Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Diaporthe foeniculina* Associated with Branch Canker of Avocado in Greece

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Avocado (*Persea americana* Mill.) is a rapidly expanding crop in the Mediterranean basin. In Greece it is mainly cultivated in Chania prefecture (Crete island), and data on fungal diseases are limited. In fall 2018, in an orchard in Alikianos district of Chania (35°27'05.03"N, 23°54'49.82"E), ~8% of 50 5- to 6-year-old avocado trees (cv. Hass) showed branch and twig cankers with brownish bark discoloration, resulting in twig dieback with brown leaves remaining attached to trees. Small symptomatic bark tissue pieces from diseased trees (10 from two branches of each of four trees) were surface disinfested in 0.5% NaOCl followed by 70% EtOH for 2 min each step, rinsed twice with sterile distilled water, dried, placed on PDA amended with 1 ml of lactic acid 10% per 100 ml of PDA (pH 4.5), and incubated in the dark at 25°C for 2 to 3 days. The 27 isolates recovered (68% isolation frequency) had identical colony morphology. Mycelial plugs from the actively growing colony margins were transferred onto fresh PDA plates to obtain pure cultures. Morphological identification was based on pure cultures. Colonies on PDA were white, slow-growing with sparse aerial mycelium, on which globose to subglobose dark brown to black pycnidia (420 to 670 µm diam.) with an elongated neck (up to 100 µm) were produced after 2 weeks. Conidiophores were hyaline, septate, and branched (18.0 to 24.0 × 1.9 to 3.0 µm). α-Conidia were aseptate, hyaline, ellipsoidal or fusiform, and 8.0 ± 0.8 × 2.5 ± 0.6 µm (*n* = 50). β-Conidia were hyaline, aseptate, filiform, slightly curved, 26.9 ± 1.8 × 1.3 ± 0.2 µm (*n* = 50), and presented in larger numbers than α-conidia. Morphological and cultural characteristics matched *Diaporthe* spp. (anamorph *Phomopsis* spp.). To confirm identification, DNA from mycelium of four isolates (Av-1, 2, 6, 8) was extracted using the NucleoSpin Plant II kit (Macherey-Nagel). The ITS1-5.8S-ITS2 region was amplified with primers ITS1/ITS4

(White et al. 1990). PCR products were sequenced, and BLASTn analysis revealed 99.8% similarity with sequences of *Diaporthe foeniculina* isolates (e.g., chestnut, LN651172; citrus, KC843294; soybean, JF430495; apple, MK370623). All sequences were identical, and a representative one (Av-1) was deposited in GenBank (MT374094). Partial amplification of β-tubulin (tub2) and translation elongation factor 1-α (EF1-α) was performed with Bt2a/Bt2b and EF1-728F/EF1-986R primers, respectively, using Av-1 (Carbone and Kohn 1999; Glass and Donaldson 1995). The amplicons were sequenced, deposited in GenBank (EF1-α, MT374092; tub2, MT374093), and showed 100% homology with sequences of *D. foeniculina* isolates (tub2, MF418578; EF1-α, LN651178). The isolate was identified as *D. foeniculina* (Sacc.) Udayanga & Castl. (Udayanga et al. 2015). Pathogenicity tests were conducted using 15 1.5-year-old avocado trees (cv. Hass) under greenhouse conditions. PDA plugs (4 mm diam.) with actively growing mycelium of a 7-day-old culture were transferred onto wounds made by a cork borer on previously sterilized trunk surfaces, covered with sterile paper, and sealed with Parafilm. Five trees inoculated with noncolonized PDA disks were controls. Five months after inoculation, lesions with brownish bark discoloration had developed on all inoculated trees; control plants remained symptomless. *D. foeniculina* was reisolated on PDA from all artificially inoculated plants. Identity was reconfirmed morphologically and by PCR amplification and sequencing of the ITS and EF-1α regions of two isolates from inoculated plants. Koch's postulates were thus fulfilled. On avocado, canker symptoms on shoots, branches, or trunks are caused by several Botryosphaeriaceae and Diaporthaceae species. In Italy, there is one report of *D. foeniculina* causing avocado canker (Guarnaccia et al. 2016). This is the first report of *D. foeniculina* causing branch canker disease on avocado in Crete, Greece. As the disease threatens avocado crops in the area, application of preventive measures to control spread should be considered.

References:

- Carbone, I., and Kohn, L. M. 1999. Mycologia 91:553.
- Glass, N. L., and Donaldson, G. C. 1995. Appl. Environ. Microbiol. 61:1323.
- Guarnaccia, V., et al. 2016. Eur. J. Plant Pathol. 146:963.
- Udayanga, D., et al. 2015. Fungal Biol. 119:383.
- 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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