

A new molecular diagnostic tool for the detection of Botryosphaeriaceae species



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Introduction

Many Botryosphaeriaceae species have been recognized as pathogens causing grapevine trunk diseases (GTD) throughout the world. Grapevines infected by Botryosphaeriaceae spp. show 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.

Objective

The goal of this work was to validate the End-Point Nested PCR kit (PCR.Botr-25/100Liq) developed by IpadLab and Qualiplante to offer a quick and easy detection of multiple Botryosphaeriaceae species. Thus the aims of this research were to:

- Verify the multiple species detection using a collection of Botryosphaeriaceae.
- Test the specificity of the kit.
- Establish the sensitivity of the method.
- Assess its functionality to evaluate the incidence of these pathogens.

Material and Methods

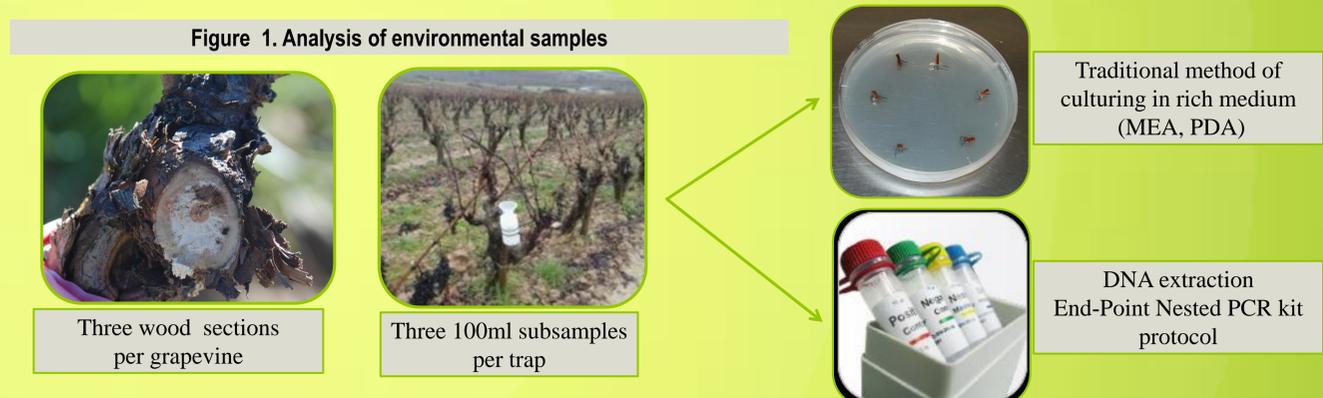
- To assess the amplification of multiple Botryosphaeriaceae spp., DNA from 24 strains belonging to 12 different species previously isolated and characterized were tested (Table 1). All strains, except those of *Diplodia corticola*, one of *Neofusicoccum australe* and one of *N. mediterraneum*, were isolated from grapevine wood in different countries.

Taxon	Isolate code	Host	Location	Collector	ITS	Kit
<i>Diplodia seriata</i>	CBS 121485	<i>Vitis vinifera</i>	Spain	J Luque	+	+
	JL538	<i>Vitis vinifera</i>	Spain	J Luque	+	+
	Ds 1	<i>Vitis vinifera</i>	Spain	L Martín	+	+
<i>Di. mutila</i>	El Pisón 1	<i>Vitis vinifera</i>	Spain	FJ Legorburu	+	+
	CBS 112553	<i>Vitis vinifera</i>	Portugal	AJL Phillips	+	+
<i>Di. corticola</i>	JL222	<i>Quercus ilex</i>	Spain	J Luque	+	+
	CBS 112070	<i>Quercus suber</i>	Spain	J Luque	+	+
<i>Neofusicoccum parvum</i>	CBS 121486	<i>Vitis vinifera</i>	Spain	J Luque	+	+
	Np1	<i>Vitis vinifera</i>	Spain	L Martín	+	+
<i>N. luteum</i>	CAP037	<i>Vitis vinifera</i>	Portugal	AJL Phillips	+	+
	CBS 121482	<i>Vitis vinifera</i>	Spain	J Luque	+	+
<i>N. australe</i>	JL835	<i>Vitis vinifera</i>	Spain	J Luque	+	+
	CBS 122027	<i>Pistacia vera</i>	Spain	J Luque	+	+
<i>N. mediterraneum</i>	JL593	<i>Pistacia vera</i>	Spain	J Luque	+	+
	NmedRR	<i>Vitis vinifera</i>	Spain	R Raposo	+	+
<i>Botryosphaeria dothidea</i>	CBS 121484	<i>Vitis vinifera</i>	Spain	J Luque	+	+
	JL807	<i>Vitis vinifera</i>	Spain	J Luque	+	+
<i>Dothiorella iberica</i>	UCD1448SLO	<i>Vitis vinifera</i>	California	JR Urbez-Torres	+	+
<i>Do. sarmentorum</i>	JL716	<i>Vitis vinifera</i>	Spain	J Luque	+	+
<i>Spercermartinsia viticola</i>	CBS 117009	<i>Vitis vinifera</i>	Spain	J Luque	+	+
	CBS 117008	<i>Vitis vinifera</i>	Spain	J Luque	+	-
	JL573	<i>Vitis vinifera</i>	Spain	J Luque	+	-
<i>Lasiodiplodia sp.</i>	6.12.2a (433)	<i>Vitis vinifera</i>	Spain	R Raposo	+	+
	JL687	<i>Vitis vinifera</i>	Spain	J Luque	+	+

CBS: Centraalbureau voor Schimmelcultures

- Specificity** of the method was tested using DNA from isolates of other GTD pathogenic fungi [*Phaeoacremonium aleophilum*, *Phaeoacremonium viticola* (Pm), *Phaeomoniella chlamydospora* (Pch), *Phomopsis viticola* (Pho), *Eutypa lata* (Ela), *Fomitiporia mediterranea* (Fom) and *Cryptovalsa ampelina* (Ca)] (Figure 3). The primer pair ITS4-ITS5 was used to verify that DNA extracts were suitable for amplification.

- Two tenfold serial dilution (100 ng to 5 fg) of purified genomic DNA from *Diplodia seriata* was used to determine the **sensitivity** of the Qualiplante nested PCR kit. All PCR assays were made in triplicate.



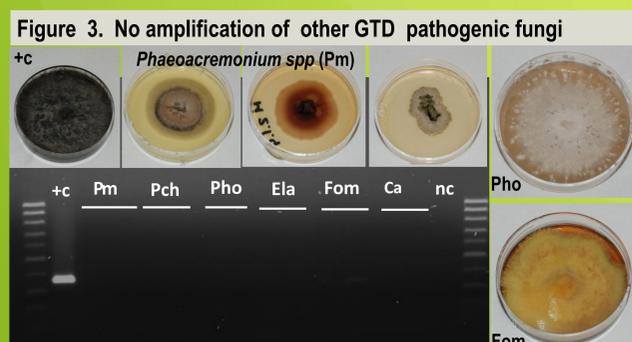
- Reliability of the diagnostic tool was verified on 12 GTD symptomatic grapevines and 12 rainwater samples collected at pruning time in a mature vineyard where GTD are known to be present. (Figure 1).

Results

- Multi-species amplification.**

A simple amplicon band of 365 bp was obtained for 12 different Botryosphaeriaceae spp. (Table 1, Figure 2).

- Specificity**



- Sensitivity**

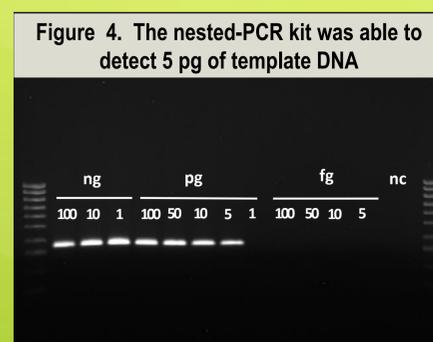


Figure 2. Detection and identification of Botryosphaeriaceae species



- Validation of the diagnostic tool for epidemiological research**

Number of positive samples	Culturing on medium				Nested-PCR	
	Botryosphaeriaceae spp.	Other GTD fungi				
		Pm	Pch	Pho	Fom	
Grapevine wood samples	10	5	5	1	4	11
Rain water samples	3	2	nd	nd	nd	7

nd: not detected

Conclusions

- 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 and to perform epidemiology research in order to prevent Botryosphaeriaceae infection of grapevines.
- Dispersion of Botryosphaeriaceae spp. throughout the rain water was demonstrated.

Acknowledgements

Authors are grateful to FJ Legorburu, AJL Phillips, JR Úrbez-Torres and R Raposo for kindly providing some of the Botryosphaeriaceae isolates.