

DISEASE NOTES



First Report of *Calonectria* Leaf Blight Caused by *Calonectria metrosideri* on *Eucalyptus benthamii* in Brazil

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Eucalyptus species are among the main hosts of *Calonectria* fungus (anamorph *Cylindrocladium*), causing blight of leaf and branch tissues. In December 2015, an outbreak of leaf blight was observed in a progeny test of *Eucalyptus benthamii* Maiden & Cabbage located in São Mateus do Sul (Paraná state) and in Mafra and Porto União (Santa Catarina state), Southern Brazil. In the two subsequent years, symptoms that started with dark brown leaf blight of variable dimensions in the lower part of crown reached all trees and resulted in more than 50% crown defoliation in 2-year-old trees. Diseased leaves were collected, and small pieces of discolored or necrotic tissues were rinsed with 70% alcohol for 10 s and 1% NaOCl for 60 s and then washed with sterile distilled water. Afterward, these pieces were plated in Petri dishes containing potato dextrose agar (PDA) and incubated for 7 days at 24°C in the dark. Colonies had white mycelium and brown growth, similar to *Calonectria*. Cultures in PDA medium were submitted to physical stress to stimulate the

production of fruiting structures. Conidiophores consisted of fertile branches in a penicillate arrangement; hyaline, smooth, septate, 146.5 to 208×3 to $4 \mu\text{m}$; with a stipe extension septate, erect to flexuous, 123.5 to $179 \mu\text{m}$ in long, 2 to $3.5 \mu\text{m}$ wide at the apical septum, ending in a spathulate to obpyriform vesicle 5 to $7.5 \mu\text{m}$. The conidiogenous apparatus was 30.5 to $78 \mu\text{m}$ wide by 29.5 to $80 \mu\text{m}$ in long, with two (to three) branches fertile; the first was 14 to 24.5×4 to $5.5 \mu\text{m}$; secondary branch 10.5 to 16×3 to $4.5 \mu\text{m}$; additional branch (three) 8.5 to 10.5×3 to $4 \mu\text{m}$, each producing two to four phialides, hyaline, aseptate, 9.5 to 12×3 to $4 \mu\text{m}$, doliform to reniform. Conidia hyaline, erect, one-septate, $(42$ to) 45 to 49 (to $50.5) \times 3.5$ to $4 \mu\text{m}$ (average $47 \times 4 \mu\text{m}$), both rounded tips; teleomorph not observed. β -Tubulin (*tub2*) and calmodulin (*cmdA*) genes were PCR amplified and sequenced using the primers T1 (O'Donnell and Cigelnik 1997)/CYLTUB1R (Crous et al. 2004) for *tub2* and CAL-228F/CAL-737R (Carbone and Kohn 1999) for *cmdA* of isolates CIS 41 (GenBank MK279422-TUB, MK279410-CAL), CIS 49 (MK279423, MK279411), CIS 24 (MK279419, MK279407), CIS 21 (MK279418, MK279406), and CIS 30 (MK279420, MK279408). These isolates were deposited in the Collection of Forest Fungi and Oomycetes, Embrapa Florestas, Colombo, Paraná State, Brazil. A phylogenetic analysis was made via Bayesian inference. Based on morphological features and phylogenetic analysis, these isolates belong to *Calonectria metrosideri* R.F. Alfenas, O.L. Pereira, P.W Crous & A.C. Alfenas (Alfenas et al. 2013). Pathogenicity was evaluated by spraying a spore suspension (10^4 conidia/ml) prepared with a 1-week-old culture of isolate CIS 30 onto 10 branches of 8-month-old seedlings of *E. benthamii*, in a greenhouse (25°C average temperature). Control consisted of 10 branches sprayed with sterile distilled water. All sprayed branches were wrapped with polyethylene bags for 48 h to create a moist chamber. Ten days after inoculation, spore-sprayed branches showed brown necrotic lesions on leaves, whereas water-sprayed branches remained symptom-free. *C. metrosideri* was reisolated from diseased leaves, fulfilling Koch's postulates. This is the first record of *C. metrosideri* on eucalypts in the world.

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