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First report of *Alternaria alternata* causing black spot on pink lapacho (*Handroanthus impetiginosus*)

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Abstract A severe leaf spot disease was observed on pink lapacho trees, *Handroanthus impetiginosus* for the first time in Buenos Aires province, Argentina during the autumn of 2013. The pathogen was identified as *Alternaria alternata* based on the morphological characteristics and sequence data from the internal transcribed spacer region of ribosomal DNA, and partial β-tubulin sequence. A pathogenicity test was performed and Koch's postulates were confirmed by re-isolating the fungus from artificially inoculated leaves. This is the first report of *Alternaria* black spot of *Handroanthus impetiginosus* trees.

Keywords Lapacho · *Alternaria alternata* · ITS bar code · Leaf spot

Handroanthus impetiginosus (common name pink lapacho, pau d'arco or taheebo), syn. *Tabebuia impetiginosa*, family Bignoniaceae, is a native tree of America. It grows from northern Mexico to northern Argentina and is a member of tropical rain forests throughout Central and South America. In South America and Europe, this blooming tree is used as an ornamental plant in landscaped gardens and public areas. It produces high-quality wood that is used to build houses, boats and/or farm tools. Traditionally the inner bark of *H. impetiginosus* has ethnopharmacological applications like the treatment of skin inflammatory diseases such as eczema, psoriasis, bacterial infections and even human cancers (Woo and Choi 2005; Kim et al. 2007; Byeon et al. 2008). During autumn of 2013, leaves of lapachos growing in the urban area of La Plata (Middle eastern, Argentina, Buenos Aires province) presented many spots that looked like disease symptoms (Fig. 1). Both leaf surfaces presented large necrotic lesions, of a brown-to-black colour that increased their size, became irregular and eventually coalesced resulting in withering, extensive drying and shedding of leaves.

Pieces of Infected leaf tissue were surface sterilized with 1 % NaOCl solution for 1 min and plated on potato dextrose agar (PDA) that were incubated at 25 °C for 7 days under a 12 h light photoperiod. Within 7 days of incubation, white fungal colonies developed which initially turned to green olivaceous colour and later turned to black as the culture aged. Conidiophores were short, septate, branched or unbranched, and had a golden brown appearance, measuring 15 μm long and 2–6 μm thick. Conidia were obclavate, obpyriform or ellipsoidal with a short conical beak, and borne in long chains, pale brown to golden brown in colour, measuring 19–39 μm long and 6–15 μm wide at the broadest point, showing 2–5 transverse septa. Based on the described morphological characters, the pathogen was tentatively identified as *Alternaria alternata* (Fr.) Keissler (Simmons 2007). This identification was confirmed by molecular data from partial β-tubulin and Internal Transcribed Spacer (ITS) (International Bar Code, Schoch et al. 2012). Genomic DNA of pure cultures was isolated by means of the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI) according to manufacturer's instruction. The ITS was amplified using ITS-4 and ITS-5 primers (White et al. 1990) and the partial β-tubulin sequence using Bt1a and Bt1b primers (Glass and Donaldson 1995). Both reactions were performed in a 15 μL final volume containing 50 ng of template DNA, 50 ng of each forward and reverse primer, 1.5 μL 10×

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Fig. 1 Alternaria black spots of pink lapacho leaves caused by *A. alternata*

Fig. 2 Detached leaf inoculation test with pathogenic (*black spot*) *A. alternata* isolates

reaction buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs and 0.5 unit Taq polymerase (all Inbio Highway®, Buenos Aires, Argentina). Amplifications were done in a PTC-0150 MiniCycler (MJ Research. Watertown, MA, USA) programmed as follows: an initial denaturation step of 4 min at 94 °C, followed by 33 cycles of 1 min at 94 °C, 45 s at 56 °C for ITS and at 58 °C for β-tubulin, 1 min at 72 °C, with a final elongation of 7 min. The amplicons obtained were purified and sequenced as described by Sanger et al. (1977) using the same set of primers. The 570 bp ITS sequence (GenBank accession N° KJ829533) and the 422 bp β-tubulin sequence (GenBank accession N° KM396706) were 100 % homologous to those of *Alternaria alternata* epitype CBS916.96 (GenBank accession N° FJ196306) and KS53-2 (GenBank accession N° KJ008699), the etiological agent of leaf spot of jujube in Xinjiang, China. Pathogenicity of the isolate was determined as described by Belisario et al. (1999). Three cultures of siblings isolated from leaves with similar symptoms were grown on PDA for 2 weeks. Leaflets were then inoculated with 5 μl of a 1×10^5 spore suspension obtained from the culture. A total of 25 leaflets were inoculated. The leaves were then placed in petri dishes with a wet, sterilized piece of cotton, and incubated at 25 °C in the dark (90 % relative humidity and a 12-h photoperiod). After 7 days, spots similar to symptoms of the disease developed (Fig. 2) and *A. alternata* was consistently re-isolated from the diseased tissues. Control leaves mock inoculated with sterile distilled water remained asymptomatic. The pathogenicity test was repeated three times and the results were similar. A culture of the isolate used in pathogenicity and taxonomic studies was deposited in the Culture Collection of the Centro de Investigaciones de Fitopatología (CIDEFI), Facultad de Ciencias Agrarias y Forestales, University of La Plata as CIDEFI AA124. *Alternaria alternata* has been reported on *Handroanthus* from Brazil on *H. serratifolia* (as *Tabebuia serratifolia*) (Farr and Rossman 2014), and seedlings of *Handroanthus* sp. (as *Tabebuia* sp.) in Mexico (Tovar et al. 2008). To the best of our knowledge this is the first report of *A. alternata* causing leaf spot on *H. impetiginosus* trees.

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