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Mycosphaerella and *Teratosphaeria* leaf spot diseases of *Eucalyptus globulus* in Ecuador

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Abstract *Mycosphaerella* and *Teratosphaeria* leaf spot diseases were detected for the first time in Ecuador. Fungi were isolated from leaf spots on *Eucalyptus globulus* plants growing in the Andean Highlands at altitudes higher than 2610 m. *Teratosphaeria molleriana* and *Mycosphaerella lateralis* were identified based on morphological characteristics and sequence analysis. Most leaf spots were associated with diverse isolates from the *Cladosporium cladosporoides* complex.

Keywords *Eucalyptus globulus* · Leaf spot · *Mycosphaerella* · *Teratosphaeria* · Ecuador

Eucalyptus trees are native to Australia, but due to their fast growth, they are now used worldwide for timber and pulp production. In Latin America, increasing numbers of plantations of *Eucalyptus* have appeared, thus the introduction of *Eucalyptus* fungal pathogens previously described in other continents has also increased. In South-America, there were reports about *Mycosphaerella* and *Teratosphaeria* leaf diseases (MLD, TLD) in Brazil (Teodoro et al. 2012) and in Uruguay (Pérez et al. 2009, 2013). These studies report severe defoliation to *Eucalyptus* plants and also indicate that introduction of plant pathogens may threaten native *Myrtaceae*. Considering these problems, the increasing severity of MLD/TLD diseases on *Eucalyptus* plantations and the reported evidence of fungal host jumps, we investigated the presence of MLD/TLD on *Eucalyptus globulus*, the predominant *Eucalyptus* species in Ecuador.

Commercial plantations of *E. globulus* have rapidly expanded during the last two decades in the province of Pichincha, which is located in the Andes. Its Mediterranean-type climate is well suited for rapid growth of *E. globulus*. Although there have not been any previous records about the impact of MLD/TLD, a rapid increase of leaf spots has been reported the last years.

During the spring and summer of 2015, *E. globulus* plantations were surveyed for the presence of leaf spots and the impact of the disease on tree growth was estimated. An evaluation of the severity and the economical impact of the disease were beyond the scope of this study. Diminished tree growth, due to a reduction in green leaf area, was reported in more severely affected plantations. Leaf spots were observed in all surveyed areas, though significant differences in the abundance of leaf spots were observed between plantations. In a few plantations, the affected leaf area covered up to 50 % of the surface. Leaf spots were observed on both juvenile and adult leaves, while lesions were more frequent on senescing leaves, which indicate the increased presence of saprophytic species or secondary invaders of diseased tissue. Most lesions were yellow to brown in colour, with irregular shape and the lesion borders had reddish margins.

In order to identify which primary pathogens cause symptoms of MLD/TLD, samples were collected during surveys of plantations in Quito and near to Quito, in the province of Pichincha. Leaf tissue samples were collected at an altitude between 2610 m and 2810 m between March and June 2015. Isolations from lesions with perithecia followed the procedure

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Fig. 1 *Mycosphaerella lateralis* (KU198344). Macroconidia. Scale bar =10 μm

described by Crous (1998). Ascospores released from perithecia were collected on malt extract agar (MEA). Petri dishes were then incubated in the dark at approximately 25 °C during the daytime and 15 °C during the nighttime. After 48 h, plates were examined for the presence of ascospores that had been ejected onto the surface of the medium. Germinating ascospores were then transferred to fresh MEA plates to generate monosporic cultures.

Several *Mycosphaerella lateralis* isolates derived from yellow spots of juvenile leaves of approximately 2 mm diameter were observed. The colonies appeared as grayish aerial mycelia with irregular shape on MEA. *Teratosphaeria molleriana* isolates appeared from small dark brown spots on juvenile leaves. The colonies were dark brown with irregular shapes on MEA. To observe conidia and conidial structures, slide culture technique (Riddle 1950) was used and cultures were grown for 2 weeks in a PDA medium before microscopic examination (Fig. 1). Conidia and conidial growth was observed as described for *Pseudocercospor*a and *Teratosphaeria* species (Crous et al. 2007). Both *T. molleriana* and *M. lateralis* are known to infect *E. globulus* leaves (Jackson et al. 2004; Maxwell et al. 2003). Diverse isolates from the *Cladosporium cladosporoides* (Genbank Accession: KU198345, KU198346, KU198347, KU198348) complex were simultaneously isolated with the mentioned MLD/TLD strains. Isolates of the fungal cultures were deposited in the culture collection of the Phytopathology Unit at Agrocalidad, Ecuador (Table 1).

For molecular characterization of the fungal isolates, DNA was extracted using a standard cetyltrimethyl ammonium bromide (CTAB) procedure, as described previously (Doyle and Doyle 1987). Polymerase chain reaction (PCR) analysis was performed by sequencing of the ITS region according to the method of White et al. (1990) with primers ITS1 (5′-TCC GTA GGT GAA CCT GCG-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT-3′). The PCR product was separated by electrophoresis on 1 % agarose gel in a 1× TBE (Tris-Borate-EDTA) buffer, stained with SYBR Safe (Invitrogen) and visualized with a transilluminator. Subsequently, the PCR products were sequenced in both directions with the same primers used for the PCR amplifications by a commercial company (Macrogen Inc., Korea). All sequences obtained in this study were deposited in GenBank and accession numbers are shown in Table 1.

Identification of *M. lateralis* and *T. molleriana* was done by BLASTn analysis of the 370-bp sequence (KU198343) and 571 bp sequence (KU198344), which resulted in >99 % homology with the *T. molleriana* strain CBS 122905 (KF901626) from Chile and several other *T. molleriana* spp. and in 100 % with the *M. lateralis* strain CPC:13,264 (GQ852741) from Australia and other *M. lateralis* spp., respectively. This is the first time that *Teratosphaeria molleriana* and *Mycosphaerella lateralis* have been found in Ecuador.

Table 1 Fungal isolates and fungal references used in that study

Fungus	Culture no.	Host	Location	Genbank accession no. (ITS)
Teratosphaeria molleriana	ANB15MIC02	*Eucalyptus globulus*	Pichincha, Ecuador	KU198344
Mycosphaerella lateralis	ANB15QP002	*E. globulus*	Pichincha, Ecuador	KU198343
Cladosporium cladosporoides	ANB15RYB02	*E. globulus*	Pichincha, Ecuador	KU198345
C. cladosporoides	ANB15RYB50	*E. globulus*	Pichincha, Ecuador	KU198346
C. cladosporoides	ANB15RYB30	*E. globulus*	Pichincha, Ecuador	KU198347
C. cladosporoides	ANB15RYB04	*E. globulus*	Pichincha, Ecuador	KU198348
T. molleriana	CBS122905	*E. globulus*	Chile	KF901626
M. lateralis	CPC:13264	*E. globulus*	Australia	GQ852741

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