First report of *Hakea sericea* leaf infection caused by *Pestalotiopsis funerea* in Portugal

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The woody shrub *Hakea sericea* (Proteaceae) is native of south-eastern Australia and has been considered as an invader of natural habitats. In northern Portugal, dense stands of this plant are rapidly spreading usually after intense forest fires (Fig. 1a). In May 2003, unusual leaf spots were observed on these naturally growing plants. Infected plants exhibit reddish leaves bearing black circular lesions with 1-3 mm in diameter (Fig. 1b,c). Leaf sections containing necrotic lesions were plated onto PDA (potato dextrose agar) and eight fungi isolates were obtained. Pure cultures exhibit pinkish mycelium bearing compact acervuli containing black and slimy spore masses (Fig. 2a). Microscopic observation revealed typical *Pestalotiopsis* sp. 5-celled spores (3 coloured median and 2 hyaline end cells) with 3-4 apical and 1 basal appendages (Fig. 2b,c). The identification of *Pestalotiopsis* at species level is difficult and has been based on morphological characteristics of conidia (Guba, 1961). Recently, molecular approaches have been reported based on sequencing the internal transcribed spacer region (ITS) of the ribosomal RNA operon and comparision of the sequenced region to its orthologs of already identified *Pestalotiopsis* species (Jeewon *et al*., 2002).

In this work, genomic DNA from fungi isolates was purified using DNeasy® Plant Mini Kit (Qiagen), and used as template in thermocyclic amplifications using Ready-To-Go™ PCR Beads (Amersham Biosciences) with ITS5 and ITS4 universal primers (White *et al*., 1990). The amplified sequences (599bp) were then analysed together with other *Pestalotiopsis* ITS sequences already edited in databases, using the programs ClustalX (alignment of sequences), GeneDoc (manual correction of the alignment) and Phylip (phylogenetic tree construction). The results showed that ITS sequences from all fungi isolates were identical to each other and 99.3% similar to *Pestalotiopsis funerea* (Fig. 3). To confirm the pathogenecity of *Pestalotiopsis funerea* towards *Hakea sericea*, 6 weeks-old plants grown *in vitro* were infected with a suspension containing 10^5 spores/ml and maintained at 22°C, under 16 h light-photoperiod. After 6 days, *in vitro* plants exhibited tissue lesions identical to those observed in field plants, bearing fungus spores identical to those from original isolates.

Members of *Pestalotiopsis* genus have been described as pathogenic for plants from different families, including Proteaceae (Taylor *et al*., 2001). In other countries where *H. sericea* invasion has become a problem (*e.g.* South Africa), the biological

control of this invader has been successfully achieved using the pathogenic *Colletotrichum gloeosporioides* (Richardson and Manders, 1985). The identification of *P. funerea* in portuguese *H. sericea* stands could allow the design of a biological control method for this invader based on a naturally occurring pathogenic fungus.

References

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Legends of Figures

Figure 1 – *Hakea sericea* stand (a). Infected leaves (b) bearing black circular lesions (c).

Figure 2 – *Pestalotiopsis* isolate from *Hakea sericea* infected leaves. Mycelium grown on PDA, 1 and 4 weeks after inoculation (a), and corresponding conidia (b). Bar = 20 µm.

Figure 3 – Phylogenetic tree, designed using Phylip package (maximum likelihood method), representing the relationship between ITS sequences of *Pestalotiopsis* sp. isolated from *H. sericea* and other *Pestalotiopsis* species.