

Disease Note

Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Pleurostoma richardsiae* Associated with Twig and Branch Dieback of Olive Trees in Spain

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Funding: Funding was provided by Río Lacerón S.L. (Badajoz, Spain), Centro para el Desarrollo Tecnológico Industrial and Consejería de Educación, Junta de Castilla y León (ORDEN EDU/601/2020). Plant Dis. 106:1981, 2022; published online as <https://doi.org/10.1094/PDIS-10-21-2127-PDN>. Accepted for publication 19 December 2021.

Pleurostoma richardsiae has been described as an olive tree pathogen causing decline and brown wood streaking (Carlucci et al. 2013). A survey was carried out in plots under olive cultivation (*Olea europaea* L., cv. Picual; 10-year-old plants) at La Garrovilla, Spain, in September 2020 in which a putative *Verticillium* wilt had been visually diagnosed. In plot 1 (2.6 ha; 741 plants), 20.4% of the plants exhibited wilt, foliar browning, leaf drop, and twig and branch dieback. The level of incidence in plots 2 (4.8 ha; 1368 plants), 3 (3.20 ha; 912 plants), and 4 (1.85 ha; 527 plants) was 25.0, 19.5, and 42.9% respectively, which meant that that harvest had an average reduction in olive production and an economic loss of 30.2%. Three trees from each plot were uprooted and analyzed. Five out of 12 intriguingly showed brown streaking under the bark extending from the root system and ascending the trunk, which is a symptom that is never associated with *Verticillium dahliae*, as it does not produce necrosis and cankers in the wood (López-Escudero and Mercado-Blanco 2011). Samples from the five tree trunks showing necrosis were taken to the lab and surface sterilized. Small pieces of discolored wood were placed onto malt extract agar plates containing chloramphenicol (0.25 g/liter) and incubated for 21 days at 25°C in darkness. The growing fungal colonies were then transferred to potato dextrose agar (PDA). Isolates were identified by micromorphological characteristics, according to Vijaykrishna et al. (2004), as *P. richardsiae*. Colonies on PDA were cottony,

brown with a whitish edge, and abundantly produced two types of conidia: brown (spherical or subglobose) or hyaline (allantoid to cylindrical) that appeared on septate and inconspicuous phialides, respectively. Identification was confirmed by amplification and sequencing of the internal transcribed spacer (ITS) region using ITS1/ITS4 primers (White et al. 1990) and partial sequencing of the β -tubulin gene using T1 (O'Donnell and Cigelnik 1997) and Bt2b (Glass and Donaldson 1995) primers. The ITS sequence showed a 99.82% identity with that of *P. richardsiae* IFM51337 (CBS406.93 type strain; GenBank AB364703.1), whereas the β -tubulin sequence exhibited a 99.77% identity with the *P. richardsiae* CBS406.93 β -tubulin gene (GenBank MT501304.1). The ITS and β -tubulin sequences were deposited in GenBank (MZ519916 and MZ542764, respectively). The *P. richardsiae* isolate has been deposited in the Spanish Type Culture Collection (CECT 21196). Pathogenicity tests were conducted on 1-year-old potted olive plants cv. Picual maintained in a growth chamber at 25°C with 12-h dark/12-h light. Twelve plants were inoculated in a wound in the stem made with a scalpel, and mycelial plugs (5 mm in diameter) from 15-day-old PDA plates were inserted into the wounds. Another set of 12 plants was inoculated with sterile agar plugs and used as negative control plants. Four months after inoculation, 66% of the plants inoculated with mycelia plugs showed wilting, necrosis under the bark, or had even died. *P. richardsiae* was successfully reisolated from necrotic areas in 75% of the plants inoculated with mycelia plugs. Ten reisolates were identified as *P. richardsiae* by the above molecular techniques to confirm Koch's postulates. No symptoms were observed in the negative control plants, and the pathogen was not reisolated from them. To our knowledge, this is the first report of *P. richardsiae* associated with twig and branch dieback of olive trees in Spain.

References:

- Carlucci, A., et al. 2013. *Phytopathol. Mediterr.* 52:517.
Glass, N. L., and Donaldson, G. C. 1995. *Appl. Environ. Microbiol.* 61:1323.
López-Escudero, F. J., and Mercado-Blanco, J. 2011. *Plant Soil* 344:1.
O'Donnell, K., and Cigelnik, E. 1997. *Mol. Phylogenet. Evol.* 7:103.
Vijaykrishna, D., et al. 2004. *Stud. Mycol.* 50:387.
White, T. J., et al. 1990. Page 315 in: *PCR Protocols: A Guide to Methods and Applications*. Academic Press, San Diego, CA.

The author(s) declare no conflict of interest.

Keywords: fungal pathogenicity, *Olea europaea*, olive disease, wood streaking

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