



First report of bacterial wilt caused by *Ralstonia solanacearum* on *Eucalyptus* sp. in Paraguay

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Planting eucalyptus forests has become an important agricultural activity in Paraguay. Large areas are cultivated for charcoal, cellulose and home construction material. Currently, eucalyptus forests represent 93% of planted forests in the country (MAG, 2009). Bacterial wilt, caused by *Ralstonia solanacearum*, is a major disease of eucalyptus and was first reported on this host in Brazil in 1983 (Sudo *et al.*, 1983). Crop losses that average 30 to 40% are frequently reported after initial outbreak reports. In March 2011, eucalyptus trees with typical symptoms of bacterial wilt were observed in Caaguazú and San Pedro states, located in the Central and Northern regions of Paraguay, respectively. Plants were wilted and foliar necrosis or defoliation of the lower portion of the canopy was common. Transverse sections of the trunk revealed browning of the xylem vessels forming dark rings (Fig. 1). On Kelman's medium (Kelman, 1954), colonies of white colour, elevated and of fluidal aspect resulted from the isolation of the pathogen from diseased tissues (Fig. 2).

The isolated bacterium was Gram-negative, grew aerobically and did not form endospores. Cells utilised trehalose and dextrose. Based on PCR amplification using the universal bacterial 16S rDNA primer set E8F/E1541R (Baker *et al.*, 2003) a single amplicon was obtained and the PCR product was sequenced (GenBank Accession No. KF006342) and exhibited 99% identity over 1096 bp with other sequences of *R. solanacearum* in GenBank. Based on all tests, isolates were identified as *R. solanacearum*. Additional characterisation with the 759/760 primer set (Opina *et al.*, 1997) amplified a 280 bp fragment typical of *R. solanacearum* and when the multiplex PCR analysis was carried out (Fegan & Prior, 2005) the bacterium was identified as *R. solanacearum* Phylotype II.

Koch's postulates were conducted under greenhouse conditions (~25°C), by inoculating five plants each of *Eucalyptus grandis*, *Solanum lycopersicum*, *S. tuberosum* and *Musa* spp. Plants were inoculated with 20 µl of a cell suspension containing 10⁷ to 10⁸ cfu/ml deposited into the leaf axilla with a syringe. Wilt occurred in eucalyptus, tomato and potato plants after three days, but no symptoms developed in banana plants. Bacterial colonies with

similar characteristic of *R. solanacearum* were re-isolated from symptomatic plants. To our knowledge, this is the first report of eucalyptus bacterial wilt in Paraguay and serves as a warning to other properties in the country and in South America.

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Figure 1



Figure 2

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