Diseases Caused by Bacteria and Phytoplasmas

First Report of *Ralstonia solanacearum* Causing Bacterial Wilt of *Eucalyptus* in Ecuador

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In January 2018, eucalyptus trees showing wilt symptoms were identified in a commercial plantation located in the Quevedo Cantón, Los Ríos province, Ecuador. Disease incidence reached 40% of the eucalyptus field, and affected plants displayed reddening and wilting of the foliage, leaf drop, branch dieback, and reduced growth, resembling bacterial wilting symptoms (Santiago et al. 2014). Transverse sections made on trunks of wilted trees revealed vascular discoloration of the wood and bacterial ooze. Wood pieces taken from discolored tissues were surface disinfected and macerated in sterilized distilled water. The resulting suspensions were spread on plates containing triphenyltetrazolium chloride medium (Kelman 1954) and incubated at 28°C for 48 h. Three mucoid white colonies with pinkish centers, characteristic of *Ralstonia solanacearum*, were purified. The isolates were gram negative in the KOH test, nonfluorescent on King's B medium, and formed cream colonies on yeast extract–dextrose–calcium

carbonate medium. Phylotype-specific multiplex PCR (Fegan and Prior 2005) and phylogenetic analysis based on partial endoglucanase (*egl*) gene sequences (Poussier et al. 2000) identified the isolates as *R. solanacearum* phylotype IIB sequevar 51. The isolates were preserved in the COAD culture collection of the Universidade Federal de Viçosa, Brazil (codes COAD 2586, 2587, and 2588), and the corresponding *egl* DNA sequences were deposited in GenBank (accession nos. MH350031, MH350032, and MH350033). Pathogenicity tests were conducted with all three isolates. Twenty microliters of bacterial suspensions (10^8 CFU/ml of saline solution) were injected at the base of the stem of eucalyptus (*Eucalyptus urograndis*), banana (*Musa* sp. cv. Maçã), and tomato (*Solanum lycopersicum* cv. Santa Clara) plants kept in a greenhouse ($28 \pm 2^\circ\text{C}$) (Fonseca et al. 2016). Three plants of each host species were inoculated per bacterial isolate, and plants injected with distilled water were used as controls. Necrotic symptoms appeared at the inoculation site on eucalyptus plants after 10 days. Wilt symptoms started at the top and progressed toward the base of the plants. Tomato plants died within 1 week, and eucalyptus seedlings died after 3 weeks. The inoculated banana seedlings and all plants from the control treatments remained asymptomatic. Bacterial ooze was observed on freshly cut transverse sections made on wilted eucalyptus seedlings, and typical colonies of *R. solanacearum* were isolated from inoculated plants, fulfilling Koch's postulates. The correct diagnosis of the pathogen is the first step in the long-term process of developing effective management tools for this disease in Ecuador.

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