DEVELOPMENT OF A SIMPLIFIED MODEL TO CHARACTERIZE PATHOGENICITY AND EVALUATE FOLIAR SYMPTOMS EXPRESSION ON BOTRYOSPHAERIA DIEBACK

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Development of a simplified model to characterize pathogenicity and evaluate foliar symptoms expression on Botryosphaeria dieback

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Introduction

Botryosphaeria dieback is a worldwide spread grapevine trunk disease, associated with several *Botryosphaeriaceae* species. The most common species isolated from grapevines around the world are *Diplodia seriata* De Not. and *Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips. They are xylem-inhabiting fungi that cause leaf and berry symptoms, and eventually the death of the plant. These symptoms include leaf spots, fruit rots, shoot dieback, bud necrosis, vascular discoloration of the wood and perennial cankers. Research has been developed to gain a better understanding of the mechanisms that are involved in symptoms expression by the artificial reproduction of this symptoms. Therefore, the aim of our work is to develop a simplified model with *D. seriata* and *N. parvum* in order to better characterize their pathogenicity by measuring the size of necrosis and evaluating the percentage of vine developing foliar symptoms.

Material and Methods

During a four years period (2011-2014), grafted grapevine cuttings of cv. Aragonez (=Tempranillo) were potted individually and placed randomly in a ventilated greenhouse at 24 °C under natural light. After one month's growth, plants were used for infection experiments using five isolates of *N. parvum* (Np) and *D. seriata* (Ds) with two different origins (Portuguese and French) and different degrees of virulence. In 2014, as positive control, the causal agent of black rot of grape *Phyllosticta ampelicida* (Engelm.) Aa (Gb), *Cladosporium* sp. and *Penicillium* sp. isolates were inoculated (Table 1). Negative controls were inoculated with 3 mm PDA plugs. Thirty replicates were made and each experiment was carried out for two years in the greenhouse. Dimension of cankers was evaluated three months after the inoculation by measuring the width and the length in order to calculate the area of the elliptical necrosis. Data obtained was analysed statistically using the STATISTICA 6.1 software package and means compared by using a Duncan's test. Eight months after the inoculation, grapevines were visually evaluated for the percentage of foliar symptoms

Table 1 – Fungi isolates used in inoculations

Isolates	Species	Virulence	Origin
Np 19	*Neofusicoccum parvum*	High	Portugal
Np 67	*Neofusicoccum parvum*	Low	Portugal
Ds 98-1	*Diplodia seriata*	High	France
Ds 99-7	*Diplodia seriata*	-	France
Ds AR	*Diplodia seriata*	-	France
Gb 17	*Phyllosticta ampelicida*	High	Portugal
Gb 32	*Phyllosticta ampelicida*	High	High
-	*Penicillium* sp.	-	France
-	*Cladosporium* sp.	-	France

Fig. 1 – A wound was produced by removing a 3 mm of bark with a corkborer at the base of the green shoots, between the second and third node.

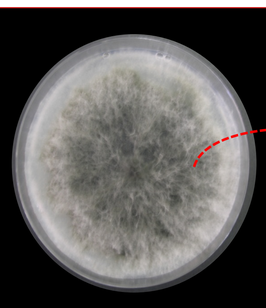

Fig. 2 – Three mm inoculation plugs were cut from the actively growing margin of *Neofusicoccum parvum and Diplodia seriata* colonies and placed on the wounds.

Fig. 3 - Inoculation points were covered with moist cotton wool and sealed with Parafilm for two weeks.

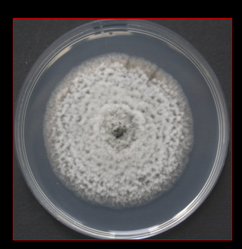

Fig. 4 – Positive control inoculation with the causal agent of black rot of grape *Phyllosticta ampelicida* (Gb), C*ladosporium* sp. and *Penicillium* sp.

Results

Table 2 - Dimensions of cankers produced by isolates of *Neofusicoccum parvum* (Np) *and Diplodia seriata* (Ds) in green shoots: width (mm), length (mm), area (mm^2)

Isolates	Years											
	Width (mm)				Length (mm)				Area (mm^2)			
	2011	2012	2013	2014	2011	2012	2013	2014	2011	2012	2013	2014
Negative control	4.2 a	5.6 a	5,7 a	5.5 a	8.7 a	5.7 a	5.8 a	5.4 a	28.7 a	25.0 a	26.0 a	23.3 a
Np 19	12.8 ab	14.7 ab	15.1 ab	14.1 ab	20.4 ab	24.6 ab	25.3ab	24.8 ab	158.5 ab	204.4 ab	299.9 ab	288,1 ab
Np 67	28.2 b	24.6 b	24.2 b	23.4 b	81.2 c	78.8 c	79.5 c	83,4 c	1797.5 c	576.9 c	1510.3d	1532.0 d
Ds 98-1	7.1 a	9.1 a	10.0 a	9.7 a	12.8 a	17.5 a	18.3 a	19.1a	71.3 ab	129.8 ab	143.7 ab	145.4 ab
Ds 99-7	-	12.8 a	12.7 a	13.1 a	-	20.4 a	19.5 ab	20.1 a	-	158.5 ab	194.4 ab	206.7 ab
Ds AR	9.9 a	13.8 a	13.9 a	12.7 a	41.3 b	37.8 b	38.2 ab	39.7 b	415.0b	314.3 b	416.8 c	395.8 c

Table 3 - Foliar symptoms produced in grapevines of cv. Aragonez by isolates of *Neofusicoccum parvum* (Np) *and Diplodia seriata* (Ds)

Isolates	Vines with foliar symptoms (%)			
	Years			
	2011	2012	2013	2014
Negative control	0.0	0.0	0.0	0.0
Np 19	60.0	66.7	77.0	73.0
Np 67	76.7	73.3	77.0	73.0
Ds 98-1	36.7	50.0	53.0	50.0
Ds 99-7	-	33.3	36.6	30.0
Ds AR	6.3	60.0	57.0	60.0

Table 4 - Dimensions of cankers produced by isolates of *Phyllosticta ampelicida* (Gb), *Cladosporium* sp. and *Penicillium* sp. in green shoots

Isolates	Years		
	Width (mm)	Length (mm)	Area (mm^2)
	2014	2014	2014
Negative control	5.5	5.4	23.3
P. ampelicida (Gb32)	11.0	28.0	103.3
P. ampelicida (Gb17)	11.0	16.0	62.0
Cladosporium sp.	5.1	5.2	20.8
Penicillium sp.	5.5	5.4	23.3

Fig. 5 - Necrosis observed in the green shoots of grafted cuttings cv. Aragonez; (A) Negative control; (B) *Neofusicoccum parvum*; (C) *Diplodia seriata*; (D) Positive control (*Phyllosticta ampelicida*).

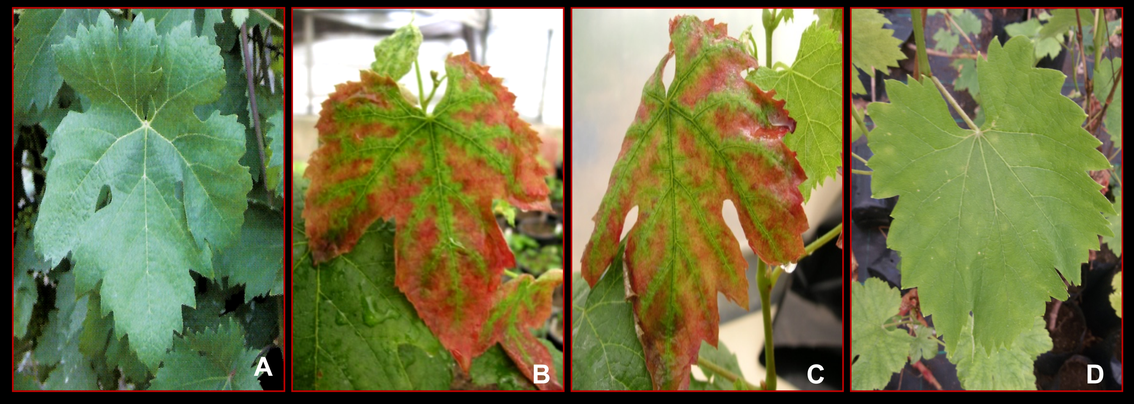

Fig. 6 - Foliar symptoms in leaves of inoculated grafted cuttings cv. Aragonez; (A) Negative control; (B) *Neofusicoccum parvum*; (C) *Diplodia seriata*; (D) Positive control (*Phyllosticta ampelicida*).

Conclusions

During the extension of this assay, means lesions surface associated with Np 67 and Ds AR were higher than those associated with the remaining isolates. One year after the infection, the percentage of infected plants showing foliar symptoms was higher in Np 67 and Np 19 than in Ds 98-1, Ds 99-7 and Ds AR, while none of the control plants showed any symptoms. Results obtained for positive control assay revealed that both *P. ampelicida* isolates produced necrosis on green shoots, while *Cladosporium* sp. and *Penicillium* sp. originated necrosis similar to those recorded for negative control. Also, no foliar symptoms could be observed for any of the isolates used as positive control. In conclusion, the developed this simplified model may allow an accurate characterization of isolates pathogenicity and connection with foliar symptoms of Botryosphaeria dieback.