New Disease Reports

Diplodia africana causing twig death on *Araucaria araucana*, a new host and first record for Chile

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Araucaria araucana (monkey puzzle tree) is an endemic conifer restricted to the temperate subantarctic forests of South America, with most of the world population concentrated in Chile, covering an area of 253,715 hectares. As a native forest species, *A. araucana* has been the object of ongoing phytosanitary surveys by the Servicio Agrícola y Ganadero (SAG), the National Plant Protection Organization of Chile. During surveillance activities in 2018, a botryosphaeriaceous fungus was isolated from a sample of partially dead branches from a tree in Conguillio National Park, located in the Andes, Araucanía Region (38°39'00" S, 71°37'6" W). Pycnidia and conidia produced *in vitro* on pine needles on water agar after 14 days were identified as *Diplodia* sp., with conidia hyaline after discharge from pycnidia, non-septate, measuring (26-)31-34.5(-36) × (7-)9.5-12(-13.5) µm (Fig. 1).

To identify the species of *Diplodia*, sequences of the rDNA ITS region, β-tubulin (BT), and translation-elongation factor 1α (TEF1) gene were obtained using the universal primers ITS1/ITS4, EF1-728/EF2 (Carbone & Kohn, 1999; O'Donnell *et al*., 1998) and TUB2Fd/TUB4Rd (Aveskamp *et al*., 2009). The sequences were submitted to GenBank with the Accession Nos. MN046380 (ITS), MN046381 (TEF1) and MN046382 (BT). BLAST analysis limited to type material showed 99.5-100% identities with *Diplodia africana* (MH863094, KF766397 and KF766129). A multi-locus phylogenetic analysis using maximum parsimony confirmed that the specimen clustered with the *D. africana* clade (bootstrap = 96%) (Fig. 2). An isolate (SAG-102744-18) was deposited in the Chilean Microbial Genetic Resources Collection, INIA Quilamapu, Chillán, Chile, with the accession number RGM 2718.

The pathogenicity of the fungi was tested by inoculating six three-year-old *A. araucana* seedlings (approximately 40 cm in height) in April 2019. The stems of the seedlings were wounded using a sterile scalpel 5 cm above the collar root and inoculated with a mycelial plug (5 mm diameter) from a seven-day-old culture grown on 2% malt extract agar. Two seedlings used as control were inoculated with sterile agar plugs. The plugs were sealed with paraffin film. Seedlings were kept outdoors with average minimum and maximum temperatures of 5.4 and 21.5°C, and watered as needed. After four weeks, brown phloem lesions ranging from 7-30 mm in length were observed on the inoculated seedlings and the fungus was consistently re-isolated from all lesions. Additionally, all inoculated seedlings showed foliar necrosis affecting between a few leaves to 50% of the foliage. Control seedlings remained healthy (Fig. 3-4).

The genus *Diplodia* includes species that are pathogens, endophytes and saprobes of mostly woody hosts. Some of the pathogenic species include *D. africana,* which was initially found on stone fruit of *Prunus* spp. in South Africa (Damm *et al.*, 2007) and later reported causing dieback on *Phoenicean juniper* in Italy (Linaldeddu *et al.*, 2011). To our knowledge, the current identification of *D. africana* on *Araucaria araucana* in Chile constitutes a new host-distribution of the fungus.

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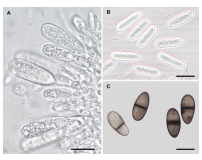

Figure 1

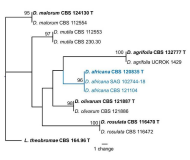

Figure 2

Figure 3

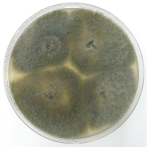

Figure 4

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