old cv. Carignan) in the province of Paphos, Cyprus, with dieback incidence of 22, 32, and 14%, respectively. More specifically, affected grapevines exhibited severe dieback symptoms in spur and cordon positions, related to perennial cankers and internal brown discoloration. Thirty symptomatic samples were surface sterilized (95% ethanol) and wood chips were plated on potato dextrose agar (PDA), amended with streptomycin (500 µg/ml) at 25°C for 3 to 5 days. Based on colony morphology (white to creamy color, with sparse aerial mycelium) and conidia production, nine *Diaporthe*-like isolates were obtained. For species identification, the internal transcribed spacer (ITS) region and β-tubulin (BT) genes were amplified using the primer pairs ITS1/ITS4 and Bt2a/Bt2b, respectively (Urbez-Torres et al. 2008). Sequences of the isolates P101b, P114c, and P289a revealed >99.8% homology to NCBI voucher specimens of *Diaporthe foeniculina* (Sacc.) Udayanga & Castl. (Udayanga et al. 2014) (ITS: CBS111553 and MH050434 and BT: KY511368 and KF778966), and were deposited in the GenBank (ITS: MT735646, MT737289, MT737287 and BT: MT903969, MT903970, MT903971). Thus, 8.3% of the collected isolates (3 of 36) were identified as *D. foeniculina*, while the rest of the *Diaporthe*-like isolates were identified as *D. ampelina*. *D. foeniculina* isolates were also transferred on 2% water agar with sterile pine needles under a near-ultraviolet regime of 12 h of light and 12 h of darkness at 25°C to induce sporulation (Guarnaccia and Crous 2017). Two weeks later, microscopic observations

revealed dark-brown to black, globose to subglobose, ostiolate pycnidia ($n = 30$) of 291 to 897 (595 ± 173) by 192 to 655 (364 ± 113) µm containing hyaline, unbranched conidiophores, bearing α- and β-conidia in the form of yellowish cirri. α-conidia were aseptate, hyaline, ovate to ellipsoidal, ranging ($n = 100$) from 5.6 to 9.9 (7.5 ± 0.8) by 1.9 to 3.3 (2.7 ± 0.3) µm in size. β-conidia were abundant, aseptate, hyaline, filiform, slightly curved ($n = 100$), and from 22.4 to 35.3 (28.1 ± 2.5) by 1.2 to 2.3 (1.6 ± 0.2) µm in size (Udayanga et al. 2014). Pathogenicity tests were conducted with isolates P101b and P289a under greenhouse conditions (24 to 32°C, 70% RH). Ten 1-year-old rooted canes of cv. Mavro were inoculated with 4-mm mycelium plugs from actively growing cultures into wounds made by drilling between two internodes at the middle of the trunk. The same number of cuttings were inoculated with sterile PDA plugs, sealed with Vaseline, and wrapped with parafilm, serving as controls. Seven months later, all inoculated cuttings developed brownish wood discolorations (average 39 ± 13 mm), like those of naturally infected plants. No symptoms were observed in the controls. Successful reisolations were made only from the inoculated cuttings and confirmed by colony morphology. Previously, *D. foeniculina* (as *D. neotheicola*) has been reported as a grapevine wood saprophyte (Urbez-Torres et al. 2013). It has also been reported to cause shoot canker and dieback in numerous hosts, including almond, avocado, citrus, and sweet chestnut (Annesi et al. 2016; Diogo et al. 2010; Guarnaccia and Crous 2017; Mathioudakis et al. 2020). This is the first record of *D. foeniculina* associated with grapevine trunk diseases (GTDs) in Cyprus. However, its relative importance as the causal agent of GTDs remains to be further investigated.

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