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Short communication. A new host and phenotypic variation of *Phytophthora hedraiandra* in Spain

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Abstract

The oomycete *Phytophthora hedraiandra* De Cock & Man in't Veld is first reported in Spain on the ornamental plant *Rhododendron catawbiense* Michx., as well as on leaves of *Viburnum tinus* L. in nurseries. The identification of these isolates was carried out by examining their morphological and cultural features, and by comparing the sequence of the nuclear rDNA ITS region and the mitochondrial *cox1* gene with those published in GenBank database. The phenotypes of the isolates fitted the species description, but a higher intraspecific variation was noticed in respect to their sporangial size, colony pattern and radial growth rates. Due to the similarities between *P. cactorum* (Lebert *et* Cohn) and *P. hedraiandra*, the taxonomy and host ranges of the *P. cactorum*/*P. hedraiandra* complex in ornamental nurseries and natural ecosystems need reviewing.

Additional key words: ornamental plant disease, phenotypic variation, plant pathogen.

Resumen

Nota corta. Nuevo hospedador y variación fenotípica de *Phytophthora hedraiandra* en España

Se cita por primera vez en España el oomiceto *Phytophthora hedraiandra* De Cock & Man in't Veld sobre la planta ornamental *Rhododendron catawbiense* Michx. Se dan además nuevas citas del patógeno sobre hojas de *Viburnum tinus* L. en viveros de Cataluña. Los diferentes aislados fueron identificados tras su examen morfológico y la comparación de las secuencias del ADNr de la región ITS, así como las del gen mitocondrial *cox1*, con datos publicados en las bases de datos del GenBank. Aunque los fenotipos de los diferentes aislados coinciden en términos generales con la descripción morfológica de *P. hedraiandra*, se observó cierta variación en relación a la dimensión de los esporangios, la tasa de crecimiento radial y el patrón morfológico de las colonias. Las similitudes con *P. cactorum* (Lebert *et* Cohn), y el hecho de que ambas ocupen nichos parecidos, indican que las relaciones filogenéticas entre ambas especies deberían ser reexaminadas.

Palabras clave adicionales: enfermedades de plantas ornamentales, patógeno vegetal, variación fenotípica.

Phytophthora hedraiandra De Cock & Man in't Veld is a new species first described from *Viburnum* sp. in the Netherlands (De Cock and Lévesque, 2004). It has since been reported in Italy (Belisario *et al*., 2006) and Mallorca (Spain) causing stem canker and root rot on potted *Viburnum tinus* L. (Moralejo *et al*., 2006), and blights on leaves and stems of various *Rhododendron* cultivars in the USA (Schwingle *et al*., 2006). In addition, it has recently been identified from a fungal culture collection in Australia (Cunnington *et al*., 2006) and isolated from *Rhododendron* in Slovenia (Munda *et al*., 2006). The occurrence of *P. hedraiandra* on *Rhododendron catawbiense* Michx. from a garden centre in Catalonia (northeast Spain) is here first reported, together with new findings on leaves of *V. tinus* in nurseries in this region.

In June 2005, a foliage lesion of a potted *R. catawbiense* was observed. The primary symptom was a round, pale-brown, water-soaked lesion on the margin of a young leaf. Small pieces of tissue from the lesion edge were plated onto P_5ARP medium (Erwin and Ribeiro, 1996) in a Petri dish and incubated at 20ºC for three days. Isolate P11935 was selected for morphological

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Table 1. Origin of the isolates of *P. hedraiandra* used in this study with their ITS and COX1 Genbank accession numbers

Isolate	Host (organ)	Location	Date	Gene region	GenBank	Amplicon[1]
P3642	*Viburnum tinus* (stem)	Mallorca	June 2002	ITS rDNA	EF050517	792 bp
P3842	*Viburnum tinus* (stem)	Mallorca	June 2002	ITS rDNA COX 1	AY881005 DQ220015	792 bp 850 bp
P3942	*Viburnum tinus* (stem)	Mallorca	June 2002	ITS rDNA COX 1	AY961608 DQ220016	792 bp 850 bp
P11935	*Rhododendron catawbiense* (leaf)	Girona	June 2005	ITS rDNA COX 1	DQ643972 DQ643973	792 bp 850 bp
P12345	*Viburnum tinus* (leaf)	Girona	Dec 2005	ITS rDNA COX 1	EF174429 EF174431	792 bp 849 bp
P12445	*Viburnum tinus* (leaf)	Girona	Dec 2005	ITS rDNA COX 1	EF174430 EF174432	792 bp 852 bp
P12545	*Viburnum tinus* (leaf)	Girona	Dec 2005	ITS rDNA COX 1	DQ648145 EF174433	792 bp 851 bp

[1] bp DNA linear sequence length of PCR products.

identification together with several other putative *P. hedraiandra* isolates collected from *V. tinus* in Girona, and compared with other previously identified isolates collected in Mallorca, Spain (Table 1). For this purpose, they were grown in 90 mm diam. Petri dishes on corn meal agar (CMA, Sigma), potato dextrose agar (PDA, Sigma), carrot agar (CA; Brasier, 1967) and malt extract agar (MEA; 48 g of malt extract plus 15 g of agar dissolved in 1,000 ml of distilled water) and their colony pattern and radial growth rates estimated after 4 and 7 days. The presence of sporangia and other asexual structures on all agar media was checked over two weeks. To induce the formation of sporangia, three 12 mm diam. disks were taken from the edge of a seven-day-old colony grown on CA and placed in a 60 mm diam Petri dish previously flooded with 10 ml of soil extract (filtered extract of 50 g of oak forest soil in 1,000 ml of distilled water, autoclaved at 121ºC for 15 min). The dishes were kept for 48-72 h at 20ºC under continuous white light. The shape, papilla formation, and caducity of sporangia were recorded. The presence of gametangia on CA was checked after 10 days growth at 20ºC in darkness.

Mycelial DNA was extracted from pure cultures grown in sterile pea broth, and checked for quality as previously described (Belbahri *et al*., 2006). Ribosomal DNA ITS amplifications were completed using the previously described universal primers ITS4 and ITS6 that target conserved regions in the 18S and 28S rDNA genes for amplification of the internal transcribed spacer region 1 (ITS1), the 5.8S rRNA gene and the internal transcribed spacer region 2 (ITS2) (Cooke *et al*., 2000). A partial sequence of the cytochrome c oxidase subunit I gene (COX1) was amplified with primers COXF4N and COXR4N developed by Kroon *et al*. (2004). PCR product purification and DNA sequencing were performed according to Belbahri *et al*. (2006). The sequences obtained were registered in GenBank (Table 1).

The culture characteristics of *P. hedraiandra* were clearly distinct from those of other homothallic *Phytophthora* species examined [e.g. *P. cactorum* (Lebert *et* Cohn) Shröter and *P. citricola* Sawada], but showed some intraspecific variation in their colony pattern and radial growth rates (Fig. 1). It is noteworthy that isolates collected from *V. tinus* leaves had lower radial growth rates, especially on MEA and PDA. Colony patterns

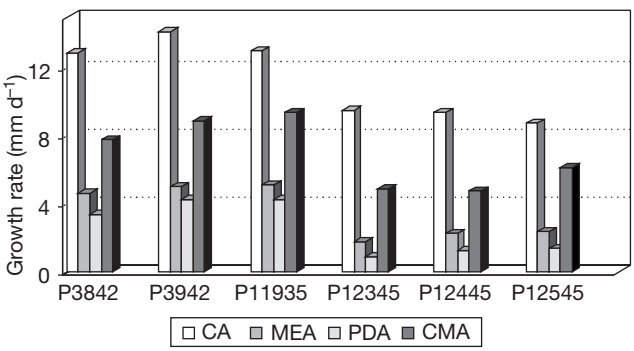

Figure 1. Average colony radial growth rates of six isolates of *Phytophthora hedraiandra* at 20°C in four agar media (CA, carrot agar; MEA, malt extract agar; PDA, potato dextrose agar, and CMA, cornmeal agar). Values averaged from measurement of two replicates.

ranged from radiate to stellate with submerged or appressed, sometimes tufted mycelium on CA; radiate with submerged and stolonate mycelium on CMA; and unpatterned (but slightly petaloid in P11935), velvet or floury, mostly with irregular margins both on MEA and PDA. Sporangia were papillate, mostly globose, ovoid or obpiriform, always with rounded bases, caducous and with a short pedicel. Oogonia were globose, smooth-walled, readily formed in fresh cultures but less so after long storage. Antheridia were mostly paragynous but sometimes amphigynous. The dimensions of the asexual and sexual structures of *P. hedraiandra* isolates are summarised in Table 2. Sporangia of P12345, P12445 and P12545 were particularly smaller than the holotype and other Spanish isolates of *P. hedraiandra*. In contrast, isolate P11935 formed large sporangia. The identity of the *P. hedraiandra* isolate P11935 from *R. catawbiense*, along with those of several new isolates recovered from leaves and stem lesions on *V. tinus* in December 2005 (Table 1), were confirmed by sequencing of the PCR amplified mitochondrial *cox1* gene (DQ643973) and the ITS region of the rDNA (DQ643972). Sequence comparisons between the ITS region and the *cox1* gene of Spanish isolates (this study) and of isolates available in GenBank did not show any polymorphism in these two DNA regions.

To assess the pathogenicity of *P. hedraiandra* on *R. catawbiense*, five leaves were placed on a metal grid in moist chambers consisting of a transparent plastic box lined on the bottom with sterile paper towels. The lower surface of four out of the five leaves was inoculated by placing a single 100 µl drop of a *ca*. 10^4 ml^{-1} zoospore suspension near the centre of the midrib. The remaining leaf was used as a control by replacing the inoculum with a drop of sterilized de-ionized water. The box was incubated at 20°C under cool white light. All leaves except the control formed large lesions and the pathogen could be re-isolated when leaf tissue was plated onto P_5ARP medium.

The emergence of *P. hedraiandra* diseases is similar to that of the early stages of the invasive *P. ramorum*. Likewise, it is spreading worldwide within nurseries through the international trade of *Viburnum* and *Rhododendron*; although *P. ramorum* was noteworthy due to the extensive damage it was causing to Californian oak trees. Maybe *P. hedraiandra* has remained unnoticed until now because of its morphological similarities with *P. cactorum*, which seems to have a wide host range (Erwin and Ribeiro, 1996). This latter fact adds further uncertainties on the actual chronology and pathology of *P. hedraiandra*, that is, whether it has been recently introduced or misidentified as *P. cactorum*. More doubts on its origin have risen after a single isolation of *P. hedraiandra* within an extensive survey of soils in natural ecosystems in Poland (Belbahri, unpublished), which suggests a recent introduction of the pathogen. In this study some evidence of phenotypic variation has been found in the small collection of isolates from Spain, which is a little surprising considering that *P. hedraiandra* is homothallic and thus a sexually inbreeding species. Further research is being carried out to determine its potential host range.

Table 2. Dimensions of sporangia and sexual structures of *P. hedraiandra* isolates grown on carrot agar (CA)

Isolate	Sporangial length (µm)	Sporangial breadth (µm)	Oogonium diameter (µm)	Antheridum length × breadth (µm)
P3842	34.7 ± 3.1	27.2 ± 1.9	31.4 ± 2.5	12.7 × 12.3
P3942	34.0 ± 2.3	27.7 ± 2.3	30.3 ± 1.5	11.8 × 12.2
P11935	51.4 ± 4.5	33.2 ± 3.0	28.7 ± 1.9	12.6 × 11.0
P12345	27.5 ± 2.9	21.5 ± 3.0	30.6 ± 2.6	12.1 × 11.7
P12445	30.7 ± 3.2	23.9 ± 2.5	29.9 ± 2.1	12.5 × 12.5
P12545	30.0 ± 4.4	25.4 ± 3.6	30.4 ± 1.5	12.1 × 12.4
CBS111725[1]	37.0 (30-53)	28.3 (23-34)	30.0 (28-36)	(9-14)

Mean values (in µm) of 20 structures measured ± standard error.
[1] Data of the holotype taken from de Cock and Lévesque (2004).
( ) range of measurements.

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