Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Eutypa lata* Causing Cankers and Dieback in Sweet Cherry in Chile

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Sweet cherry (*Prunus avium*) is one the most important fruit crops in Chile. Its production has significantly grown in recent years, reaching 228,448 tons exported in 2019/2020, to 47 countries. One of the main threats for this expanding crop are fungal pathogens, especially those that cause wood diseases. Cherry orchards ($n = 35$) located in the central area of Chile, from Curicó ($34^{\circ}57'58''\text{S}$ $71^{\circ}18'02''\text{W}$) to Angol ($37^{\circ}47'56''\text{S}$, $72^{\circ}39'09''\text{W}$), were surveyed during 2020. Wood samples were collected ($n = 72$) from living branches and trunks showing dieback, cankers, and dark necrosis, mostly wedge shaped. Small wood sections (0.5 cm) were cut off from the margin of the necrosis and surface disinfected using 0.5% v/v sodium hypochlorite. Sections were plated on a quarter-strength potato dextrose agar amended with 1 mg/liter tetracycline (PDA-tet). Plates were incubated at 25°C until mycelial development and subsequently the isolates were purified, transferring excised fungal tips to PDA. Colonies ($n = 21$) developed white cottony mycelia, which turned slightly grayish and flatter after 10 days at 25°C . Isolates developed black pycnidia that released beige conidial matrices after a subsequent 15 days at $25 \pm 2^{\circ}\text{C}$ and a 12-h photoperiod. Conidia were hyaline, curved and filiform, measuring 19.8 (27.9) to 36.7 μm length (lineal) $\times$ 1.2 (1.7) to 1.9 μm width ($n = 70$), fitting the description of *Eutypa lata* (Rappaz 1984). DNA was extracted from mycelia of the representative isolates HMCe30a, HMCe41a, HMCe109c, and HMCe110a. The partial β -tubulin gene was amplified using bt2A/bt2B primers (Glass and Donaldson 1995) and the internal transcribed spacer region was amplified using ITS1/ITS4 primers (White et al. 1990). Sequences were BLAST analyzed, finding

that ITS shared 99% and βTUB 100% identity with *E. lata* strain CBS 208.87 (Rolshausen et al. 2006). Sequences were deposited to GenBank (MW363035, MW363034, MW363033, and MW363032 [ITS]; and MW366820, MW366819, MW366818, and MW366817 [βTUB]). The isolates were inoculated on healthy sweet cherry plants cv. Kordia, produced by rooting scions in tap water amended with 500 ppm of indole-butyric acid for 30 days. An injury was made in the upper third of the shoots using a sterile 0.5-cm diameter cork borer. Mycelial plugs were placed on the injuries and covered with plastic film, using sterile agar for controls ($n = 25$). Plants were incubated in aerated tap water for 60 days at $23 \pm 3^{\circ}\text{C}$. After incubation, plants were cut, exposing dark-brown necrotic lesions, while control plants remained asymptomatic. Moreover, 2-year-old potted plants cv. Lapins were inoculated ($n = 3$ per isolate) with mycelial plugs, on fresh cuts of their main lateral branches, on 20 January, and remained under partial shade for 72 days. After incubation, bark was removed from inoculated branches and the necrotic lesion lengths were measured. HMCe109c was the most virulent isolate (3.6 cm), followed by HMCe30a (2.1 cm), HMCe41a (1.9 cm), and HMCe110a (1.1 cm), while symptoms were not reproduced in controls. Filling Koch's postulates, fungi were reisolated from all inoculated plants in both pathogenicity tests and no fungus was recovered from controls. To our knowledge, this is the first report of *E. lata* causing wood decay in sweet cherry in Chile. The pathogen was recently reported causing dieback of grapevines in Chile (Lolas et al. 2020). These are significant findings due to the frequent proximity of sweet cherry orchards and vineyards, which facilitates cross infections.

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e-Xtra

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