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NEW DISEASE REPORT

First report of *Phytophthora ilicis* causing leaf spot, shoot blight and bleeding canker on *Ilex aquifolium* in Slovenia

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English holly (*Ilex aquifolium*) is a small, evergreen tree that grows naturally in temperate and mediterranean mountain forests in Europe, and is grown throughout the world for its ornamental characteristics. Ecologically, holly is a shade-tolerant and very slow-growing plant that populates the undergrowth in temperate deciduous forests and woodlands dominated by oak or beech (Guerrero Hue et al., 2016). In Slovenia *I. aquifolium* shows an irregular distribution in wet and shady places with quite mild climates such as the Vipava and Osilniška valleys. Due to illegal removal from the wild, holly has been protected by national legislation in Slovenia.

In October 2023, moderate defoliation was found on several young English holly trees located in a mixed forest near Nova Gorica in western Slovenia (Gozd Panovec, 45°56'47.7"N 13°40'06.0"E). Diseased plants exhibited leaf necrosis, shoot blight, defoliation and occasionally stem bleeding cankers and inner bark discolouration (Fig. 1). Field surveys conducted in spring 2024 revealed a disease incidence of 60% along two linear transects of 25 m.

Fifteen necrotic leaves and three inner bark (stem) samples were collected from six diseased plants chosen at random in the study area. Samples were processed as described by Bregant et al. (2023) and cultured on carrot agar at 18°C in the dark. A typical slow-growing homothallic *Phytophthora* species was isolated consistently from the diseased samples (Fig. 2). Isolates produced semi-papillate mostly caducous sporangia (mean size 41.8 ± 6.6 × 26.4 ± 3.6 µm, n = 25) with long pedicels (mean length 17.3 ± 5.4 µm, n = 25) after 36–48 hours on carrot agar flooded in non-sterile pond water (Fig. 2). Based on these morphological data, 14 isolates appeared compatible with the species *Phytophthora ilicis* Buddenh. & Roy A. Young. The morphological identification was confirmed by molecular analysis based on the sequence of the ITS and *cox*1 regions obtained following the methodology reported in Bregant et al. (2023).

Phylogenetic reconstructions for *cox*1 sequences of two Slovenian isolates of *P. ilicis* and nine other isolates together with the other species belonging to ITS clade 3 were performed with MEGA-X 10.1.8, including all gaps in the analyses and choosing the best model determined automatically by the software (Kumar et al., 2018). Maximum likelihood analysis was performed with a neighbour-joining starting tree generated by the software (Fig. 3). Two additional *P. ilicis* strains isolated in the Marche region (Castelfidardo, Italy) were included in the phylogenetic analysis and a sequence of *P. alpina* (CBS 146801) was used as an outgroup. The sequences of *P. ilicis* generated in this study were deposited in GenBank (isolate SLO35 (Slovenia): GenBank Accession Nos.: PQ452714 & PQ472567; isolate SLO43 (Slovenia): PQ452715 & PQ472568; isolate CB1100 (Italy): PQ452716 & PQ472569; isolate CB1101 (Italy): PQ452717 & PQ472570). Mitochondrial *cox*1 phylogeny shows that the Slovenian population of *P. ilicis* is identical to those in other parts of the Mediterranean and in North America (Fig. 3).

FIGURE 1 Overview of the main symptoms observed on English holly in Slovenia: (a) necrotic spot on leaf, (b,c) necrotic leaf with fresh lesion around an axillary node, (d) shoot blight, (e,f) twig canker, (g) stem bleeding lesions and (h) under bark canker with wood discolouration.

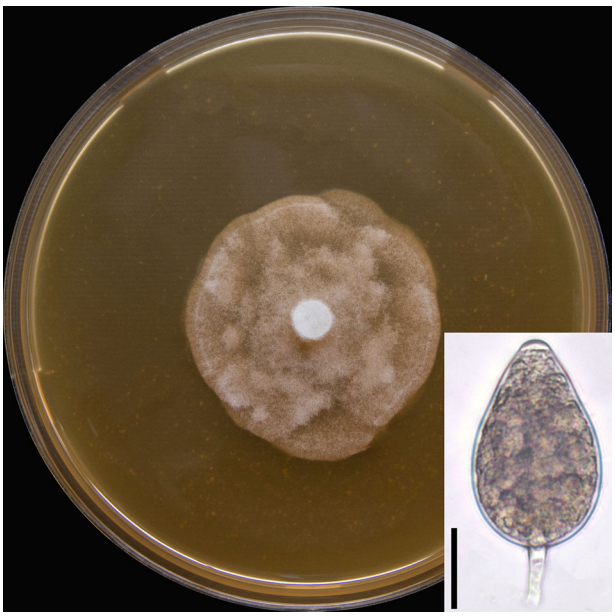

FIGURE 2 Colony morphology of *Phytophthora ilicis* (isolate SLO35) after seven days on carrot agar at 18°C in the dark, and (inset) mature sporangium with long caducous pedicel (bar = 20 μm).

The pathogenicity of *Phytophthora ilicis* was verified by inoculating asymptomatic young leaves of *Ilex aquifolium* with a representative isolate (SLO35). The upper surface of the leaf was disinfected with 70% ethanol and inoculated (without wounding) by an agar-mycelium plug (3 mm Ø) cut from the margin of a four-day-old colony on potato dextrose agar (PDA). Eight leaves were inoculated with the pathogen in the trial and another five were inoculated with a sterile PDA plug as a control. Inoculated leaves were maintained in humid chamber at 18 ± 1°C for 72 hours. *Phytophthora ilicis* proved to be pathogenic on holly leaves, causing extensive necrosis (mean lesion length 710 ±313 mm^2) (Fig. 4). Control leaves remained asymptomatic. *Phytophthora ilicis* was successfully re-isolated from all inoculated leaves, thus fulfilling Koch's postulates.

Phytophthora. ilicis is one of the few known host-specific *Phytophthora* species. It was initially described in the western United States and is also recorded on ornamental holly trees in Canada, UK, the Netherlands, and Spain (Buddenhagen & Young, 1957, Pintos et al., 2012). In 2014 *P. ilicis* was reported for the first time in natural holly stands in Sardinia and Corsica, where it probably represents an endemic species (Scanu et al., 2014). To our knowledge, this is the first report of *P. ilicis* on *I. aquifolium* in Slovenia. A study is currently underway to study the distribution and populations of this species in the rare and fragmented formations of *I. aquifolium* in continental Italy and Slovenia.

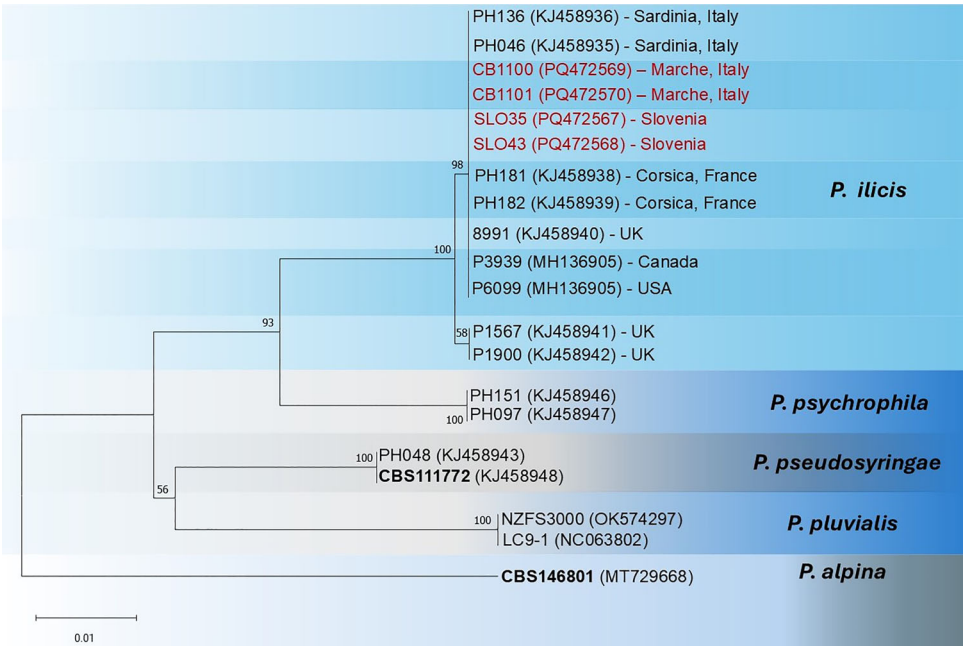

FIGURE 3 Maximum likelihood tree obtained from *cox*1 sequences of *Phytophthora* species belonging to clade 3. *Phytophthora alpina* sequence is used as outgroup. Data are based on the General Time Reversible model. A discrete Gamma distribution was used to model evolutionary rate differences among sites. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Bootstrap support values in percentage (1000 replicates) are given at the nodes. Ex-type cultures are bold, and isolates of this study in red. GenBank accession numbers are in brackets.

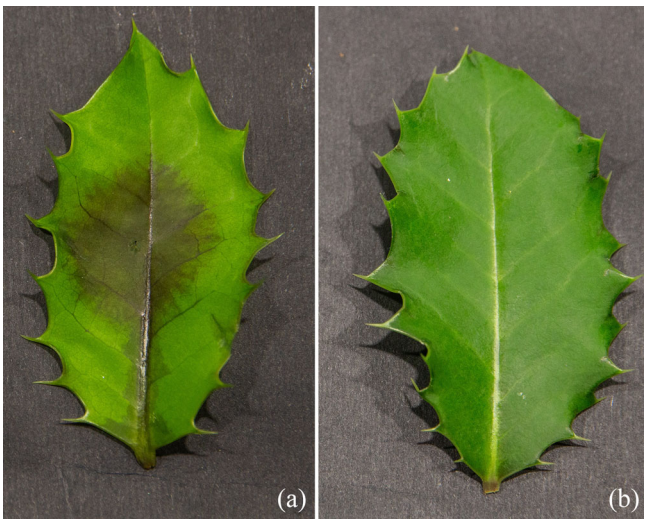

FIGURE 4 (a) Lesions recorded on English holly leaves 72 hours after inoculation with isolate SLO35 of *Phytophthora ilicis* and (b) asymptomatic control leaf.

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