First report of *Pseudophaeomoniella oleae* causing wood streaking and decay on olive trees in Greece

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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, globose to oval pycnidia with dimensions 120–330 × 90–300 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, turning into beige-pinkish in the centre after 21 days of growth on PDA. Microscopic observations revealed hyaline, smooth, ampulliform conidiophores, bearing conidia solitary or in slimy heads. Conidia were 1-celled, hyaline, smooth, subcylindrical with obtuse ends, 1.25–5.75 × 0.75–2.00 μm (average 3.12 × 1.16 μm) in size. Light to dark brown pycnidia, semi-immersed in PDA, with dimensions 150–490 × 90–320 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, actin (ACT) and translation elongation factor 1-alpha (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-MZ854243, OK143463-OK143464 and OK143465-OK143466). BLAST search revealed high similarity to GenBank sequences of the ex-type strain of *P. oleae* for ITS

(≥99.50%, NR_137966.1 and KP635972.2), ACT (100%, KP635974.1), and TEF-1a (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 cvs Amfissis and Koroneiki were artificially inoculated by drilling a 3-mm-diameter hole into the trunk and injecting 50 µl of a 1×10^7 ml^{-1} conidia suspension of the isolate DRAGVR1 (Markakis et al. 2017). Another ten trees of each cv treated similarly with sterilized distilled water served as controls. Potted trees were kept under ambient conditions. Fourteen months post inoculation, 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 steadily re-isolated 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 result in severe crop losses. Hence, effective management practices should be investigated and applied.

***References*:**

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Crous, P. W., et al. 2015. Persoonia 34:224.

Markakis, E. A., et al. 2017. Eur. J. Plant Pathol. 149:277.

1 White, T. J., et al. 1990. Page 315 in: PCR Protocols: A Guide to Methods and
2 Applications. Academic Press, San Diego.

Supplemental material

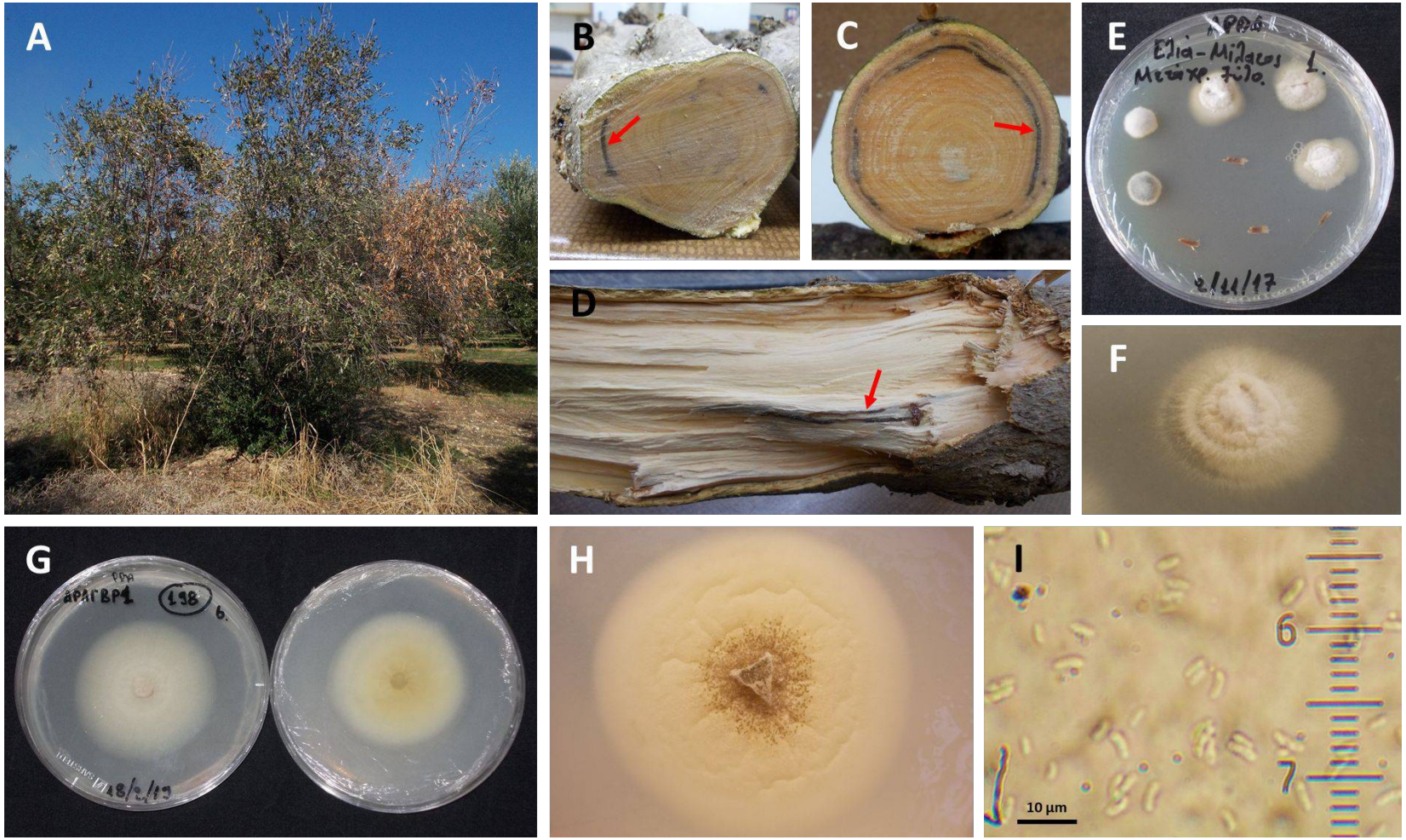

Figure 1. Symptoms of wood streaking and decay on olive tree as occurred in the field and morphological characteristics of the causal agent *Pseudophaeomoniella oleae*. **A:** leaf wilting, twig and branch dieback, **B-D:** brown wood streaking and discoloration, **E:** *P. oleae* isolation on acidified PDA, **F:** yeast-like appearance of a fungal colony emerged from tissue excision, **G:** morphological characteristics of *P. oleae* (isolate DRAGVR1) grown on PDA culture upper- and under-side, **H:** pycnidia formed in a 22-day-old PDA culture, and **I:** *P. oleae* conidia.

Supplemental material

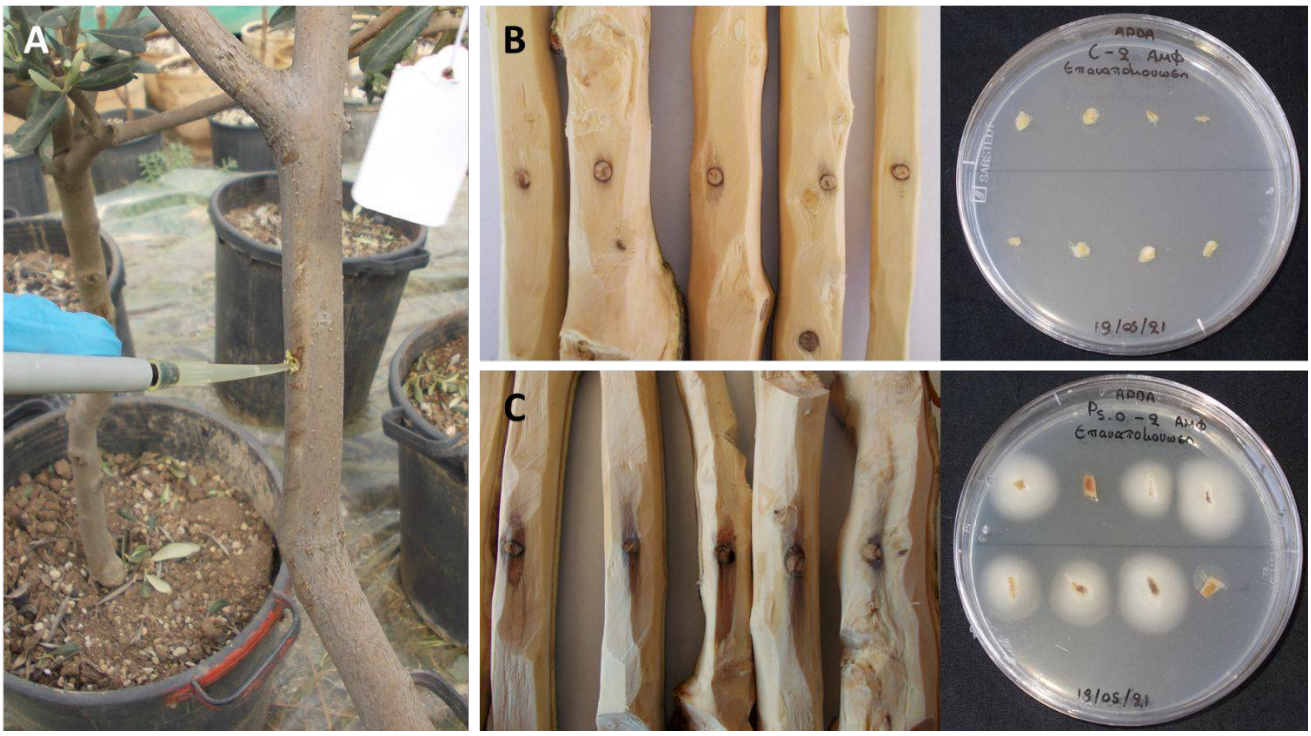

Figure 2. Disease symptoms on olive tree cv Amfissis artificially inoculated with *Pseudophaeomoniella oleae*. **A:** artificial inoculation of a 3-year-old tree, **B:** absence of wood discoloration along with no pathogen re-isolation from control trees, and **C:** symptoms of wood discoloration along with pathogen re-isolation from trunks inoculated with *P. oleae* (isolate DRAGVR1).

Supplemental material

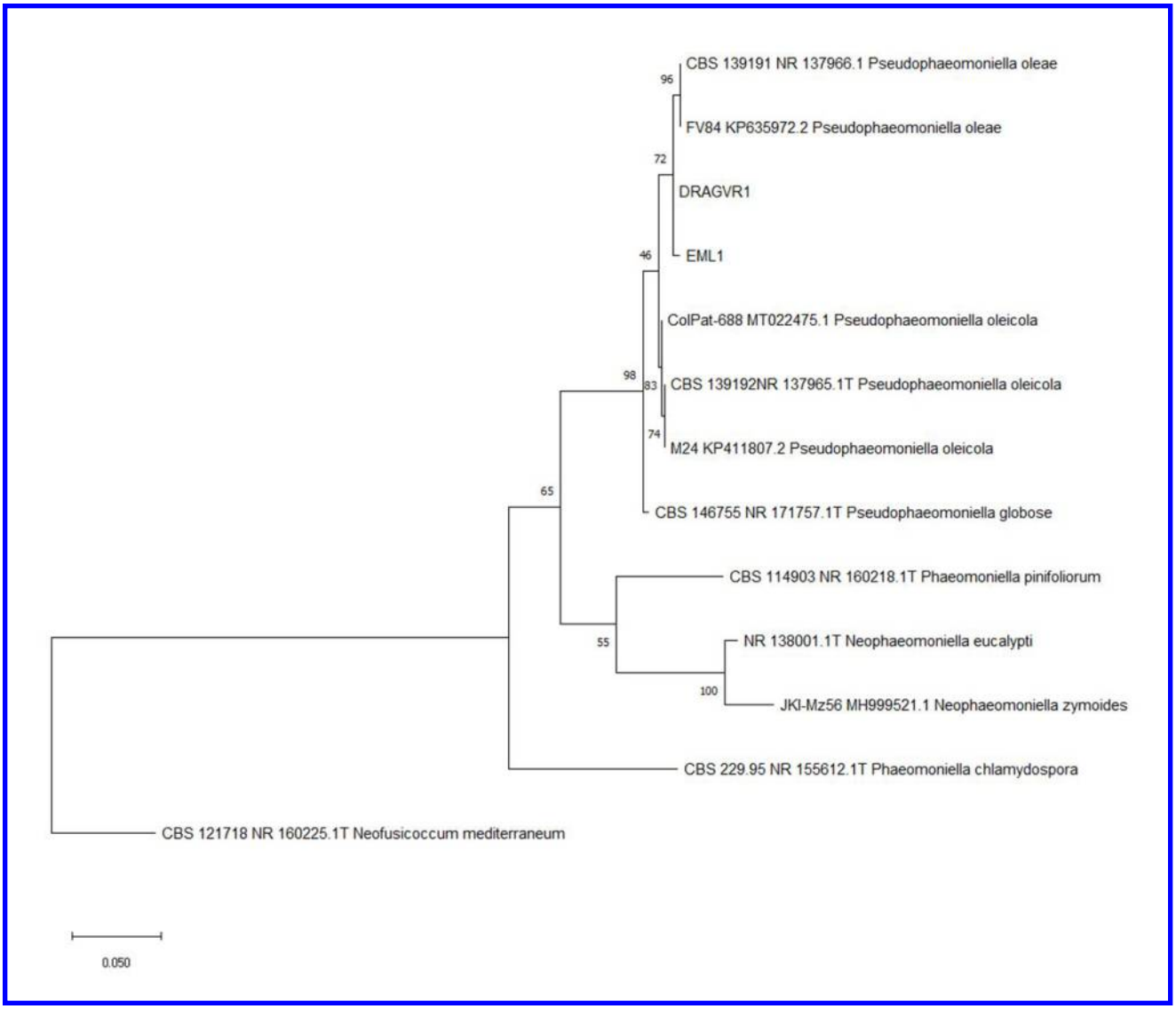

Figure 3. Phylogenetic tree presenting the evolutionary history of *Pseudophaeomoniella oleae* isolates (DRAGVR1 and EML1) originated from olive trees with symptoms of wood discoloration and decay, based on the analysis of their *internal transcribed spacer regions of ribosomal DNA (rDNA-ITS)* gene sequences. The construction of the tree was conducted by using the Maximum Likelihood method and a bootstrap resampling analysis for 1000 replications. 'T', indicates the ex-type and ex-epitype strains.

Supplemental material

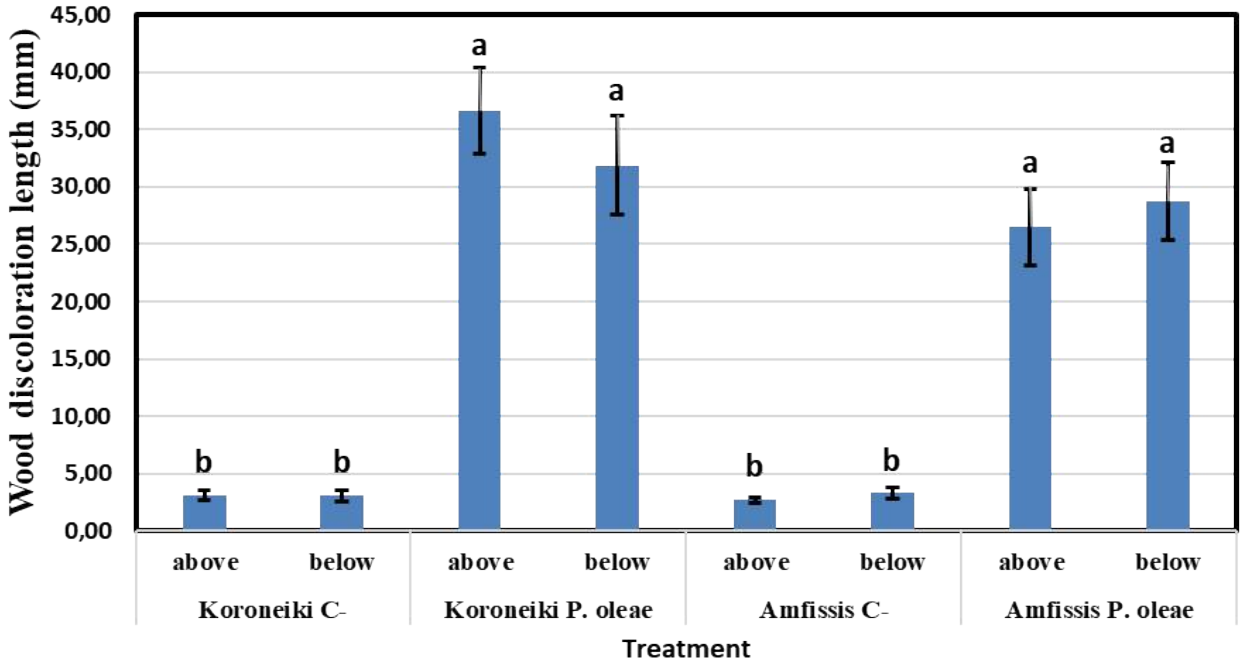

Figure 4. Length of vascular tissue discoloration above and below the inoculation point of Koroneiki and Amfissis olive cultivars, 14 months after injecting *Pseudophaeomoniella oleae* (isolate DRAGVR1) conidial suspension into the trunk vessels. Control trees (C-) were mock-inoculated with sterilized distilled water. Columns followed by the same letter are not significantly different according to Tukey honestly significant difference test ($P \leq 0.05$). Each column represents the mean of 10 trees and vertical bars indicate standard errors.