

# Disease Note

## Diseases Caused by Fungi and Fungus-Like Organisms

### First Report of *Diaporthe ambigua* Associated with Dead Arm Disease on Grapevine in Chile

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Grapevine (*Vitis vinifera* L.) is one of the most important fruit crops in Chile based on economic value. *Phaeoemoniella chlamydospora* and Botryosphaeriaceae species have been reported as the major causal agents associated with dieback symptoms in Chile commercial vineyards (Besoain 2018; Díaz and Latorre 2014; Larach et al. 2020). Recently, *Eutypa lata* has been reported attacking Chilean vineyards with dieback symptoms (Lolas et al. 2020). In this study, two commercial cultivar Cabernet Sauvignon vineyards in the O'Higgins Region of Chile showing dead cordons, dead spur with a grayish color, canker, and vascular necrosis were sampled in fall 2018, and a high incidence of symptoms was observed. Four symptomatic wood samples were analyzed from these vineyards. Pieces of wood (<1 cm<sup>2</sup>) were taken from the advanced zone of the canker lesions, disinfected with 70% ethanol, rinsed in sterile distilled water, dried, and transferred to two media in Petri plates, potato dextrose agar acidified with 0.5 ml of 96% lactic acid (APDA) and malt extract agar, and incubated for at least 7 days at 24°C in darkness. From mycelium obtained from monosporic culture, two isolates were selected and morphologically identified as *Diaporthe* sp. To induce sporulation, these two isolates were grown in APDA under near-ultraviolet light ( $\lambda = 320$  nm) at room temperature. After 30 days, the development of pycnidia was observed. Both *Diaporthe* sp. isolates presented ellipsoidal alpha-conidia with an obtuse apex and were biguttulate ( $n = 30$ ) and  $6.7 \pm 0.33 \times 3.3 \pm 0.32$   $\mu$ m. No beta-conidia or perithecia were observed. DNA was extracted from the monosporic mycelium. The ribosomal internal transcribed spacer (ITS),  $\beta$ -tubulin (BT) gene, and elongation factor (EF) gene were amplified using

ITS4/ITS5, Bt2a/Bt2b, and EF1-728F/EF1-986R primer pairs, respectively. PCR products were sequenced and identified as *Diaporthe ambigua* Nitschke (PUCV2140 and PUCV2141), showing 100% sequence identity with ITS MH864620.1, 99.8% with BT MG281142.1, and 100% with EF KC343738.1 sequences from *D. ambigua*. Sequences were deposited in GenBank (ITS: MW301136, MW301137; BT: MW323445, MW323446; and EF: MW308305, MW308306). Two pathogenicity tests were performed with PUCV2140 and PUCV2141 using 2-year-old *V. vinifera* cultivar Cabernet Sauvignon. In each test, three plants were used per isolate, considering one plant as an experimental unit. In the first test, a 5-mm-diameter mycelial plug from a 6-day-old APDA culture was inoculated using an oblique cut made in the bark with a sterile scalpel at the middle of the trunk. In the second test, the trial was done under the same conditions described previously, but 1-year-old semilignified shoots were inoculated between two internodes using mycelial plugs, one shoot for each plant. Injured plants treated with sterile APDA plugs were used as controls. Plants were placed in natural conditions, and 3 months after inoculation, they showed a cortical canker and brown vascular lesions. Noninoculated plants remained asymptomatic. The lengths of the cankers were  $22.0 \pm 1.8$  mm and  $10.5 \pm 0.6$  mm after inoculations of the trunk and cane, respectively. The vascular lesions were  $37.0 \pm 3.3$  mm and  $18.0 \pm 2.0$  mm in trunk and cane inoculations, respectively. *D. ambigua* was reisolated and reidentified morphologically from the inoculated symptomatic plants, confirming Koch's postulates. Also, the plants inoculated on the trunk showed premature leaf drop. To our knowledge, this is the first report of *D. ambigua* associated with dieback affecting grapevines in Chile. Previous *D. ambigua* was reported causing fruit rot (Auger et al. 2013; Díaz et al. 2017) and cordon dieback in kiwifruit (Díaz and Latorre 2018) and stem canker and dieback in blueberry (Elfar et al. 2013) in Chile. This study reports a new species of fungi for Chile associated with dead arm in vineyards. *D. ambigua* is a pathogen in essential crops in Chile. Therefore, it is important to further study its prevalence.

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