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Disease Notes

First Report of "*Cylindrocarpon*" *pauciseptatum* Associated with Black Foot Disease of Grapevine in Brazil

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e-Xtra

Since 1999, the decline of American grapevines (*Vitis labrusca* L.) has been common in Rio Grande do Sul, Brazil (1). In August 2012, *V. labrusca* with black foot symptoms were collected in vineyards in the Serra Gaúcha Region. Symptomatic plants had low vigor, vascular lesions, delayed budding, and decline and death of vines. Symptomatic roots had necrotic lesions and reduced biomass. Fungal isolations were made from necrotic root and crown fragments (own-rooted cultivar) on potato dextrose agar (PDA) medium amended with 0.5 g L^{-1} streptomycin sulfate. Putative colonies of "*Cylindrocarpon*" *pauciseptatum* Schroers & Crous were obtained from single macroconidia isolations. Two isolates were used to confirm the identity of isolated colonies: Cy12UFSM and Cy13UFSM. After incubation in the dark for 10 days at 20°C, the isolated mycelial colonies, which were cottony white to felty in texture, became dark orange to brown. Both isolates produced chlamydospores in chains at 40 days. Chlamydospores of Cy12UFSM and Cy13UFSM were 9 to 12 µm and 5 to 11.5 µm in diameter. Sporodochia formation on carnation leaf agar (CLA) medium was observed after 30 days. To encourage development of conidia, the isolates were grown on spezieller nährstoffarmer agar (SNA) medium for five weeks at 20°C with addition of two pieces of 1 cm^2 filter paper. Microconidia of Cy12UFSM were 4 to 8.5 × 3.5 to 5 µm and those of Cy13UFSM were 3.5 to 7.5 × 3 to 5 µm. Macroconida were predominantly 3-septate (Cy12UFSM was 36 to 45 × 7.5 to 9 µm and Cy13UFSM was 30 to 38 × 7.5 to 8 µm), but 1-, 2- septate macroconidia were observed. The sizes of the three spore types and colony morphology for our isolates were similar to those described by Schroers et al. (3) for "*C.*" *pauciseptatum*. To further confirm the identity of Cy12UFSM and Cy13UFSM, multi-gene DNA sequence analysis (rDNA-ITS, β-tubulin, and histone H3) was conducted using primer pairs ITS1 and ITS4 (4), Bt2a and Bt2b, and H3-1a and H3-1b (2), which amplify the ITS1-5.8S rRNA-ITS2 genes, part of the β-tubulin gene, and the histone H3 gene, respectively. Sequences of these three regions had 99, 99, and 97% similarity with references sequences of "*C.*" *pauciseptatum* (isolate Cy238; accessions ITS [JF735307]; β-tubulin [JF735435], and histone H3

[JF735582], respectively). To evaluate pathogenicity, 4-month-old rooted cuttings of *V. labrusca* cv. Bordô were inoculated with two isolates by immersing them in a conidial suspension (10^6 conidia ml^{-1}) for 60 min. Ten single-vine replicates were used for each isolate, and 10 water-inoculated vines were included as controls. Thirty days after inoculation, vines were re-inoculated with 40 ml of a 10^6 conidia ml^{-1} suspension to ensure root infection. After 4 months, the inoculated plants had reduced root mass relative to controls (39.18% for Cy12UFSM and 18.27% for Cy13UFSM). Inoculated plants also had root and crown necrosis, vascular lesions, shoot decline, and vine mortality (60 and 80% mortality for Cy12UFSM and Cy13UFSM, respectively). All water-inoculated control plants remained symptomless. The fungi Cy12UFSM and Cy13UFSM were re-isolated from infected woody tissues, confirming Koch's postulates. To our knowledge, this is the first report of "*C.*" *pauciseptatum* associated with black foot disease of grapevine in Brazil, which may potentially impact the sustainability of grapevine nurseries and vineyard productivity.

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