



## First report of *Phytophthora ilicis* causing twig blight on holly in Spain

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During *Phytophthora ramorum* monitoring surveys at nurseries, parks and public gardens, a range of ornamental plants susceptible to infection with *Phytophthora* were extensively surveyed. In 2011, during these surveys in Santiago de Compostela (Galicia, NW Spain), several holly plants (*Ilex aquifolium*) were observed exhibiting black leaf spots, defoliation, twig blight, cankers and shoot dieback. Excised tissue from the leading edge of lesions on twigs and stems was plated on V8 agar semi-selective medium (Erwin & Ribeiro, 1996) and incubated for four days at 20°C in the dark. A *Phytophthora* sp. was consistently isolated. This was transferred to carrot agar (CA) and potato dextrose agar (PDA) (Fig. 1). Isolates exhibited optimal growth at 16-18°C and maximum growth at 25°C. Sporangia formed sympodially, were semipapillate, caducous with medium length pedicels 5-15 µm long (Fig. 2a) and had a length/width ratio of 1.2-1.6. Chlamydospores were not observed. Isolates were homothallic. Oogonia ranged from 17-27 µm in diameter (average 22.67 µm). Oospores were plerotic 14.6-23.6 µm in diameter (Fig. 2b-d). Antheridia were amphigynous, round, and occasionally elongated (Fig. 2b-d). Based on these morphological characters, cultures were identified as *Phytophthora ilicis* (Buddenhagen & Young, 1957). Identity was confirmed by DNA sequences analysis. DNA from isolates was extracted and the internal transcribed spacer (ITS) and translation elongation factor 1 alpha (EF-1 alpha) gene regions were amplified using primers DC6, ITS4 and ITS6 (Cooke *et al.*, 2000) and ELONGF1-ELONGR1 (Kroon *et al.*, 2004). PCR amplicons were sequenced and deposited in GenBank (Accession Nos. JQ609277 and JQ609278, respectively). Comparison of the sequences with others sequences available in GenBank showed 100% homology with those previously identified as *P. ilicis* (FJ8001947 and AY564127).

A pathogenicity test of one representative isolate of *P. ilicis* was conducted using five detached stems of holly. A shallow wound was made with a scalpel on the middle of the stem. A 5 mm mycelial plug, from the margin

of a seven-day-old culture growing on V8 agar, was inserted in every wound and sealed with Parafilm. The inoculated shoots were maintained at room temperature and 75% humidity. After 10 days stem blackened cankers and defoliation were observed (Fig. 3). Control stems, inoculated with sterile V8 agar, remained symptomless. *P. ilicis* was re-isolated from all inoculated stems but not from controls. To our knowledge this is the first report of *Phytophthora ilicis* causing leaf and twig blight on *Ilex* in Spain. *P. ilicis* is an invasive aerial *Phytophthora* characteristically found in cool temperate regions and it is host specific on holly. All cultivars of *I. aquifolium* are susceptible, although some other *Ilex* species and hybrids show resistance (Hall, 1991). *P. ilicis* was first reported and described in the western United States and is also recorded in Canada, U.K, the Netherlands and France.

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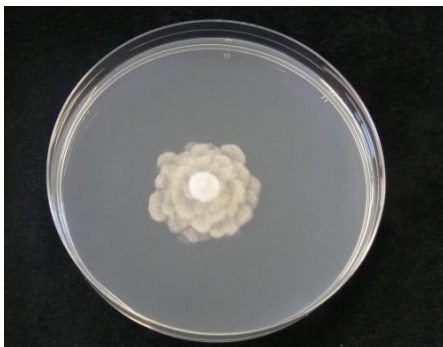


Figure 1



Figure 2



Figure 3

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