

First report of *Dactylonectria alcacerensis*, *Dactylonectria macrodidyma* and *Ilyonectria liriodendri* associated with black-foot disease of grapevine in Argentina.

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Black-foot is a fungal disease that affects young vines and planting material in the countries where grapevines are cultivated. During the 2018 grape-growing season, symptoms of reduced vigor, short internodes, leaf chlorosis, root rot and necrosis in the base of plants were observed in vines between 2 to 10 years old from 400 ha of vineyards of Mendoza and Salta regions. A total of 30% of the vineyards of cultivars Malbec, Cabernet Sauvignon and Aspirant Bouchet, and rootstocks 101-14 Millardet et de Grasset and 1103 Paulsen, had vines with disease symptoms. Vines had 15% disease incidence and 30% disease severity. These symptoms have been described as characteristic of black-foot, caused by “*Cylindrocarpon*”-like asexual morph fungi. Isolations were made from roots and basal part of 30 symptomatic plants. Fragments of advanced necrotic tissue were washed with running water, surface sterilized with 70% alcohol for 20 seconds, 2% NaOCl solution for 4 minutes and rinsed in sterile distilled water twice. These fragments were placed onto Potato Dextrose Agar (PDA) and incubated at 25°C in the dark for 10 days to isolate fungal pathogens. A total of 50 colonies resembling black-foot disease pathogens were subcultured onto fresh PDA in order to obtain single spore cultures. The isolates developed buff to cinnamon and dark brown, and felty mycelium. Colonies subcultured in synthetic nutrient agar (SNA) produced hyaline, cylindrical, straight and/or slightly curved with one to three septate (35.6 × 5.3 µm) macroconidia and abundant, hyaline, ellipsoidal and zero to one septate (12.5 × 6.0 µm) microconidia. Cultures and conidia morphological characteristics were similar to those of *Ilyonectria* or *Dactylonectria* genera (Cabral et al. 2012a; Lombard et al. 2014). DNA sequence analysis of the partial histone H3 gene was obtained for isolates INTA SC1 (*I. liriodendri*), INTA LC2 (*D. alcacerensis*) and INTA LC1 (*D. macrodidyma*) and deposited in GenBank (accession nos. OK338901, OK338900 and OK338899). The BLAST search was conducted against type specimens. Sequences showed high similarity (99% to 100%) to the sequences of *Ilyonectria liriodendri* (Halleen, Rego & Crous) Chaverri & Salgado (GenBank accession no. JF735509), *Dactylonectria alcacerensis* (A. Cabral, Oliveira & Crous) L. Lombard & Crous (GenBank accession no. JF735630) and *Dactylonectria macrodidyma* (Halleen, Schroers & Crous) L. Lombard & Crous (GenBank accession no. JF735647). Pathogenicity tests were conducted under greenhouse conditions on 1-year-old rooted canes cv Malbec using the three isolates. Ten rooted cuttings with pruned roots were immersed in a suspension of 10⁶ conidia ml⁻¹ of each isolate for 60 min (Cabral et al. 2012b)

while control cuttings were immersed in sterile distilled water. Immediately they were planted in pots and kept in a greenhouse at a temperature between 25 to 30°C. Symptoms developed on all plants 4 months after inoculation and consisted in necrotic lesions of roots and in the base of the canes, with a reduction in root biomass. Plants did not develop aerial symptoms. All fungi were re-isolated only from necrotic lesions of root and base of inoculated canes, fulfilling Koch's postulates. No symptoms were observed on the control plants. To our knowledge, this is the first report of the species *I. liriiodendri*, *D. alcacerensis* and *D. macrodidyma* associated with black-foot disease of grapevine in Argentina. Black-foot disease has a negative effect on the viability of planting material and young vines, and this report will assist with monitoring distribution of the disease as well as developing management recommendations to nurseries and grape growers in Argentina.

References:

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