

Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Diplodia seriata*, *Diplodia mutila*, and *Dothiorella omnivora* Associated with Apple Cankers and Dieback in Río Negro, Argentina

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Apple (*Malus domestica* Borkh.) is an important fruit crop in Río Negro, Argentina. In recent years, the frequency of canker and dieback symptoms has increased, affecting different apple cultivars. In September 2014, a higher occurrence of cankers (29%) and dieback (9%) was observed in a commercial orchard of 10-year-old apple trees ($n = 210$) cultivar 'Ital Red' in General Roca, Río Negro, Argentina (39°2'36.73"S, 67°32'44.55"W). Symptoms initially appeared as necrotic bark lesions on tree trunks and branches often associated with pruning wounds. Superficially, papyrus detachment of the bark and cracked bark were observed on the affected area. When the bark was removed, the diseased wood showed a dark brown color. Cross sections of diseased branches revealed necrotic lesions that progressed to branch death. Samples were collected from different symptomatic trees ($n = 30$) and were superficially disinfected with 70% ethanol. Internal tissues (0.5 cm²) were excised from the advance margin of the necrotic lesions, plated on potato dextrose agar (PDA), and incubated at 22°C. Pycnidia were induced on sterilized pine needles overlaid on 2% water agar under near-UV light. Optimum temperature of culture growth on PDA was studied. According to their morpho-cultural characteristics, three different morphotypes were identified. The first showed optimum growth at 30°C, had moderately dense white

aerial mycelia, and turned dark gray after 7 days. Conidia were ovoid, mostly aseptate, 20.8 to 25.6 × 8 to 11.4 μm ($n = 50$), and hyaline to brown. The second exhibited optimum growth from 25 to 30°C, was white to gray, with sparse to moderate aerial mycelium that turned dark olive green. Conidia were ovoid, one-septate, 17.6 to 22.4 × 8.1 to 11.2 μm ($n = 50$), and brown. Finally, the third showed optimum growth at 25°C, and mycelium was gray to dark olive green. Conidia were oblong to ovoid with both ends rounded, aseptate and one-septate at maturity, 20.8 to 24 × 11.2 to 14.4 μm ($n = 50$), hyaline turned brown. Genomic DNA was extracted from one representative isolate of each morphotype, and the ITS and EF1- α loci were amplified with the primer sets ITS1/ITS4 (White et al. 1990) and EF1-728F/EF1-986R (Carbone and Kohn 1999), respectively. The nucleotide sequences indicated ≥99% identity to *Diplodia seriata* (CBS 114796 and CBS 112555), *Diplodia mutila* (CBS 302.36 and CBS 112553), and *Diplodia juglandis* (CBS 188.87), reclassified as *Dothiorella omnivora* (Linaldeddu et al. 2016), for both DNA regions. The sequences were deposited in the GenBank database (MW596418, MW598375; MH665432, MK955889; MH665413, MK937229). To confirm pathogenicity, healthy 1-year-old twigs of adult apple trees were pruned, and wounds of attached twigs were immediately inoculated with 20 μl of conidial suspension (10³ conidia/ml, $n = 9$ per isolate) or sterile distilled water (control, $n = 9$) and then wrapped with Parafilm. The experimental design was randomized, and the experiment was repeated once. After 90 days, the area of lesion on all twigs inoculated was determined. *D. mutila* and *Do. omnivora* produced mean canker areas (65 and 73 mm², respectively) significantly larger ($P < 0.005$) than *D. seriata* (48 mm²). No lesion occurred in the negative controls. Fulfilling Koch's postulates, fungi were reisolated from all inoculated twigs, and no fungus was recovered from controls. To our knowledge, this is the first report of *D. seriata*, *D. mutila*, and *Do. omnivora* associated with apple canker and dieback in Argentina, which shows the need to study the role of these fungi in orchard health.

References:

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