



## NEW DISEASE REPORT

# First report of *Phytophthora hibernalis*, *P. multivora* and *P. niederhauserii* causing root rot and bleeding cankers on *Eucalyptus globulus* in Portugal

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Email: [carlo.bregant@phd.unipd.it](mailto:carlo.bregant@phd.unipd.it)**KEYWORDS**

disease, emerging, oomycetes

During spring 2022 *Phytophthora* related-disease symptoms were observed in three *Eucalyptus globulus* plantations in central Portugal (Aveiro district). Affected trees displayed a range of symptoms including root rot, bleeding cankers and extensive canopy dieback (Figure 1). Average disease incidence and tree mortality rate were estimated as 67 and 20% along three linear transects of 50 m, respectively.

In order to isolate the causal agents, rhizosphere and root samples were collected from 20 diseased trees chosen at random in the monitored plantations. Samples were processed as described by Bregant et al. (2023) and cultured on carrot agar at 20°C in the dark. *Phytophthora* colonies were examined and identifying features recorded and compared with published morphological records of *Phytophthora* species. The following three species were identified: *Phytophthora multivora* (12/20 isolates), *P. hibernalis* (6/20) and *P. niederhauserii* (2/20) (Figure 2). The identity of all isolates was confirmed by analysis of the internal transcribed spacer region using the universal primers ITS1 and ITS4. DNA extraction, PCR amplification and sequencing were performed according to Bregant et al. (2023). A BLASTn search against the GenBank database showed 100% identity with sequences of the ex-type cultures of *P. hibernalis* (CBS 114104), *P. multivora* (CBS 124094) and *P. niederhauserii* (WPC P10616). The ITS sequence of a representative isolate of each species was deposited in GenBank (*P. hibernalis*: GenBank Accession No. OQ520314; *P. multivora*: OQ520315; and *P. niederhauserii*: OQ520316).

The pathogenicity of a representative strain of each *Phytophthora* species was assessed on one-year-old *E. globulus* seedlings as described by Bregant et al. (2023). For each isolate ten seedlings were inoculated with an agar-mycelium plug (3 mm diameter) cut from the margin of a four-day-old colony on potato dextrose agar (PDA), whereas ten seedlings used as controls were inoculated with a sterile PDA plug. Seedlings were kept in controlled conditions at 21°C and watered regularly for 30 days. All seedlings inoculated with *Phytophthora* isolates showed inner bark necrotic lesions spreading up and down from the inoculation point (Figure 3). Average lesion size varied significantly among isolates: *P. niederhauserii* 28 ± 3 mm (average ± SD), *P. hibernalis* 10 ± 3 mm and *P. multivora* 7 ± 3 mm. The control seedlings remained asymptomatic. All *Phytophthora* species were successfully re-isolated from all inoculated seedlings, thus fulfilling Koch's postulates.

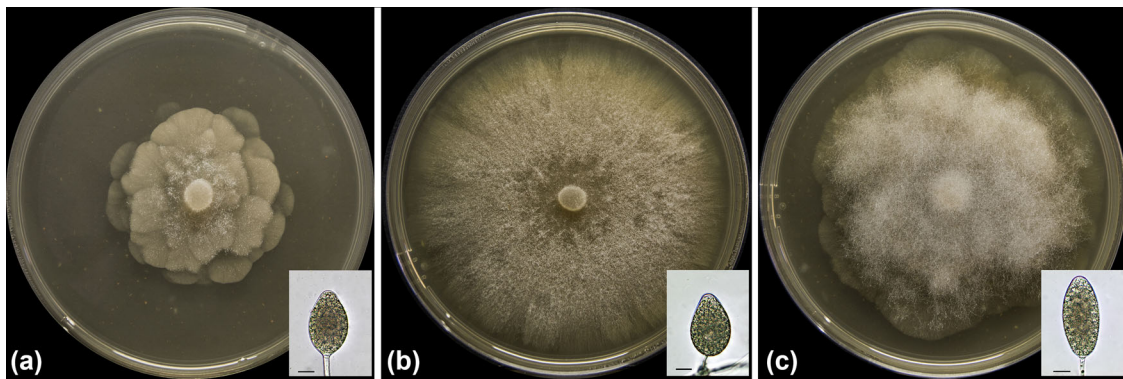
This study represents the first report of *P. hibernalis*, *P. multivora* and *P. niederhauserii* on *E. globulus* in Portugal and in Europe. Recently, two other species, *Phytophthora cinnamomi* and *P. alticola*, have been reported as root rot agents of *E. globulus* in several areas of Portugal (Diogo et al., 2023). The involvement of multiple *Phytophthora* on *E. globulus* plantations is of particular concern due to the invasiveness of the species detected and the potential spread of these pathogens into natural ecosystems adjacent to eucalypt plantations.

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**FIGURE 1** Overview of one of the *Eucalyptus globulus* plantations with severe *Phytophthora* disease symptoms (a); progressive canopy dieback symptoms (b); and bleeding cankers at the collar (c).



**FIGURE 2** Colony morphology after seven days on carrot agar at 20°C in the dark and mature sporangia of *Phytophthora hibernalis* (a), *P. multivora* (b) and *P. niederhauserii* (c). Scale bars = 20 μm.



**FIGURE 3** Inner bark necrotic lesions observed on *Eucalyptus* seedlings 30 days after inoculation with *Phytophthora hibernalis* (a), *P. multivora* (b) and *P. niederhauserii* (c). Asymptomatic control seedling (d).

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