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MOLECULAR IDENTIFICATION OF A NEW PATHOGENIC FUNGUS AGAINST THE ALIEN *HAKEA SERICEA*

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INTRODUCTION

Hakea sericea Schrad., a species belonging to the Proteacea family, is expanding all over the Minho region and has recently been considered as one of the most invasive in our country (Fig.1). This alien woody species has experimented a successful expansion due mainly to the effects of forest fires that stimulates the opening of the woody follicles and the release of the large accumulated seed store (Fig.2). In fact, as the number of forest fires in our country increases, vast mountain areas have been occupied by dense Hakea shrubs stands thus replacing the native forest species of oaks, chestnuts and pines (Fig.3).

Traditional methods used to control *Hakea* proliferation have revealed themselves inadequate. In order to develop other strategies for *Hakea* control, a new pathogenic fungus was isolated from field plants.

Fig.1 - *Hakea sericea* Schrad. shrub.

Fig.2 - *Hakea sericea* follicle.

Fig.3 - Mountain area invaded by *Hakea sericea* (Serra d'Arga).

When scouting an area invaded by *H. sericea*, it was noticed that some specimens had a slightly different colour and presented some leaf spots (Fig. 4). These apparently infected specimens were recovered from the field and then observed with the help of a magnifying glass. It was clear that these spots were necrotic lesions possibly caused by some pathogenic fungus (Fig. 5).

Fig.4 - Apparently infected specimen recovered from the field.

Fig.5 - Necrotic lesion present in a leaf of *H. sericea* Schrad. amplified with a magnifying glass

With the purpose of isolating the pathogenic fungus responsible for these wounds, the injured leaves were placed in PDA (potato dextrose agar). After several medium transfers, it was possible obtain pure cultures of the fungus (Fig. 6.a.). After 20 days of mycelium growth, acervules started to appear on its surface (Fig. 6.b). The spores produced in these structures depicted the same morphologic characteristics of the fungi belonging to the genus *Pestalotipsis* (Fig. 7).

Fig. 6 - Isolated fungus grown in PDA, 5(a) and 20 days(b) after inoculation.

Fig. 7 - *Pestalotiopsis* spores colected from the isolates grown in PDA. Image amplified 400x in a light microscope.

After fungal genomic DNA extraction, a thermocyclic amplification of the ITS and 5.8 rRNA subunit regions was performed using universal primers for the conserved 28S and 18S rRNAs (Fig. 8). The ITS sequences (598bp) were then compared with orthologs of already identified *Pestalotiopsis* species. The results indicate that all the fungi isolates are very similar to *Pestalotiopsis funerea* (Fig. 9).

Fig.8 - Scheme of the amplified region used to determine the *Pestalotiopsis* species. The primers based on conserved sequences are represented by red arrows.

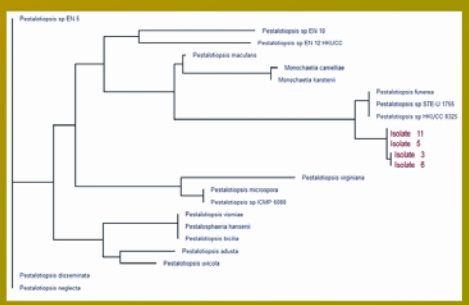

Fig.9 - Phylogenetic relationship between already characterized *Pestalotiopsis* species and the fungi isolated from the infected Hakea specimens found in Serra d'Arga (highlighetd in red).

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