



First report of *Phytophthora gonapodyides* involved in the decline of *Quercus ilex* in xeric conditions in Spain

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Over the last three decades an intense dieback of holm oak (*Quercus ilex*) has been recorded in southwest Spain, with *Phytophthora cinnamomi* and water stress believed to be the major factors involved (Romero *et al.*, 2007; Solla *et al.*, 2009). In 2009, *P. cinnamomi* and *Pythium spiculum* were recovered during all seasons from soil and roots from trees showing characteristic symptoms in five declining *Q. ilex* stands in the province of Cáceres, Extremadura, SW Spain. In October 2009, a different *Phytophthora* species was isolated from roots and from rhizosphere soil of a single tree located in Malpartida de Plasencia (39°58'N 6°5'W, 443 m above sea level), using young *Q. robur* and *Q. ilex* leaves as baits and V8-PARPH agar as a selective medium (Jung *et al.*, 1996). The heterothallic isolates formed irregularly branched hyphae, but no chlamydo-spores or hyphal swellings were observed. Nonpapillate elongated-ovoid to obpyriform sporangia (28-58 x 25-40 µm) with exit pores of 10-20 µm were produced by flooding one cm squares from the growing margin of a V8-agar culture for 24 h in non-sterile soil-extract. The colony pattern on V8 agar was stellate, and the average radial growth rates at 20, 25 and 30°C were 2.5-2.7, 2.5-2.7 and 2.2-2.3 mm/day, respectively. All these features are typical of *P. gonapodyides* (Erwin & Ribeiro, 1996; Jung *et al.*, 1996). The identity was confirmed by sequencing the internal transcribed spacer region of the rDNA with the primers ITS4/ITS6 (GenBank Accession No. GU724194).

Because *P. gonapodyides* causes root rot and stem lesions in *Q. robur* (Jung *et al.*, 1996; Balci & Halmschlager, 2003), pathogenicity tests on one-year-old *Q. ilex* seedlings were performed. Thirty plants were grown on 250 ml pots containing a mixture of sand and peat (1:1). For inoculum preparation (Romero *et al.*, 2007), the isolate was grown in petri dishes containing 20 ml of carrot broth at 20°C in darkness. After four weeks of incubation, the liquid medium was discarded, and the mycelium was washed, added to sterile water, shaken and mixed for three minutes. Each pot was inoculated with the mycelium harvested from one petri dish. Plants were kept at an average temperature of 25°C in natural daylight. Three months after inoculation, mortality of infected plants was 53%, and mean survival time (±SD) of infected plants was 71±15 days. For comparison, additional plants were inoculated in the same way with *P. cinnamomi*. After three months, mortality of *Q. ilex* seedlings was 94% and mean survival time 28±7 days. The pathogens were consistently re-isolated from the roots of the dead plants. Control plants did not show any symptoms of disease. To our knowledge, this is the first report of *P. gonapodyides* in Spain. This pathogen has always been associated with moist sites (Hansen & Delatour, 1999; Balci & Halmschlager, 2003), in

contrast to our findings, in which mean volumetric soil moisture values at 30 and at 100 cm depth (loam soil) were 11.4 and 22.1% respectively, and the mean soil water table depth was 4.6 m. Under field conditions, further research about the involvement of this pathogen in *Q. ilex* decline will be undertaken

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