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RESEARCH PAPER

Effects of *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum* on grapevine rootstocks

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Abstract

G. A. Díaz, M. Esterio, and J. Auger. 2009. Effects of *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum* on grapevine rootstocks. Cien. Inv. Agr. 36(3):381-390. Cuttings of five grapevine (*Vitis vinifera*) rootstocks were wounded and immediately inoculated with suspensions (approximately $5x10^3$ conidia·mL^{-1}) of either *Phaeomoniella chlamydospora*, *Phaeoacremonium aleophilum* or a mixture of both species. The presence of these endophyte fungi affected the quality of each of the five rootstocks. Among the roostocks investigated in this study, 1103P and 101-14 MG were less susceptible to the infection caused by *Pa. chlamydospora* and *Pm. aleophillum*.

Key words: Endophytes fungi, fungal diseases, Petri disease, *Vitis vinifera*, wood fungi.

Introduction

Chile is a major exporter of table grapes (*Vitis vinifera* L.) and wines, and regional viticulture has expanded considerably in recent years. The rapid growth of this industry has considerably increased the demand for nursery plants, and particularly the demand for grafted grapevines, and with increasing demand, the quality of the available propagation material has declined. The problems associated with the production of the grafted grapevines include inhibition of the basal callus formation, decrease in root emission, poor formation of the grafting callus and graft failures, and symptoms of incompatibility.

Petri disease is recognized as one of the fungal diseases related to the decreasing quality of propagation plant materials. This disease affects young grapevine plants and causes plant loss in many countries. Research focused in Chile (Auger *et al*., 2004b), Italy (Mugnai *et al*., 1999), USA (Morton, 1995), France (Larignon and Dubos, 1997), South Africa (Ferreira *et al*., 1994) and Australia (Pascoe and Cottral, 2000) has attributed the symptoms of Petri disease to the presence of vascular endophytic fungi (Mugnai *et al*., 1999). The causal agents associated with Petri disease are *Phaeomoniella* (*Pa*) *chlamydospora* and species of *Phaeoacremonium* (*Pm*) (Mugnai *et al*., 1999; Eskalen *et al*., 2001; Whiting *et al*., 2001; Fourie and Hallen, 2004; Santos *et al*., 2005). *Phaeoacremonium aleophilum* is the species of *Phaeoacremonium* most commonly identified in diseased grapevines (Wallace *et al*., 2002; Auger *et al*., 2005a). Numerous studies have suggested that *Pa. chlamydospora* and *Pm. aleophilum* are the causal agents of Petri disease (Feliciano *et al*., 2004; Gaforio *et al*., 2005; Mostert *et al*., 2006).

The symptoms of Petri disease include general

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weakness, slowed growth, delayed bud burst, short internodes, reduction of the diameter of trunks and arms, small leaves, and early leaf senescence. The formation of dark brown streaks in the vascular tissue of the xylem, close to the pith, is observed in longitudinal sections of the trunk or stems. Cross-sections of trunk or stem material have revealed black punctuated patterns corresponding to the occlusion of the xylem vessels (Scheck *et al*., 1998; Khan *et al*., 2000; Eskalen *et al*., 2001; Stamp, 2001; Whiting *et al*., 2001; Wallace *et al*., 2002; Auger *et al*., 2005b; Díaz, 2008).

Phaeomoniella chlamydospora and *Pm. aleophilum* were reported recently in Chile and were primarily associated with young grapevine cvs. Several varieties in vineyards located in Regions V, VI, VII and the Metropolitan Region that were grafted on 3309 C and Kober 5BB (Cabernet Sauvignon, Merlot, Pinot Noir, Red Globe, Flame Seedless, Thompson Seedless and Ruby seedless) were all affected by decline symptoms (Auger *et al*., 2004b; Auger *et al*., 2005c). *Pa. chlamydospora* and *Pm. aleophilum* have occasionally been detected along with different species of *Botryosphaeriaceae* in Red Globe grapevines (Auger *et al*., 2004a).

The previously discussed species *Phaeoacremonium* and *Pa. chlamydospora* are part of the fungus complex that causes dieback of adult grapevines, increasing the incidence of chlorotic leaf roll associated with *Fomitiporella vitis* (Auger *et al*., 2005a). In Europe, *Pa. chlamydospora* and *Phaeoacremonium* spp. have also been isolated from grapevines affected by esca and eutipiosis (Larignon and Dubos, 1997).

In Chile, the use of grafted grapevines, primarily SO4 (*V. berlandieri* x *V. riparia*), has increased in recent years, but the its behavior or response against Chilean isolates *Pa. chlamydospora* and *Pm. aleophilum* is unknown. The rootstock SO4 is known to be very susceptible to these two endophytic fungi (Eskalen *et al*., 2001). Therefore, the objective of this study was to determine the effects of *Pa. chlamydospora* and *Pm. aleophilum* on grafted grapevines on the major rootstock used for wine grapes in Chile.

Materials and methods

Rootstocks

The effects of *Pa. chlamydospora* and *Pm. aleophilum* on basal callus formation, root emission, grafting callus and bud burst was characterized for *V. vinifera* cv. Carménère grafted on 3309 C (*V. riparia* x *V. rupestris*), 1103 P (*V. berlandieri* x *V. rupestris*), 101-14 MG (*V. riparia* x *V. rupestris*), Kober 5BB (*V. berlandieri* x *V. riparia*), and SO4 (*V. berlandieri* x *V. riparia*). The effects were studied in 480 30-cm-long woody cuttings of each rootstock.

Inoculum and inoculation

Conidia of *Pa. chlamydospora* (pach01-2004) and *Pm. aleophilum* (pmal01-2004) were obtained in potato dextrose agar (PDA) incubated at 25ºC for 15 days in darkness. The inoculum concentration was adjusted to $5x10^3$ conidia mL^{-1} (Wallace *et al*., 2002). The inoculation method involved injecting 20 µL of conidial suspension into the bases of woody cuttings of grapevine rootstocks. The following inoculation treatments were performed: i. *Pa. chlamydospora*, ii. *Pm. aleophilum* or iii. a mixture of *Pa. chlamydospora* and *Pm. aleophilum*. An equal number of cuttings from each rootstock were injected with 20 µL of sterile deionized water and used as control treatment (Eskalen *et al*., 2001). This assay was performed twice using 100 cuttings per inoculation treatment (Test 1) and it was repeated (Test 2) using 20 cuttings.

Effects of Phaeomoniella chlamydospora *and* Phaeoacremonium aleophilum *on the root formation, basal callus, grafting callus and bud burst (Test 1)*

We arranged 400 previously inoculated cuttings of each rootstock (n = 2000) in 100 L hydration pools during a 48 h period. *V. vinifera* cv. Carménère (5 cm long) were grafted on to the rootstocks using a graft machine. To avoid

dehydration, grafted cuttings were immediately immersed in wax (70-72ºC). Cuttings were subsequently placed in a callus growth chamber at 28-31ºC and 85% relative humidity (RH) for 20 days. To promote growth and root development, cuttings were maintained for 28 days in a greenhouse at 24- 28ºC and 65-75% RH. The basal callus formations as well as the callus formations on the grafting areas were evaluated after the callus formations process, using the scale described by Fourie and Hallen (2004). Root emissions and bud burst were evaluated at the end of the greenhouse growing period, and were subjectively graded based on the visible proportion of callus formation on the cutting base grafting area (0 = 0%, 1 = 1 to 25%, 2 =26 to 50%, 3 = 51 to 75%, 4 = 76 to 99% and 5 = 100%). Bud burst and root emission were scored from 1 (no bud burst or roots emission) to 2 (bud burst or roots emission > 2 cm in length).

*Effects of P*haeomoniella chlamydospora *and* Phaeoacremonium aleophilum *on the vascular system (Test 2)*

In this test, 80 cuttings from each rootstock (n = 400) were inoculated with the conidial suspensions described above. The cuttings were placed in an incubation chamber with 12 h of light and 12 h of darkness, immersed in water (5 cm from the base) at 25-28ºC, and continuously agitated for 49 days. This procedure was designed to faster bud burst and the roots emission in the cuttings, and to stimulate the production of auxins synthesized by leaves (Hartman and Kester, 1997), a process which occurs generally 21 days post-budburst. After this treatment period, cuttings were inoculated according to Eskalen *et al*. (2001). We measured the length of the vascular tissue damage (streak) 7 months post-inoculation. To re-isolate the inoculated pathogens, samples of diseased tissue were isolated from the margins of the necrotic lesions and were plated in PDA and incubated at 25ºC in continuous darkness for 14 days. Pathogen identification was based on the description and taxonomic key described by Crous *et al*. (1996) and Crous and Gams (2000).

Design and statistical analysis

Inoculated treatments were distributed as a completely randomized design with 100 and 20 replicates, in Tests 1 and 2, respectively. A two-factor analysis of variance (ANOVA) was performed (rootstock x inoculated treatments), and when data was non-parametric, a Kruskal-Wallis test was applied. Means were assessed using Tukey's multiple range test ($p \leq 0.05$). When a significant ($p \leq 0.05$) interaction between inoculated treatments and rootstocks was obtained, Tukey's multiple range test was used to assess rootstocks.

Results

Effects of Phaeomoniella chlamydospora *and* Phaeoacremonium aleophilum *on root formation, basal callus, grafting callus and bud burst (Test 1)*

Inoculation of the cuttings with *Pa. chlamydospora* and *Pm. aleophilum* negatively affected the quality of grapevine rootstock cuttings. For the base callus development, the interaction between inoculated treatments and rootstocks was not statistically significant ($p = 0.5652$). In the case of the parameter grafting callus, we identified a significant interaction between the rootstock and inoculated treatment factors ($p = 0.0076$) (Table 1). With respect to the development of the basal callus, the ANOVA showed significant effects of inoculation treatment and rootstock type ($p < 0.0001$) (Table 2). For root emission, interaction between the treatments and the rootstock factors was not significant ($p = 0.9652$). The ANOVA showed significant differences between inoculated treatments ($p < 0.0001$) and rootstocks ($p < 0.0001$) in the root emission (Table 3). For bud burst, inoculation treatments and rootstock types showed no significant interaction ($p = 0.3271$). The ANOVA showed a significant effect of both treatments ($p < 0.0001$) and rootstocks ($p = 0.0002$) (Table 4).

Table 1. Effect of *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum* on grafting callus developments on grapevine rootstocks.

Inoculation treatments	Grafting callus developments (0 to 5)[1] on grapevine rootstocks				
	Kober 5BB	SO4	3309 C	101-14 MG	1103 P
Sterile water	4.0 b[2]	3.0b[2]	4.0 c[2]	4.0 b[2]	3.0 b[2]
Pa. chlamydospora	2.0 a	1.0 a	3.0 bc	3.0 a	2.0 a
Pm. aleophilum	2.0 a	1.0 a	1.0 a	3.0 a	2.0 a
Pa. chlamydospora+*Pm. aleophilum*	2.0 a	1.0 a	2.0 b	3.0 a	2.0 a
Analysis of variance:					
Source	df	F	p		
Rootstocks (R)	4	22.93	< 0.001		
Inoculation treatments (T)	3	31.52	< 0.001		
R x T	12	2.26	0.0076		

[1]Using a 0 to 5 scale, callus formation was visually assessed as the percentage of the circumference of the graft union with visible callus tissue (0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51- 75%, 4 = 76- 99% and 5 = 100%).
[2]Mean of one hundred replicates. Results followed by the same letters in the column and rows show non-significant differences according to Tukey's multiple range test ($p \leq 0.05$).

Effect of Phaeomoniella chlamydospora and *Phaeoacremonium aleophilum on the vascular system (Test 2)*

The cuttings inoculated with *Pa. chlamydospora*, *Pm. aleophilum* or a mixture of both pathogens showed a reddish brown to dark brown vascular discoloration. In each rootstock, the presence of these fungi was consistently associated with the treated sample and was consistently absent from the cuttings that had not been inoculated. The results indicated a significant interaction ($p < 0.0001$) between the rootstocks and inoculation treatments.

Discussion

Our study results confirmed that *Pa. chlamydospora* and *Pm. aleophilum* partially or completely inhibited the formation of the basal callus and the development of the grafting callus (Figure 1), as previously reported for other grapes cultivars (Khan *et al*., 2000). However, we found that *Pm. aleophilum* showed little effect on callus formation in rootstocks of Kober 5BB, 1103 P, 101-14 MG, SO4, Ramsey, and 99 Richter grapevines (Wallace *et al*., 2002). These differential responses may be due to the use of less virulent strains of *Pm. aleophilum*. Differences in virulence have been demonstrated by Santos *et al*., (2005); theses authors compared different strains of *Phaeoaremonium* spp., and *Pa. chlamydospora* and detected different levels of aggressiveness.

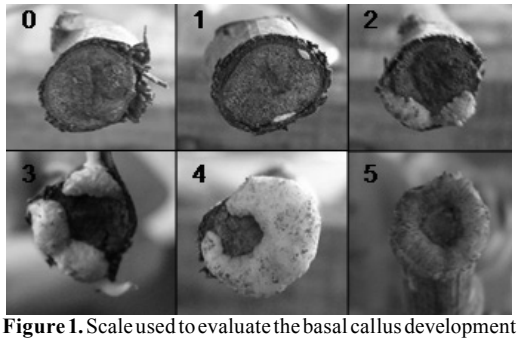

Figure 1. Scale used to evaluate the basal callus development in grapevine cuttings inoculated with *Phaeomoniella chlamydospora*.

Corroborating previous studies, the basal callus was consistently larger in grapevines inoculated with *Pa. chlamydospora* than with those inoculated with *Pm. aleophilum* (Bertelli *et al*., 1998; Khan *et al*., 2000) (Table 2). With the exception

of rootstock 3309 C, we detected significant differences in grafting callus formation between all control plants and inoculated plants (Table 1). In terms of the developing grafting callus (Table 1), we found that the interface was formed when the rootstock and graft have a close interaction. Previous studies by Hartmann and Kester (1997) demonstrated that the entire graft interface is complex, and that the formation of the interface becomes increasingly complicated with the introduction of any interfering factor. The presence of *Pa. chlamydospora, Pm. aleophilum* or other endophytic fungi may faster the accumulation of tylosis and phenolic compounds in the colonized tissues. Further, the toxic metabolites (exopolysacarides) produced by *Pa. chlamydospora* and *Pm. aleophilum* can adversely affect the formation of the grafting callus (Sparapano *et al*., 2000; Lorena *et al*., 2001). These results were similar to those obtained in South Africa using rootstock 99-Richter grafted with 'Chenin Blanc' (Ferreira *et al*., 1994).

Santos *et al*. (2005) examined callus formation under laboratory conditions and demonstrated the inhibitory effect of *Pa. chlamydospora* and *Phaeoacremonium* spp. for the rootstock 3309 C, the Baga and María Gomes cultivars. Additionally, they determined that this inhibition was different for different cultivars and rootstocks, demonstrating the differential susceptibility between the grapevine genotypes. We found similarly diverse results, and showed that the rootstock SO4 was consistently more susceptible than other rootstocks (Table 2). The rootstock 1103 P and the rootstock 101-14 MG developed the best basal and grafting calluses. The rootstock that showed the least susceptibility to the pathogens was 101-14 MG (Table 2).

Table 2. Effects of *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum* on basal callus development on grapevine rootstocks.

Inoculation treatments	Basal callus development (0 to 5) [1] on grapevine rootstocks					
	Kober 5BB	SO4	3309C	101-14 MG	1103 P	Mean[2]
Sterile water	4	3	4	5	5	4 c
Pa. chlamydospora	2	1	2	3	3	2 a
Pm. aleophilum	3	1	3	4	3	3 b
Pa. chlamydospora + *Pm. aleophilum*	3	2	3	3	3	3 b
Mean[3]	3 B	2 A	3 B	4 C	4 C	
Analysis of variance:						
Source	df	F	p			
Rootstocks (R)	4	88.70	< 0.0001			
Inoculation treatments (T)	3	106.57	< 0.0001			
R x T	12	0.88	0.5652			

[1] Calluses were visually assessed on a 0 to 5 scale as the percentage of the circumference of the basal callus with visible callus tissue (0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51- 75%, 4 = 76- 99% and 5 = 100%).
[2] Mean of one hundred replicates. Results followed by the same letters in the column and rows show non-significant differences according to Tukey's multiple range test ($p \leq 0.05$).

Inoculation with *Pa. chlamydospora* greatly inhibited root emission (Table 3). Our results with this fungus differ from the previous findings of Khan *et al*. (2000), which did not conclusively demonstrate differences in root emissions between *Pa. chlamydospora* and species de *Phaeoacremonium*. The rootstock SO4 showed the most susceptibility with respect to root emissions, and emissions were significantly lower than the rest of the rootstocks (Table 3).

The inoculated treatments showed severely re-

Table 3. Effects of *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum* on root emissions.

Inoculation treatments	Roots emission (%) on grapevine rootstocks					
	Kober 5BB	SO4	3309C	101-14 MG	1103 P	Mean[1]
Pa. chlamydospora	58	47	61	60	59	57 a
Pm. aleophilum	73	57	65	82	72	70 b
Pa. chlamydospora + *Pm. aleophilum*	75	60	79	79	76	74 b
Mean[2]	69 B	55 A	68 B	74 B	69 B	
Analysis of variance:						
Source	df	F	P value			
Rootstocks (R)	4	20.35	< 0.0001			
Inoculation treatments (T)	3	41.38	< 0.0001			
R x T	12	0.40	0.9652			

[1]Mean of one hundred replicates. Results followed by the same letters in the column and rows show non-significant differences according to Tukey's multiple range test (p = 0.05).

duced grafting bud bursts when rootstocks were inoculated with *Pa. chlamydospora* or *Pm. aleophilum* (Table 4). Significant differences in bud bursts were not detected between SO4, 3309 C, Kober 5BB and 101-14 MG, but the rootstock 1103 P showed significantly higher rates of bud burst for treated samples (Table 4). Notably, during the process of bud burst, symptoms of incompatibility were observed in the inoculated treatments, and the joint between the rootstock and the scion was observed to be very weak.

Significant differences in the stem streaks were observed between the control samples and samples inoculated with fungi, consistent with the results obtained by Ferreira *et al*. (1994), Khan *et al*. (2000), Eskalen *et al*. (2001), Laukart *et al*. (2001) and Feliciano *et al*. (2004) (Figure 2). The rootstock SO4 inoculated with a mixture of *Pa. chlamydospora* and *Pm. aleophilum* showed a shorter streak length compared to inoculation with either *Pa. chlamydospora* or *Pm. aleophilum*. Independently of the rootstock type, cut-

Table 4. Effects of *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum* on bud burst of grapevine cv. Carmenere grafted in five different grapevine rootstocks.

Inoculated treatments	Bud burst (%)[1] on 'Carmenere' grafted on:					
	Kober 5BB	SO4	3309C	101-14 MG	1103 P	Mean[2]
Pa. chlamydospora	36	27	44	33	37	35 a
Pm. aleophilum	24	31	24	38	36	31 a
Pa. chlamydospora + *Pm. aleophilum*	55	45	41	52	60	51 b
Mean[2]	38 A	34 A	36 A	41 AB	44 B	
Analysis of variance:						
Source	df	F	P value			
Rootstocks (R)	4	5.63	0.0002			
Inoculation treatments (T)	3	34.40	< 0.0001			
R x T	12	1.13	0.3271			

[1]Percentages determined relative to the non inoculated control plants.
[2]Mean of one hundred replicates. Results followed by the same letters in the column and rows show non-significant differences according to Tukey's multiple range test (p = 0.05).

tings inoculated with *Pa. chlamydospora* or *Pm. aleophilum* developed streaks similar in length. The rootstock SO_4 showed a longer streak than the other tested rootstocks, demonstrating the high susceptibility to *Pa. chlamydospora* isolate and *Pm. aleophilum* isolate (Table 5). The results suggest that there are considerable differences between rootstock types with respect to streak formation inside the cuttings. These results are similar to the observations made by Eskalen *et al*. (2001), and may be due to genetic differences between the rootstocks. Similar response has been observed in grapevine cultivars (Khan *et al*. 2000; Feliciano *et al*. 2004).

Table 5. Effects of *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum* on length of the streak formed inside of each cutting in five different grapevine rootstocks.

Treatments	Length of the streak (mm) in rootstocks[1]				
	Kober 5BB	SO4	3309 C	101-14 MG	1103 P
Sterile water	5.2 a	7.2 a	5.2 a	6.2 a	5.4 a
Pa. chlamydospora	35.4 b	71.5 c	35.5 bc	21.0 b	18.3 bc
Pm. aleophilum	33.8 b	77.0 c	43.2 c	23.4 b	22.6 c
Pa. chlamydospora +Pm. aleophilum	24.3 b	37.8 b	25.6 b	22.3 b	17.4 b
Analysis of variance:					
Source	df	F	p		
Rootstocks (R)	4	77.62	< 0.0001		
Treatments (T)	3	135.75	< 0.0001		
R x T	12	12.93	< 0.0001		

[1]Mean of twenty replicates. Results followed by the same letters in the column and rows show non-significant differences according to Tukey's multiple range test (p = 0.05).

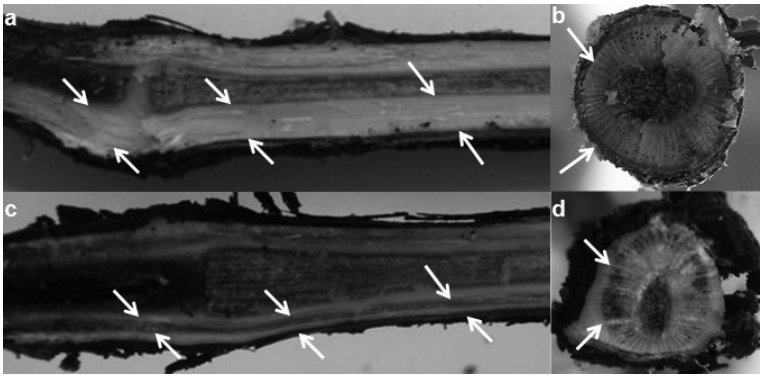

Figure 2. Longitudinal section (a, c) and cross section (b, d) of cuttings of grapevines Kober 5BB. (a, b) Control treatment (sterile water) without internal dark streaking and (c, d) cuttings inoculated with Pa. chlamydospora with internal dark streaking of the wood. Arrows indicate apparently healthy tissue (above) and damage tissue (below).

Resumen

G.A. Díaz, M. Esterio y J. Auger. 2009. Efecto de *Phaeomoniella chlamydospora* y *Phaeoacremonium aleophilum* sobre portainjertos de vid. Cien. Inv. Agr. 36(3):381-390. Se inocularon cinco portainjertos de vid con una suspensión conidial (20 µL de aproximadamente $5x10^3$ conidia·mL^{-1}) de *Phaeomoniella chlamydospora*, *Phaeoacremonium aleophilum* y una mezcla de ambos patógenos, mediante una perforación en la base de cada estaca. Estos hongos endófitos afectaron todo los parámetros de calidad en cada uno de los portainjertos, siendo menos susceptible el 1103 P y 101-14 MG. El portainjerto SO4 fue el que presentó la mayor susceptibilidad a estos hongos endófitos. Los portainjertos Kober 5BB y 3309 C presentaron una susceptibilidad intermedia y los portainjertos 1103 P y 101-14 MG se comportaron como los menos susceptibles bajo las condiciones de este estudio.

Palabras clave: Enfermedad de Petri, enfermedades fungosas, hongos de la madera, hongos endófitos, *Vitis vinifera*.

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