Development of molecular tools for the detection and quantification of Eutypa and Botryosphaeria dieback pathogen inoculum in Australian vineyards.

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Eutypa dieback (ED) and Botryosphaeria dieback (BD) are caused by several Diatrypaceae and Botryosphaeriaceae species, respectively and are considered important grapevine trunk diseases worldwide. Their spores (ascospores and/or conidia) are primarily dispersed by rain splash and wind and infect susceptible pruning wounds leading to cankers, dieback and eventually death of vines. The objective of this study was to develop molecular tools to detect and quantify Diatrypaceae and Botryosphaeriaceae spores from the environment. These tools are essential for investigating spore dispersal patterns, thus, high risk infection periods of ED and BD pathogens in Australian vineyards. Four DNA extraction protocols were evaluated and one was found suitable for extracting DNA from artificially-inoculated tapes and Burkard spore trap tapes collected from the vineyards. Two quantitative PCR (qPCR) protocols using two sets of multi-species primers were further developed to detect and quantify Diatrypaceae and Botryosphaeriaceae spores from a single environmental sample. Specificity tests showed that the two multi-species primers were able to amplify the DNA of their corresponding target Diatrypaceae and Botryosphaeriaceae species (nine each) while none of the 20 non-target species were amplified. The two qPCR methods were suitable for amplifying purified DNA, synthetic DNA fragments (gBlocks®) and mixed DNA from spore trap tapes. The Diatrypaceae primers that amplify a fragment of the rRNA gene had a limit of detection (LOD) of ~20 fg of purified DNA which is equivalent to less than one spore. The LOD for Botryosphaeriaceae primers, that amplify a fragment of the β-tubulin gene, was ~300 fg of purified DNA which is equivalent to seven spores. The qPCR methods developed in this study were shown to be rapid and sensitive in detecting ED and BD pathogens from environmental samples and are currently being used to analyse spore trap samples from different viticulture regions in Australia.