Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Phaeoacremonium oleae* and *P. viticola* Associated with Olive Trunk Diseases in Italy

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Over 300 trunk, branch, and stem samples with vascular discoloration, necrotic wood, and shoot death were collected from olive (*Olea europaea*) orchards in Lecce, Brindisi, Bari, and Foggia provinces (Apulia region, Italy) from October to May from 2013 to 2019. Small chips of symptomatic wood samples were surface sterilized (5% NaOCl, 3 min; 70% ethanol, 30 s), rinsed (sterile distilled water, ×3), and placed onto potato dextrose agar (PDA) plates amended with 500 ppm of streptomycin sulfate. After 14 days at 25°C in the dark, hyphal tips of growing fungi, including different taxa, for instance *Phaeoacremonium* and Botryosphaeriaceae spp., were transferred to new PDA plates and incubated until sporulation. Monoclonal colonies resembling *Phaeoacremonium*-like genus (Mostert et al. 2006) were selected for further study, and genomic DNA of 59 representative isolates was extracted (Carlucci et al. 2013). Partial actin and β -tubulin genes were amplified with primers ACT-512F/ACT-783R (Carbone and Kohn 1999), and T1 (O'Donnell and Cigelnik 1997) and Bt2b (Glass and Donaldson 1995), respectively. The sequenced amplicons were compared by BLAST algorithms with reference strains of *Phaeoacremonium* spp. retrieved from GenBank. Forty-four isolates showed 99 to 100% similarity with reference strains *P. italicum*, *P. minimum*, *P. parasiticum*, *P. scolyti*, and *P. sicilianum* (Carlucci et al. 2015), nine with *P. oleae*, and six with *P. viticola*. Actin and β -tubulin sequences of *P. oleae* (Pm14) and *P. viticola* (Pm34) were submitted to GenBank (MW714561, MW714563; MZ318697, MZ318696). Microscopy of *P. oleae* isolates showed the following: conidiophores branched and unbranched, (18.7 to 21.9 to 57.1 (to 67.8) × (2.9 to) 3.3 to 4.7 (to 5.2) (mean, 38.9 × 4.1) μ m ($n = 30$); conidia oblong-ellipsoidal to obovoid or subcylindrical, 3.4 to 5.5 μ m long, and 1.5 to 2.4 (mean, 4.6 × 2.2) μ m wide

($n = 30$). Microscopy of *P. viticola* isolates showed the following: conidiophores subcylindrical, branched at base (6.7 to) 8.9 to 27.2 (to 29.3) × (2.0 to) 2.6 to 3.3 (to 3.7) (mean, 21.4 × 3.2) μ m ($n = 30$); conidia oblong-ellipsoidal to obovoid or subcylindrical, 3.3 to 6.8 μ m long, and 1.1 to 2.2 (mean, 4.2 × 1.6) μ m wide ($n = 30$). In spring 2020, artificial inoculations were carried out with *P. oleae* (Pm14, Pm46) and *P. viticola* (Pm34, Pm43) strains on 10 healthy, 2-year-old olive seedlings of cultivar 'Coratina'. Agar plugs (diameter, 0.3 to 0.5 cm) from 10-day-old cultures grown on water agar at 23 ± 2°C were inserted under the bark of small wounds in the stems (length, 0.4 to 1.0 cm) made with a sterile scalpel. After inoculation, the wounds were wrapped with wet sterile cotton wool and sealed with Parafilm. Ten control olive seedlings were inoculated with sterile agar plugs. The experiment was replicated three times. All inoculated young olive plants were grown in pots in a greenhouse without temperature control. After 120 days, inoculated plants showed decline symptoms, and when cut longitudinally, brown streaks were observed in the wood. For *P. oleae* these streaks measured 3.0 to 5.5 cm long (standard deviation [SD], 0.9 cm), and for *P. viticola* they were 1.8 to 3.5 cm (SD, 0.62 cm). Both fungal species were reisolated from the symptomatic wood from 85 and 80%, respectively, of these inoculated olive seedlings, fulfilling Koch's postulates. No symptoms were observed from olive seedlings used as control. *P. oleae* was first described as a fungal pathogen of wild olive (*O. europaea* subsp. *cuspidata*) in South Africa by Spies et al. (2018) and *P. viticola* as a fungal pathogen of grapevine in France by Dupont et al. (2000). To the best of our knowledge, this is the first report of *P. oleae* associated with olive trunk disease in Italy and the first report of *P. viticola* associated with olive trunk disease worldwide.

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The author(s) declare no conflict of interest.

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