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Disease Notes

First Report of *Ilyonectria macrodidyma* Associated with Black Foot Disease of Grapevine in Brazil

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Cultivated grapevine (*Vitis labrusca* and *V. vinifera*) is of considerable economic importance to the Brazilian fruit industry for both fresh market consumption and for the production of wines, sparkling beverages, and juices. Black foot disease is caused by fungi of the genera *Ilyonectria* P. Chaverri & C. Salgado (anamorph: *Cylindrocarpon* Wollew.), *Campylocarpon* Halleen, Schroers & Crous, and *Cylindrocladiella* Boesew. In 2012, 4- to 40-year-old grapevines (*Vitis* spp.) showing reduced vigor, vascular lesions, necrotic root lesions, delayed budding, vine decline, and death were collected from seven locations at Rio Grande do Sul state, Brazil. Fungal isolations were made from root fragments and crown lesions (at least 2 cm above the bottom) on potato dextrose agar (PDA) medium added with 0.5 g L^{-1} streptomycin sulfate. Eight isolates were obtained and identified on the basis of morphological features and multi-gene analysis (rDNA-ITS, β-tubulin, and histone H3) as *Ilyonectria macrodidyma* (Halleen, Schroers & Crous) P. Chaverri & C. Salgado. One representative isolate (Cy5UFSM) was used for more detailed morphological and molecular characterization, and pathogenicity confirmation. When incubated in the dark at 20°C for 7 to 10 days, colonies of felty straw-colored mycelium (3) 4.79 cm diameter on average were observed. No sporodochia or other fruiting bodies were produced on carnation leaf agar (CLA) medium after 30 days. Microconidia that were produced after 5 weeks on spezieller nährstoffarmer agar (SNA) medium with addition of two pieces of 1 cm^2 filter paper showed ovoid and ellipsoid shape (6.4 × 3.6 µm) and one-septate macroconidia (17.3 × 4.1 µm). To confirm the species, primer pairs ITS1 and ITS4 (4); Bt2a and Bt2b; and H3-1a and H3-1b (2) were used to amplify the ITS1-5.8S rRNA-ITS2, part of the β-tubulin and histone H3 genes, respectively. Sequences of these three regions showed 99, 100, and 100% of homology with *I. macrodidyma*, respectively. To confirm pathogenicity, 4-month-old rooted cuttings of *V. labrusca* cv. Bordô were inoculated by immersing them in a conidial suspension of the isolate (10^6 conidia ml^{-1}) for 60 min (1). Thirty days later, inoculation was performed again by drenching the crown with 40 ml of 10^6 conidia ml^{-1}

suspension to ensure infection of the roots. In the control treatment, plants were inoculated with sterile distilled water. Plants inoculated with *I. macrodidyma* showed necrosis of the leaf ribs, reduction in root mass, root and crown necrosis, browning of vessels, drying of shoots, and death. *I. macrodidyma* was re-isolated from the crown necrosis and vascular lesions, confirming Koch's postulates. To our knowledge, this is the first report of *I. macrodidyma* associated with black foot disease of grapevine in Brazil, which poses considerable threat to the industry unless management options are realized.

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