

A new molecular diagnostic tool for the detection of Botryosphaeriaceae species L. MARTÍN¹, F.J. CASTAÑO¹, G. DURANTE², C. CHATILLON³ and J. LUQUE⁴. ¹Fundació Parc Tecnològic del Vi (VITEC), Ctra. de Porrera km 1, 43730 Falset, Spain. ²International Plant Analysis and Diagnostics srl Via Einstein - Loc. Cascina Codazza -26900 Lodi, Italy. ³Qualiplante, Cap Alpha - Avenue de l'Europe - 34830 Clapiers (France). ⁴Institut de Recerca i Tecnologia Agroalimentàries (IRTA) Patologia Vegetal, Ctra. de Cabrils km 2, 08348 Cabrils, Spain.

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Botryosphaeriaceae spp. have been reported as major pathogens causing grapevine trunk diseases (GTD) worldwide. On grapevines, Botryosphaeriaceae spp. infection cause internal wood necrosis, cankers, dieback, cane bleaching and graft union failure. Isolation of these pathogens is time consuming and morphological identification can be difficult to species level. The development of culture independent PCR based methods could help to promote an accurate control in grapevine nurseries as well as new epidemiology research. This work showed a reliable and optimized molecular tool that allowed a quick and easy detection of multiple Botryosphaeriaceae species in a few hours. A collection of Botryosphaeriaceae isolates, was used to verify the multiple species detection of the *Botryosphaeria* spp. Qualiplante SAS End-Point Nested PCR kit (PCR.Botr-25/100Liq). DNA from 12 different tested Botryosphaeriaceae species., including all of the main species pathogenic to grapevines, showed a single positive amplicon band of 365 bp. A specificity *in vitro* test of the molecular kit was performed using DNA from pure culture of other GTD pathogens fungi that may occur in grapevines (i.e. *Phaemoniella chalmydospora*, *Phaeoacremonium* spp., *Eutypa lata*, *Cryptovalsa ampelina* and *Phomopsis viticola*). Results confirmed that the kit was specific for Botryosphaeriaceae spp. The sensitivity threshold of the Qualiplante nested PCR kit was evaluated using purified genomic DNA from mycelia of a *Diplodia seriata*. The assay could detect as little as 5 pg DNA of *D. seriata*. The effectiveness of the assay was validated with environmental samples from naturally infected wood and rainwater traps collected in a mature vineyard where GTDs are known to be present. The viability and virulence of Botryosphaeriaceae pathogens was confirmed through isolation from water and wood material. The specificity of the assay was demonstrated even in the presence of competing DNA templates since other microorganisms were isolated from environmental samples. Detection of Botryosphaeriaceae DNA in rainwater revealed dispersion of these pathogens throughout the rain and the applicability of the assay in epidemiological studies. The results of this study showed that the specificity and sensitivity of this new molecular tool could be used to establish sanitary controls over the nursery propagation process in order to possibly prevent Botryosphaeriaceae infection.

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