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RESEARCH PAPERS - 10TH SPECIAL ISSUE ON GRAPEVINE TRUNK DISEASES

Endotherapy of infected grapevine cuttings for the control of *Phaeomoniella chlamydospora* and *Phaeoacremonium minimum*

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Summary. The pathogens *Phaeomoniella chlamydospora* and *Phaeoacremonium minimum* are associated with different syndromes of the esca disease complex affecting grapevine propagation material, and young and adult plants. Infections by these fungi occur in grapevine nurseries and in vineyards, with disease control strategies providing limited protection in both cases. Several chemicals are effective *in vitro* against these two pathogens, but treatment of infected plants, especially endotherapy, has not yet proven satisfactory. Five chemicals (elemental silver, fosetyl-Al, glutaraldehyde, hydrogen peroxide and Blad-containing oligomer) were tested *in vitro*, with the first four also tested *in planta*, by means of endotherapy, against *Pa. chlamydospora* and *Pm. minimum*. All chemicals were effective *in vitro* for preventing growth of both pathogens, at different concentrations. Endotherapy of rooted grapevine cuttings (cv. Touriga Nacional) was effective against *Pa. chlamydospora* for all the tested chemicals, with reductions in the frequency of re-isolation of this pathogen of 91–95% (glutaraldehyde), 68–96% (hydrogen peroxide), 68–77% (elemental silver) and 58–59% (fosetyl-Al) when compared with the water-treated experimental controls. The only treatment that was effective against *Pm. minimum* was glutaraldehyde, providing a 75–83% reduction in re-isolation frequency. These results indicate that endotherapy of young grapevines during early stages of infection may be an effective control strategy, especially against the wood pathogen *Pa. chlamydospora*.

Key words: tracheomycosis, grapevine trunk diseases, fungicides, chemical control, endotherapy.

Introduction

Phaeomoniella (*Pa.*) *chlamydospora* and *Phaeoacremonium* (*Pm.*) *minimum* are tracheomycotic ascomycetes involved in the esca disease complex, a major trunk disease of grapevines (*Vitis vinifera* L.) (Gramaje *et al.*, 2018). They are directly responsible for three syndromes, affecting rooted cuttings (brown wood streaking), young grapevines (Petri disease) and adult plants (grapevine leaf stripe disease, GLSD). They also play a role in the development of the 'esca proper' syndrome, in which their presence occurs simultaneously with wood rotting basidiomycetes (e.g. *Fomitiporia mediterranea*) (Surico, 2008; 2009). Primary internal symptoms develop in grapevine wood, where these fungi cause brown wood streaking, black dots and necroses. However, plants may remain externally asymptomatic for a number of years after infections have taken place (Mugnai *et al.*, 1999; Sparapano *et al.*, 2001). Overall, affected plants suffer reduced vigour, shortened lifespan, and reduced grape quality and yield (Bertsch *et al.*, 2013; Fontaine *et al.*, 2016).

Over the last 20 years, several studies have identified fungicides, biocontrol agents and natural compounds that are capable of inhibiting growth of *Pa. chlamydospora* and *Pm. minimum in vitro* and in grapevines. However, to date, control methods have only achieved partial protection through integrated management approaches, in which sanitation methods are also of major relevance (Bertsch *et al.*, 2013; Gramaje *et al.*, 2018; Mondello *et al.*, 2018). There are two main disease management strategies. The first is against tracheomycotic fungi inside the wood of grapevines,

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which is a challenging task, as it is difficult to reach the pathogens with fungicides and other compounds. The second is to prevent new infections, due to the widespread occurrence of these pathogens as epiphytes or in the endosphere of grapevine propagation material (Larignon and Dubos, 2000; Rego *et al.*, 2000; Zanzotto *et al.*, 2001; Ridgway *et al.*, 2002; Fourie and Halleen, 2004), as well as in the air (Gramaje *et al.*, 2018), soil (Rooney *et al.*, 2001; Whiteman *et al.*, 2002), arthropods (Edwards *et al.*, 2001) and other plant hosts (Díaz and Latorre, 2014). For the control of tracheomycotic/esca infections in the field, moderate results have been obtained by spraying leaf symptomatic grapevines with a copper formulation (Di Marco *et al.*, 2011a) and with a mixture of calcium chloride, magnesium nitrate and seaweed extract (Calzarano *et al.*, 2014), achieving reduced expression of the frequency of foliar symptoms. However, trunk injections of active ingredients performed in adult plants with established infections gave mainly negative results (Calzarano *et al.*, 2004; Sentenac *et al.*, 2005; Loskill *et al.*, 2006; Darrieutort and Pascal, 2007). These studies that focused on endotherapy, based observations exclusively on the appearance of leaf symptoms to assess treatment effects, and did not examine changes in the presence of pathogens in the wood pre- or post-treatment, leaving questions on the true efficacy of endotherapy.

The aims of the present study were: (i) to test promising chemicals *in vitro* against *Pa. chlamydospora* and *Pm. minimum*, and (ii) to test endotherapy for the control of these pathogens in the wood of artificially infected rooted grapevine cuttings.

Materials and methods

Fungal isolates, chemicals tested and plant material

The fungal isolates used in this study were *Pa. chlamydospora* CBS 161.90 and *Pm. minimum* CBS 110713, from the CBS culture collection (Westerdijk Fungal Biodiversity Institute, Netherlands). Stock cultures were maintained in Petri dishes containing potato dextrose agar (PDA; Difco™), at 25°C, in the dark.

The chemicals examined in this study, and the range of tested concentrations, are listed in Table 1.

Rooted cuttings of *Vitis vinifera* L. cv. Touriga Nacional were used in greenhouse experiments. For the first year experiment (EXP 1), one-year-old canes were sampled in a vineyard in the Azeitão region (Portugal), and left in a cold-room (4°C) for 2 months. Three-bud cuttings were rooted in a warm bench, at 24°C, and then potted in a mixture of peat and sand. For the second year experiment (EXP 2), three-bud cuttings, rooted and potted in a mixture of peat and sand, were provided by the Viveiros VitiOeste nursery (Pó, Portugal). Rooted cuttings were grown and maintained under greenhouse conditions at an average temperature of 24°C. Plants were treated fortnightly with meptyldinocap (35.7% w/w) or sulfur wettable powder (80% w/w) to prevent powdery mildew (*Erysiphe necator*).

Minimum inhibitory concentration (MIC) of the chemicals tested

To determine the lowest concentration of active ingredient required to prevent visible fungal growth, experiments were performed as a modified version of that described by Kuipers *et al.* (1999).

(i) Separate conidia suspensions of *Pa. chlamydospora* or *Pm. minimum* were prepared, by flooding 14-d-old cultures of each pathogen with sterile distilled water (SDW), and dislodging the conidia from the mycelium with sterile glass rods. The suspension of each fungus was filtered through a double layer of cheesecloth, the conidia concentration was determined using a hemocytometer, and then adjusted to 1×10^5 conidia mL^{-1} with sterile distilled water (SDW).

(ii) The liquid growth medium was a solution of potato dextrose broth (PDB).

(iii) The five chemicals examined in this study as antifungal agents were tested at 11 concentrations, and their respective dilution ranges are presented in Table 1. Each tested concentration was a sequential three-fold dilution of the previous solution, commencing from the most concentrated solution (e.g. 1.000, 0.333, 0.111, 0.037 … 1.69×10^{-5} g a.i. L^{-1}).

Fungal growth occurred in 96-well culture plates (flat bottom). Each well contained equal volumes (80 µL) of pathogen, liquid growth medium and candidate chemical. Positive controls contained a combination of equal volumes (80 µL) of conidia suspension, PDB and SDW; negative controls contained equal volumes (80 µL) of PDB, candidate chemical at its greatest concentration and SDW.

The 96-well culture plates were sealed with Parafilm®, and the cultures left to grow at 25°C, in the dark, for 72 h, during which the conidia would

Table 1. Chemicals tested against *Phaeomoniella chlamydospora* (Pch) and *Phaeoacremonium minimum* (Pmin), the range of concentrations of active ingredients (a.i.) tested *in vitro*, and the minimum inhibitory concentrations (MICs) of each chemical.

Active Ingredient	Trade Name	Manufacturer	Formulation	*In vitro* conc. max – min (g a.i. L^{-1})	MIC (g a.i. L^{-1})	
					Pch	Pmin
Blad-containing oligomer (BCO)	Fracture®	CEV/ CONVERDE	20% (v/v) BCO	$1.00 – 1.69\times10^{-5}$	0.037	0.111
Elemental silver	BioBac®	M.H.I Compania de Ingenerie	1000 ppm Elemental silver	$0.012 – 2.00\times10^{-7}$	0.004	0.012
Fosetyl-Al	Aliette Flash®	Bayer	74.6% (w/w) Fosetyl-Al	$0.667 – 1.13\times10^{-5}$	0.222	0.222
Glutaraldehyde*	-	VWR chemicals	25% (v/v) glutaraldehyde	$14.88 – 2.52\times10^{-4}$	0.061	0.020
Hydrogen peroxide	-	Sigma-Aldrich	30% (v/v) hydrogen peroxide	$11.10 – 1.88\times10^{-4}$	0.015	0.046

* The glutaraldehyde solution was activated by increasing pH to 8, using a solution of $NaHCO_3$ (0.93% w/v) (Gorman and Scott, 1977).

be mostly germinated (Pierron *et al.*, 2016). Fungal growth was measured spectrophotometrically, targeting turbidity of the solution (optical density [$\lambda = 630$ nm]; Kuipers *et al.*, 1999). Samples were analysed in a microplate reader (BIO-TEK Synergy HT) with BioTek GEN5 Data Analysis Software. Each treatment was applied in four replicates, and the experiment was repeated once.

Pathogen inoculation and endotherapy

In greenhouse assays, each grapevine stem was surface-disinfected with 70% ethanol. An artificial wound was then made by drilling a hole in the bark of the stem (4 mm diam., 4 mm depth), below the upper bud (Figure 1a).

Inoculation of *Pa. chlamydospora* or *Pm. minimum* was performed by inserting in the wound either a mycelium plug (EXP 1) or a conidia suspension (EXP 2). In the first case, the mycelium plug (4 mm diam.) was cut from the margin of a 2-week-old colony, to minimize the presence of conidia, and was then placed in the stem hole with the mycelium facing the inner part of the stem. The inoculated wound was covered with moist cotton and wrapped tightly to the plant with Parafilm®. In the second experiment, 50 µL of conidia suspension (1×10^5 conidia mL^{-1}), prepared from 2-week-old colonies, were deposited in the stem hole, which was then covered with Parafilm® for protection during the incubation period.

Three months after inoculation, each grapevine cutting was injected with 1 mL of candidate chemical solution (or SWD for the controls), with the aid of a modified syringe, into a hole made 3 cm below the pathogen inoculation point. Each hole (4 mm diam., 5 mm deep; Figure 1, b-c) was made using a drill. The drill bit was disinfected with ethanol (70%) and NaClO solution (0.5% w/w active chlorine) after each use. Each treatment consisted of ten biological replicates. Preliminary tests, using a food-colouring agent dissolved in water, showed that the injected liquid diffused throughout the xylem vessels of the plants (data not shown). Fluids were absorbed into the stem wood, on average, within 5 h from the moment the syringes were applied to the rooted cuttings.

Injection of BCO was unsuccessful, probably due to the large size of this oligomer, which prevented the BCO solution from entering the xylem of the plants. Application of BCO in endotherapy will not be further discussed in this paper.

Examination of rooted cuttings, and re-isolation of inoculated pathogens

One month after treatment, the green shoot length of each plant was measured, and the plants were in-

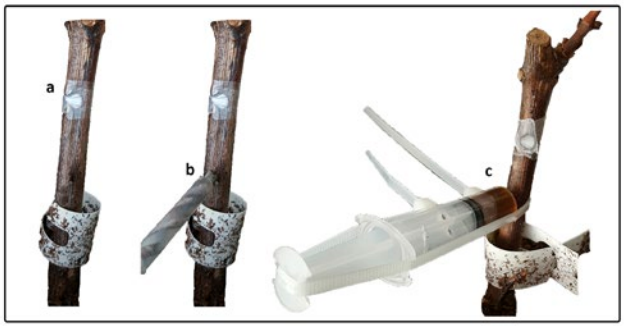

Figure 1. Schematic illustration of treated grapevine cuttings. Pathogen inoculation, via mycelium plugs or conidia suspensions, occurred at point (a). For endotherapy, a drill hole (b) was made 3 cm below point (a) and a modified syringe was placed in the hole (c) to force chemicals into the stem xylem.

spected for the appearance of symptoms attributable to pathogens infection or phytotoxicity from the treatments applied, both in leaves and in the stem. Symptoms such as brown streaking and other wood alterations were recorded.

Pieces of wood were collected 1.5 cm below the pathogens inoculation point, they were surface sterilized using flame, immersed in NaClO solution (0.5% w/w active chlorine) for 1 min, then double rinsed in sterile water, and plated onto PDA supplemented with chloramphenicol (250 mg L^{-1}) in Petri dishes. Resulting fungus colonies emerging from the wood pieces were identified, and their frequency of re-isolation was calculated as follows: 100 × (number of wood pieces from which a pathogen was re-isolated / total number of pieces plated).

Wounded but non-inoculated and non-treated plants, representing negative controls, were screened for the presence of wood discoloration and background infections of *Pa. chlamydospora* and/or *Pm. minimum*.

Data analyses

Minimum inhibitory concentrations (MICs) were determined as outlined by Kuipers *et al.* (1999). The least concentration of each active ingredient capable of preventing fungal growth was selected as the MIC. The MICs were recorded for both pathogens, and in all treatments these were identical among replicates and between the repetitions of the test, so no averaging was necessary.

All other data were compared using analyses of variance (ANOVA) followed by Tukey´s *post hoc* tests (at $P < 0.05$; GraphPad Prism 7.05). Data obtained from EXP 1 and EXP 2 were analyzed separately, as they used different inoculum types and were carried out in different years. In both cases, three different parameters were examined: shoot length, length of brown wood streaking, and frequency of re-isolation of the inoculated pathogens. For each parameter, a two-way ANOVA was performed, examining the factors 'pathogen' (*Pa. chlamydospora* or *Pm. minimum*) and 'treatment' (water control, elemental silver, fosetyl-Al, glutaraldehyde or hydrogen peroxide). Data expressed as percentages, as for frequency of re-isolation, were arcsine-square root transformed before analyses.

Results

Minimum inhibitory concentrations

All the chemicals studied prevented *in vitro* growth of *Pa. chlamydospora* and *Pm. minimum*, but at different concentrations (Table 1). MICs indicated that *Pa. chlamydospora* is more sensitive than *Pm. minimum* to most chemicals tested, with the exception of fosetyl-Al (identical MICs for this fungicide) and glutaraldehyde (lower MIC for *Pm. minimum*). The *in vitro* inhibitory effects of the tested chemicals against these pathogens is reported in here for the first time.

Examination of grapevine cuttings

Four months after inoculation of the rooted grapevine cuttings with *Pa. chlamydospora* or *Pm. minimum*, and 1 month after treatment with each of the chemicals under study, except for BCO, no leaf symptoms attributable to the wood pathogens were observed. In addition, no phytotoxicity symptoms in leaves were observed for any treatment, except for glutaraldehyde, where 10% of the plants exhibited leaf wilting on the two lower internodes of the green shoots. The shoot lengths of grapevine plants were not statistically affected ($P > 0.05$) by the 'treatment' and 'pathogen' factors analyzed, both for EXP 1 and EXP 2, so data were pooled for presentation in Table 2.

For lengths of brown wood streaking, two-way ANOVA followed by Tukey's *post hoc* tests revealed no statistically significant differences ($P > 0.05$) for factors 'pathogen' and 'treatments'. However, the

Table 2. Mean shoot lengths, extents of brown wood streaking and wood discolouration, and reductions in frequencies of pathogen re-isolation, after injection of different chemicals into rooted grapevine cuttings (cv. Touriga Nacional) that had been inoculated with *Phaeomoniella chlamydospora* (Pch) or *Phaeoacremonium minimum* (Pmin).

Treatment	Conc. (g a.i. L^{-1})	Shoot length* (mm)		Brown wood streaking (mm)				Wood discoloration near injection point* (mm)		Reduction in frequency of re-isolation (%)			
				EXP 1		EXP 2				EXP 1		EXP 2	
		EXP 1	EXP 2	Pch	Pmin	Pch	Pmin	EXP 1	EXP 2	Pch	Pmin	Pch	Pmin
Water control	-	1000 a	928 a	102 b	120 b	62 c	69 c	<1.0	<1.0	-	-	-	-
Blad-containing oligomer (BCO)	-	-	-	-	-	-	-	-	-	-	-	-	-
Elemental silver	0.250	1028 a	938 a	103 b	118 b	59 c	69 c	<1.0	<1.0	68.4	12.4	77.3	0.00
Fosetyl-Al	0.250	1019 a	941 a	-	-	62 c	63 c	<1.0	<1.0	57.9	0.00	59.1	12.5
Glutaraldehyde	0.318	1029 a	933 a	111 b	118 b	64 c	67 c	12.4 d	12.2 d	94.7	75.0	90.9	83.3
Hydrogen peroxide	0.333	990 a	934 a	109 b	108 b	62 c	67 c	6.6 e	7.0 e	68.4	56.3	95.5	37.5

* Data presented for 'Shoot length' and 'Wood discoloration near injection point' are averages for the two inoculated pathogens, as no statistically significant differences ($P > 0.05$) were detected between them.
Numbers followed by the same letter in each column do not statistically differ ($P > 0.05$), according to Tukey's test.

different inoculum types that characterized EXP 1 (mainly fresh mycelium) and EXP 2 (conidia only) produced different lengths of streaking (Table 2), which was presumably due to the different wood colonization rates by mycelium and conidia. Negative controls, non-inoculated and non-treated, did not present wood streaking attributable to wood pathogen infections.

Examination of the wood surrounding the chemicals injection point revealed no wood discoloration or necrosis for the injections with water, fosetyl-Al or elemental silver, while different degrees of wood discoloration were evident after treatments with hydrogen peroxide or glutaraldehyde (Table 2), highlighting some phytotoxicity effects of these chemical at the tested concentrations.

Re-isolation of pathogens

The frequency of re-isolation of the inoculated pathogens was chosen to determine how effective each treatment had been. Two-way ANOVA showed that the factors 'pathogen' (*Pa. chlamydospora*, *Pm. minimum*; $P < 0.05$) and 'treatments' ($P < 0.01$) had statistically significant effects on the re-isolation of the pathogens, for both EXP 1 and EXP 2, and the two factors interaction effect was also significant ($P < 0.05$) in EXP 2. *Pa. chlamydospora* was affected by all treatments, and its frequency of re-isolation was significantly lower than in the water-treated plants for three of the four treatments. The most effective chemical for the control of *Pa. chlamydospora* was glutaraldehyde, resulting in 90.9–94.7% reduction in re-isolation frequency, although this was not significantly different from hydrogen peroxide (68.4–95.5% reduction) or elemental silver (68.4–77.3%) (Table 2; Figure 2 A, B). The fosetyl-Al treatment also reduced the re-isolation of *Pa. chlamydospora*, although not to a statistically significant extent.

Pm. minimum was unaffected by elemental silver or fosetyl-Al, and only mildly affected by hydrogen peroxide, while a significant reduction in frequency of re-isolation of this pathogen was observed from the glutaraldehyde treatment. In this case, treatment with glutaraldehyde resulted in a 75.0–83.3% reduction in re-isolation of the pathogen (Table 2; Figure 2 C, D).

The examination of negative controls did not reveal background infections of *Pa. chlamydospora* or *Pm. minimum*.

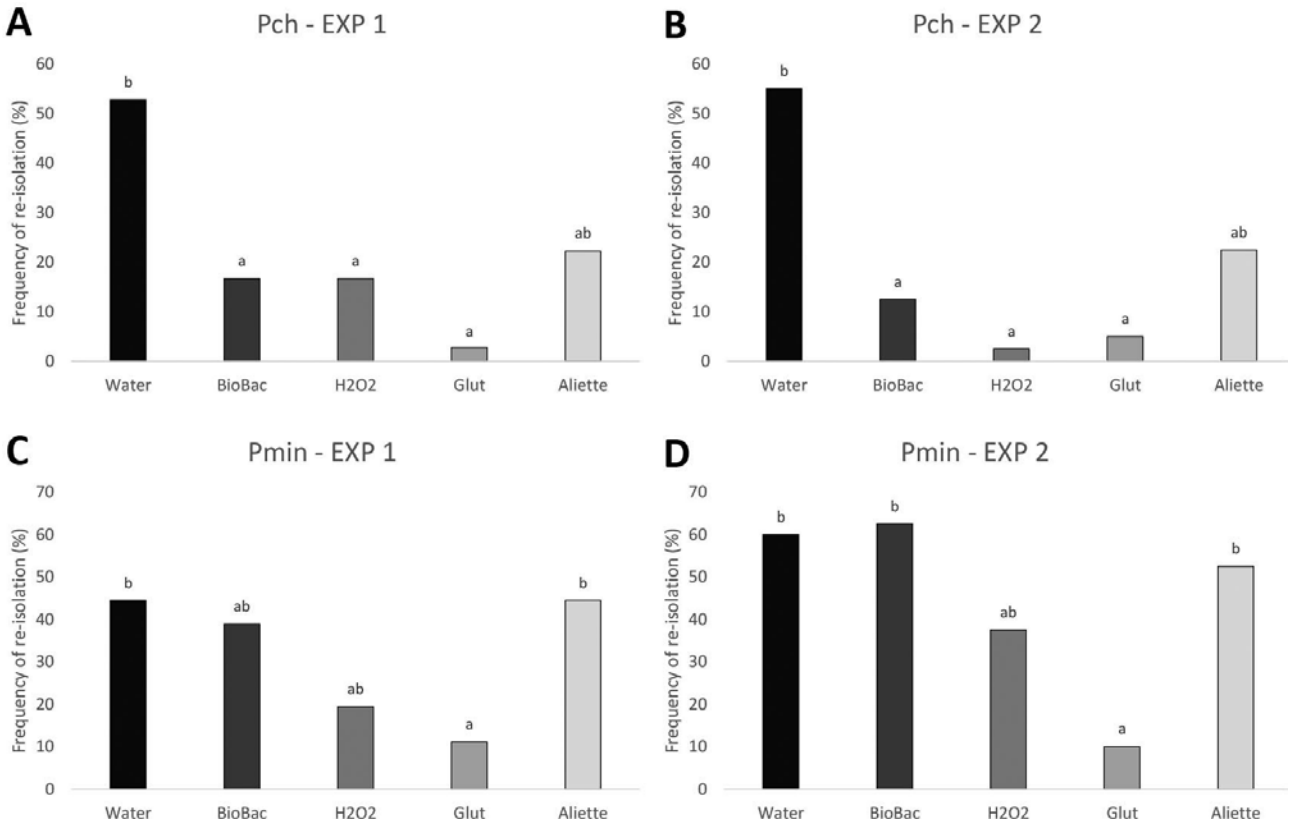

Figure 2. Frequency of re-isolation (%) of *Pa. chlamydospora* (Pch; A, B) or *Pm. minimum* (Pmin; C, D), 1 month after endotherapy with either water (negative control), elemental silver (BioBac), hydrogen peroxide (H_2O_2), glutaraldehyde (Glut) or fosetyl-Al (Aliette). The experiment conducted in 2016 (EXP 1) used mycelium plug inoculum (A, C), while that in 2017 (EXP 2) used conidia inoculum (B, D). Bars accompanied by the same letter do not differ significantly ($P < 0.05$) according to Tukey's test.

Discussion

A considerable number of chemicals and biocontrol agents have been tested against esca-related tracheomycotic pathogens over the last 20 years (Mondello *et al.*, 2018). Many chemicals have been identified as effective *in vitro*, against conidia germination, and for reduction of mycelium growth of *Pa. chlamydospora* and *Pm. minimum*, with follow-up studies on the application of chemicals under greenhouse conditions and in the field. Despite these attempts, effective control of esca-associated tracheomycotic pathogens has not been achieved.

Screening of chemicals as antifungal agents *in vitro*

The majority of *in vitro* studies performed to test fungicidal activity against *Pa. chlamydospora* or *Pm. minimum* have been conducted on solid growth media, measuring the growth inhibition of inoculated mycelium (Mondello *et al.*, 2018). In the present study, a different approach was selected, to deal with chemicals whose activity is negatively affected by dilution in solid media or long incubation periods (e.g. hydrogen peroxide, glutaraldehyde or BCO).

This study expands the list of chemicals assayed against grapevine trunk diseases (Gramaje *et al.*, 2018; Mondello *et al.*, 2018) with new active ingredients capable of inhibiting visible growth of *Pa. chlamydospora* and *Pm. minimum*.

Blad-Containing oligomer (BCO) is a high molecular weight oligomer (210 kD) extracted from the cotyledons of *Lupinus albus* L. plantlets. It is non-toxic and it has a broad-spectrum fungicidal activity (Monteiro *et al.*, 2015; Carreira *et al.*, 2018), having multisite activity (Pinheiro *et al.*, 2016; Pinheiro *et al.*, 2017).

With these characteristics, it was included in the 2017 FRAC code list as BM 01.

Silver nanoparticles-based chemicals have been shown to be effective against bacteria (Morones *et al*., 2005) and several plant pathogenic fungi (Kasprowicz *et al*., 2010; Kim *et al*., 2012; Patel *et al*., 2014). Their modes of action are yet to be fully understood but they are known to negatively interact with fungal cell membrane structure and functions (Lamsal *et al*., 2011).

Fosetyl-aluminium (fosetyl-Al) acts primarily as elicitor of plant defence responses (Di Marco *et al*., 2011b). This fungicide has also been tested, *in vitro*, against *Pa. chlamydospora*, but no fungicidal activity has been recorded for concentrations up to 5 mg a.i. L^{-1} (Groenewald *et al*., 2000). In the present study we have demonstrated that fosetyl-Al prevents the growth of *Pa. chlamydospora* and *Pm. minimum*, albeit at a higher concentration (222.2 mg a.i. L^{-1}).

No studies describe fungicidal activity of glutaraldehyde against *Pa. chlamydospora* or *Pm. minimum*, although this is a well-known antimicrobial agent (Gorman and Scott, 1977; Baldry, 1983; Migneault *et al*., 2004).

The use of hydrogen peroxide against *Pa. chlamydospora* and *Pm. minimum* is known, but has not been assayed *in vitro*, and results were inconsistent when this compound was tested as a soak-treatment of propagation grapevine material in nursery conditions (Fourie and Halleen, 2006). Despite unpredictable results, some winegrowers are already using injections of hydrogen peroxide into grapevine trunks to control grapevine trunk diseases, but more testing is required before this technique can be fully recommended (Prezman, 2017).

Endotherapy of infected rooted grapevine cuttings

Despite the promising results *in vitro*, previous *in planta* tests have been discouraging, especially for endotherapy (Calzarano *et al*., 2004; Sentenac *et al*., 2005; Loskill *et al*., 2006; Darrieutort and Pascal, 2007). The present study focused on applying some of the tested chemicals as endotherapy treatments, for the control of two grapevine pathogens in their wood environment. Injecting bioactive molecules into plant xylem allows direct interaction between the chemicals and mycelia and conidia of the pathogens, but application to adult plants with established infections finds limitations. The internal xylem surface area is large, the wood is dense and it cannot be easily penetrated by the active ingredients. Moreover, if pathogens are not completely eradicated from plants, they may re-colonize the wood when active ingredient concentrations diminish. Endotherapy of young grapevines allows increased penetration of active ingredients into internal xylem tissues, and control of pathogen infections before the hosts become heavily colonized.

Our results show that colonization by *Pa. chlamydospora* in the wood can be reduced, at least up to one month after treatment, using selected chemicals (Figure 2). Hydrogen peroxide and glutaraldehyde, the most efficient chemicals, caused wood discoloration near the injection points, indicating the need to optimize their use concentrations in future trials. The elemental silver-based chemical (BioBac) considerably reduced the pathogen presence and did not cause phytotoxicity, making it a suitable candidate for the control of *Pa. chlamydospora* in plant wood. *Pm. minimum* was more difficult to control, with glutaraldehyde being the only treatment capable of significantly reducing the presence of this pathogen.

Different responses to endotherapy were observed for the two pathogens, with presence of *Pa. chlamydospora* considerably reduced by all treatments, while *Pm. minimum* was unaffected by most of them. This behaviour is probably not attributable to differences in sensitivity to the injected chemicals, as their concentrations were always equal or greater than their *in vitro* MICs. Histological studies unveiled the different wood colonization strategies of *Pa. chlamydospora* and *Pm. minimum*. *Pa. chlamydospora* occurs mainly in the lumen of xylem vessels and in xylem fibers, having limited capacity to degrade cell wall polymers (Valtaud *et al*., 2009; Pouzoulet *et al*., 2017). *Pm. minimum* can colonize bark, pith, phloem, xylem fibers and vessels, vessel-associated cells, rays, metaxylem and protoxylem (Valtaud *et al*., 2009; Pierron *et al*., 2015). Therefore, endotherapy may be particularly efficient against *Pa. chlamydospora* because the chemicals are transported in host xylem vessels, where the fungus was located. Concerning *Pm. minimum*, although the chemicals may have effectively interacted with this pathogen in the xylem vessels, its presence in several other areas of the stem tissues may have allowed it to remain present in the wood, making endotherapy less efficient against this pathogen.

Conclusions

Although not addressed in this study, the results obtained indicate that some of the tested chemicals

may find application during the grapevine propagation processes in nurseries, for control of external viable propagules of *Pa. chlamydospora* and *Pm. minimum*. These compounds could be used in plant material, on pruning shears and on grafting machines (Retief *et al*., 2006; Gramaje *et al*., 2018), to replace other chemicals reported to be ineffective against *Pa. chlamydospora* and *Pm. minimum*, such as Chinosol (hydroxyquinoline sulfate), one of the most commonly used fungicides in nurseries (Gramaje *et al*., 2009).

We are aware that the process of injecting chemicals into host plants (endotherapy), as described in this study, is time-consuming and may not find application in large-scale nursery production systems or young vineyards. It is necessary to improve chemical delivery technology, to make it rapid and reliable, as could be the case for the instrument developed by Montecchio (2013). Testing whether different concentrations of active ingredients, and/or multiple or mixture treatments, may lead to a complete and long-lasting eradication of these important grapevine pathogens.

Conflict of interest

The authors declare that they have no relevant conflicts of interest.

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