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Disease Notes

First Report of *Lasiodiplodia theobromae* Associated with Decline of Grapevine Rootstock Mother Plants in Spain

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A field of Richter 110 rootstock mother plants in Valencia Province (eastern Spain) was surveyed during November 2006 to study the mycoflora of declining plants. Two canes with stunted leaves were collected from a plant with a reduced number of shoots. No cankers or vascular lesions were observed in the collected canes. Six wood chips (1 to 2 mm thick) were taken from one basal fragment (3 to 4 cm long) of each cane, surface sterilized in 70% ethanol for 1 min, and plated on malt extract agar supplemented with 0.5 g L⁻¹ of streptomycin sulfate. Petri dishes were incubated for 7 days at 25°C. A fungus was consistently isolated from all samples that showed the following characteristics: colonies grown on potato dextrose agar (PDA) at 25°C developed a white, aerial mycelium that turned gray after 4 to 6 days and produced pycnidia after 1 month on sterile grapevine slivers of twigs placed on the PDA surface; conidia from culture were ellipsoidal, thick walled, initially hyaline, nonseptate, and measuring 20 to 25 (22.5) × 12 to 14 (13) µm; aged conidia were brown, 1-septate with longitudinal striations in the wall; and pseudoparaphyses variable in form and length were interspersed within the fertile tissue. The fungus was identified as *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl. from the above characteristics (2). Identity was confirmed by analysis of the nucleotide sequences of the internal transcribed spacer (ITS) region from the rRNA repeat and part of the translation elongation factor 1- α (EF1- α) and the β -tubulin (B-tub) genes, as done elsewhere (1,3). BLAST searches at GenBank showed a high identity with reference sequences (ITS: 100%, EF1- α : 97%; B-tub: 99%). Representative sequences of the studied DNA regions were deposited at GenBank (Accession Nos.: ITS: EU254718; EF1- α : EU254719; and B-tub: EU254720). A pathogenicity test was conducted on 1-year-old grapevine plants cv. Macabeo grafted onto Richter 110 rootstocks maintained in a greenhouse. A superficial wound was made on the bark of 10 plants with a sterilized scalpel, \approx 10 cm above the graft union. A mycelial plug obtained from the margin of an actively growing fungal colony (isolate JL664) was placed in the wound and the wound was wrapped with Parafilm. Ten additional control plants were inoculated with sterile PDA plugs. All control plants grew normally, and the inoculation wound healed 3 months after inoculation. Plants inoculated with *L. theobromae* showed no foliar symptoms in the same period, but developed cankers variable in size surrounding the inoculation sites. Vascular necroses measuring 8.4 ± 1.5 cm (mean \pm standard error) developed in the inoculated plants that were significantly longer than the controls (0.3 ± 0.2 cm). The pathogen was reisolated from all inoculated plants and no fungus was reisolated from the controls. These results

confirmed the pathogenicity of *L. theobromae* to grapevine and points to a possible involvement of *L. theobromae* in the aetiology of grapevine decline as previously reported (3,4). To our knowledge, this is the first report of *L. theobromae* isolated from grapevine in Spain.

References: (1) J. Luque et al. *Mycologia* 97:1111, 2005. (2) E. Punithalingam. No. 519 in: *Descriptions of Pathogenic Fungi and Bacteria*. CMI, Kew, Surrey, UK, 1976. (3) J. R. Úrbez-Torres et al. *Plant Dis.* 90:1490, 2006. (4) J. M. van Niekerk et al. *Phytopathol. Mediterr.* 45(suppl.):S43, 2006.

Cited by

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