



First report of *Phytophthora hydropathica* in river water associated with riparian alder in Spain

C. Pintos*, C. Rial, O. Aguín, V. Ferreiroa and J.P. Mansilla

Estación Fitopatológica Areiro. Deputación Pontevedra. Subida a la Robleda s/n. 36153 Pontevedra, Spain

*E-mail: cristina.pintos@depo.es

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Phytophthora hydropathica has been commonly reported from riparian sites in southeastern USA, on watersheds and nursery sites in Tennessee (Hulvey *et al.*, 2010) and in nursery irrigation reservoirs in Virginia (Hong *et al.*, 2010). Recently it was also recovered from soil associated with *Viburnum tinus* in Italy (Vitale *et al.*, 2014). *P. hydropathica* causes leaf necrosis and shoot blight on *Rhododendron catawbiense*, collar rot on *Kalmia latifolia* (Hong *et al.*, 2010), and has been associated with wilting and shoot dieback on *V. tinus* (Vitale *et al.*, 2014).

In spring 2011, a survey of common alder and waterways was conducted to determine the *Phytophthora* species associated with alder decline in riparian woodlands in Galicia (northwest Spain). Alder stands showed yellowish and small leaves, tarry spots on the stem and eventually mortality of trees. *P. alni* subsp. *alni* and subsp. *uniformis* were recovered from bark and root tissue (Pintos *et al.*, 2012). Another *Phytophthora* species was consistently recovered from the waters of the Arnoia and Avia rivers. Water samples were baited with carnation petals. Cultures were grown on V8 agar, carrot-agar and potato dextrose agar (PDA). The plates were incubated in the dark at 23°C. The colony pattern on PDA was petaloid. Growth occurred from 6 to 39°C, with an optimum at 30°C. Chlamydozoospores were terminal and 37.1 µm in diameter. Obovate hyphal swellings were present. Sexual structures were not observed. Nonpapillate, noncaducous spherical to ovoid sporangia, measuring 52.0 x 33.70 µm were produced in non-sterile soil extract solution. Nesting and internal proliferation occurred. The morphological characteristics observed match those described for *Phytophthora hydropathica* (Hong *et al.*, 2010). DNA from *Phytophthora* isolates was extracted. Two nuclear DNA regions, the ITS (DC6-ITS6/ITS4) and β-tubulin gene (TUBUF2/TUBUF1), were amplified. DNA sequences showed 99% to 100% homology with multiple *P. hydropathica* isolates in GenBank (Hong *et al.*, 2010; Hulvey *et al.*, 2010). DNA sequences were deposited in GenBank (Accession Nos. JX258850 and JX258851).

Pathogenicity was confirmed by inoculating one representative isolate of *P. hydropathica*, on five three-year-old *A. glutinosa* seedlings growing in pots. A colonised 5 mm mycelial plug, removed from the margin of an actively growing colony, was inserted in a shallow wound made at the collar level,

and sealed with plastic paraffin film. Ten detached *A. glutinosa* leaves (wounded and non-wounded) were also inoculated. Colonised agar plugs were placed on the leaves. Five control plants received only non-colonised agar plugs. Detached leaves and seedlings were maintained at 24°C and 95% humidity for six days and one month, respectively. Foliar lesions on wounded leaves, and stem necrosis on inoculated *A. glutinosa* seedlings progressing from the wound, were observed. *P. hydropathica* was reisolated from inoculated alder seedlings and wounded leaves but not from the controls.

To our knowledge, this is the first report of *Phytophthora hydropathica* in Spain and the first time that its pathogenicity has been evaluated on *A. glutinosa*.

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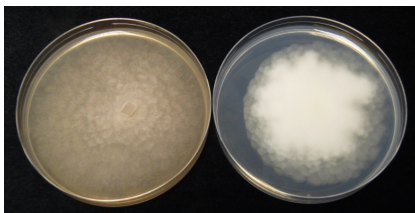


Figure 1

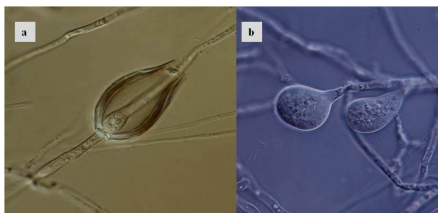


Figure 2



Figure 3

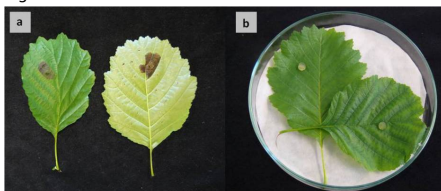


Figure 4

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