Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Pseudophaeomoniella oleae* Causing Wood Streaking and Decay on Olive Trees in Greece

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Plant Dis. 106:2263, 2022; published online as <https://doi.org/10.1094/PDIS-10-21-2353-PDN>. Accepted for publication 21 January 2022.

A survey was conducted in olive orchards (*Olea europaea* L., cv. Koroneiki) showing severe decline in Milatos and Ierapetra (Lasithi, Crete, Greece) in November 2017 and January 2019, respectively. Diseased trees exhibited wilting, yellowing of leaves, twig and branch dieback, and internal discoloration of vascular tissue. Insect infestations were commonly associated with these symptoms. A yeast-like fungus was consistently isolated from discolored vessels previously surface-disinfested with 95% ethanol on acidified potato dextrose agar (APDA). The fungus yielded several dark brown to black and globose to oval pycnidia with dimensions 120 to 330 × 90 to 300 μm (average 179.6 × 143.9 μm) after 25 days of growth on APDA. Emerging colonies were transferred to new PDA and their growth rate was 2.21 mm/day at 24°C in the dark. The sparse aerial mycelium was initially white and turned beige-pinkish in the center after 21 days of growth on PDA. Microscopic observations revealed hyaline, smooth, ampulliform conidiophores, bearing solitary conidia or in slimy heads. Conidia were one-celled, hyaline, smooth, subcylindrical with obtuse ends, and 1.25 to 5.75 × 0.75 to 2.00 μm (average 3.12 × 1.16 μm). Light to dark brown pycnidia, semi-immersed in PDA, with dimensions 150 to

490 × 90 to 320 μm (average 297.7 × 231.0 μm) were evident in 3-week-old cultures. Colony morphology and microscopic features were similar to *Pseudophaeomoniella oleae* (Crous et al. 2015). DNA from two representative isolates (EML1 and DRAGVR1) was extracted, and their internal transcribed spacer region (ITS) of ribosomal DNA and actin (*ACT*) and translation elongation factor 1- α (*TEF1- α*) genes were amplified using the primer pairs ITS1/ITS4 (White et al. 1990), ACT-512F/ACT-783R, and EF1-728F/EF1-986R (Carbone and Kohn 1999), respectively. PCR products were sequenced and deposited in GenBank (accession nos. MZ854242 and MZ854243, OK143463 and OK143464, and OK143465 and OK143466). BLAST search revealed high similarity to GenBank sequences of the ex-type strain of *P. oleae* for ITS ($\geq 99.50\%$, NR_137966.1 and KP635972.2), *ACT* (100%, KP635974.1), and *TEF1- α* (98.32%, KP635968.1). Based on morphology and phylogenetic analysis of the ITS region, the fungus was identified as *P. oleae*. Ten 3-year-old olive trees of each of the cultivars Amfissis and Koroneiki were artificially inoculated by drilling a 3-mm-diameter hole into the trunk and injecting 50 μl of a conidia suspension (1×10^7 ml⁻¹) of the isolate DRAGVR1 (Markakis et al. 2017). Another 10 trees of each cultivar treated similarly with sterilized distilled water served as controls. Potted trees were kept under ambient conditions. Fourteen months postinoculation, longitudinal and transverse sections of inoculated trunks revealed wood discoloration extending above and below the inoculation point in both cultivars, whereas no leaf symptoms were observed. *P. oleae* was consistently reisolated from symptomatic wood tissue and identified by colony morphology. Neither symptoms nor positive isolations were observed in controls. To our knowledge, this is the first report of wood streaking and decay caused by *P. oleae* in olive trees in Greece. Although the fungus has been isolated previously from decayed olive trees showing vascular wilt in Italy (Crous et al. 2015), this is the first experimental evidence of the pathogenic potential of the species on olive trees worldwide. This disease could potentially be an increasing problem in olive tree growing areas and could result in severe crop losses, so effective management practices should be investigated and applied.

References:

- Carbone, I., and Kohn, L. M. 1999. Mycologia 91:553.
- Crous, P. W., et al. 2015. Persoonia 34:167.
- Markakis, E. A., et al. 2017. Eur. J. Plant Pathol. 149:277.
- White, T. J., et al. 1990. Page 315 in: PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego.

The author(s) declare no conflict of interest.

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Keywords: actin, decline, ITS, *Olea europaea*, *Pseudophaeomoniella oleae*, TEF1- α

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