Biological Control 119 (2018) 59–67

Contents lists available at ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon

Efficacy of *P. oligandrum* affected by its association with bacterial BCAs and rootstock effect in controlling grapevine trunk diseases

L. Daraignes[a], J. Gerbore[a,b], A. Yacoub[a], L. Dubois[a], C. Romand[c], O. Zekri[c], J. Roudet[a], P. Chambon[b], M. Fermaud[a,*]

[a] UMR SAVE, INRA, Bordeaux Science Agro, ISVV, 33882 Villenave d'Ornon, France
[b] BIOVITIS, 15400 Saint Etienne de Chomeil, France
[c] NOVATECH, 16 Rue de la Chaignée, 85770 Vix, France

ARTICLE INFO

Keywords:
Biocontrol agents (BCAs)
Oomycete
Phaeomoniella chlamydospora
Neofusicoccum parvum
Pantoea agglomerans
Bacillus pumilus

ABSTRACT

The development of biological control agents (BCAs) is a promising and environmentally friendly method to control plant pathogens. In a grapevine nursery greenhouse and for the first time on grafted cuttings (scion cv. Cabernet sauvignon), this two-year study demonstrated the significant efficacy of the oomycete *Pythium oligandrum* as a BCA against two major aggressive fungal pathogens, *Neofusicoccum parvum* and *Phaeomoniella chlamydospora*, which are involved in grapevine trunk diseases (GTDs). By considering the reduction in necrosis lengths within the scion stem, treatments with *P. oligandrum* alone showed the greatest efficacy against the two pathogens (overall average efficacy of 48.3%). This major result was obtained during the two-year bioassay and in cuttings grafted on the two widely used rootstocks, 101-14 and SO4. The biocontrol efficacies of two bacterial strains previously isolated from vineyards, *Pantoea agglomerans* and *Bacillus pumilus*, were also assessed, separately or in combination with *P. oligandrum*. Treatments with each bacterial strain were less effective than treatments with *P. oligandrum*, and the efficacies were not improved when they were applied in combination with the oomycete.

1. Introduction

Grapevine trunk diseases (GTDs), such as Esca, markedly impact the vine and grape industry worldwide. In France, it has been estimated that GTDs are the reason that approximately 11% of vineyards became unproductive in 2008, and approximately 13% in 2012 (Grosman and Doublet, 2012). Because Esca is the most prevalent GTD in Europe, numerous recent studies have focused on this ancient and ubiquitous disease. Esca is currently generally referred to as Esca Complex because the disease is considered to result from the pathogenic activity of several fungal species, including the ascomycete species *Phaeomoniella chlamydospora*, *Phaeoacremonium minimum* and *Neofusicoccum parvum* and basidiomycete species *Fomitiporia mediterranea* (Larignon, 2012). These pathogenic fungi mostly damage wood tissue, causing various types of necrosis and, in most cases, the death of the plant. As the most distinctive symptoms of Esca are central and black punctuate necrosis and discolored xylem (Lecomte et al., 2012; Maher et al., 2012), necrosis length is the main criteria used to assess the attack rates of pathogens associated with GTDs (Laveau et al., 2009; Pouzoulet et al., 2013). Whereas most studies have focused on non-grafted models of grapevine (Fourie and Halleen, 2004; Haidar et al., 2016a, 2016b; Yacoub et al., 2016a), we proposed to study grafted plantlets because they are the most popular system cultivated in French vineyards.

Developing nursery management strategies that protect cuttings are an essential step in reducing the transmission of grapevine trunk diseases during propagation. Although healthy mother plants are used in nurseries, the grafting process itself poses a risk of contamination (Gramaje and Armengol, 2011). Therefore, a method offering protection against GTDs early in the cultivating process could be of great interest.

Biological control with microbial antagonists can offer an environmentally friendly solution and the use of such organisms isolated from vineyards can limit the negative impacts of other products such as chemicals or hot water treatments (Fourie and Halleen, 2004). Moreover, due to their toxicity to the environment and humans, specific pesticides have been withdrawn from the grapevine market. This notably includes the active fungicide ingredient sodium arsenite, banned in Europe in 2001. Since then, no efficient chemical control measure has been developed against GTDs in vineyards (Alabouvette et al., 2006). One alternative strategy is the use of beneficial microbial agents

* Corresponding author.
E-mail address: marc.fermaud@inra.fr (M. Fermaud).

https://doi.org/10.1016/j.biocontrol.2018.01.008
Received 28 November 2017; Received in revised form 11 January 2018; Accepted 16 January 2018
Available online 31 January 2018
1049-9644/

as biological control agents (BCAs). Some prior studies have shown the direct or indirect antagonistic activity of several species of bacteria, fungi and/or yeasts towards GTD fungal pathogens (Compant et al., 2012; Bertsch et al., 2013). However, only a few are currently registered as BCAs and commercialized in Europe, according to the EU pesticides database (Ec.europa.eu., n.d.). To select and use a BCA, it is important to note that (i) its efficiency can vary depending on how it is tested and/or screened (laboratory *versus* field conditions); (ii) selected strains must be able to survive and remain within the system (ecosystem, host tissues) they help to protect; and (iii) they need to be compatible with other cultural techniques, notably chemical agricultural inputs (Alabouvette et al., 2006). To maximize the effect of a BCA, an interesting approach is to aim for a synergetic effect between species or strains that display different types of interaction (Compant et al., 2012).

Pythium oligandrum is an oomycete that is naturally present in the rhizosphere of vines (Gerbore et al., 2014b) that has been identified as a helpful organism because of its ability to protect plants from pathogenic attacks. This ability has been demonstrated in several reports since 1986 on a range of crop plants in small-scale field, soil and greenhouse trials (Rey et al., 2008; Benhamou et al., 2012; Gerbore et al., 2014a). The biological control exerted by the chromista species *P. oligandrum* is the result of a complex process, which includes direct effects on pathogenic fungi (mycoparasitism and antibiosis) and plant defense stimulation (Rey et al., 2008; Benhamou et al., 2012). Recently, Yacoub et al. (2017) explored the interaction between grapevine and *P. oligandrum*, showing that significant changes in the grapevine root transcriptome occurred following root colonization by the oomycete. The main changes concerned genes associated with responses to stimuli, and the observed relationship presented similarities with a symbiotic microorganism/root interaction. Moreover, a preliminary greenhouse assay demonstrated that necrosis caused by *P. chlamydospora* in Cabernet Sauvignon grapevine cuttings was reduced by approximately 35% following *P. oligandrum* colonization of the root system (Yacoub et al., 2016a). However, this result was not statistically significant, and the authors did not compare different rootstocks as possibly interacting with *P. oligandrum* BCA efficacy.

Previous recent studies characterized and screened various bacterial strains isolated from Bordeaux vineyards for their antagonistic activity against *N. parvum*, *P. chlamydospora* and *B. cinerea* (Haidar et al., 2016a, 2016b). Among them, *P. agglomerans* (S1) was able to significantly reduce the necrosis length caused by *N. parvum* on ungrafted cutting stems, associated with an intermediate efficiency profile against *B. cinerea*. In similar experimental conditions, *Bacillus pumilus* (S32) and *Paenibacillus* sp (S19) were at the origin of a significant reduction of approximately 30% of the necrosis size caused by *P. chlamydospora* (Haidar et al., 2016b). Therefore, *B. pumilus* (S32) was described as triggering a systemic immune response in grapevine. However, these studies did not investigate potential synergy, interference and/or interaction between two different BCA bacterial strains and they were always carried out in ungrafted plant material.

The major objectives of this study were to quantify and compare the BCA efficacy of *P. oligandrum* under advanced experimental nursery greenhouse conditions: 1) against two aggressive GTD pathogens of prime importance, *N. parvum* (Np) and *P. chlamydospora* (Pch), notably because Po has never been tested against Np; and 2) using two types of grafted plant materials, either the SO4 or the 101-14 rootstock genotype. Similarly, the study aimed at confirming, quantifying and comparing the BCA efficacy of two bacterial strains against these two pathogens, either separately or in combination with *P. oligandrum*. The last complementary objective was to assess the ability of *P. oligandrum* to colonize the root system of the grafted grapevine cuttings following its soil application. This experiment was carried out over two years in a commercial nursery greenhouse.

Table 1
Properties of selected bacterial strains.

Code	Species identification	Origin from grapevine host organ	Targeted pathogen species
S1	*Pantoea agglomerans*	Berries surface	*N. parvum*
S32	*Bacillus pumilus*	Trunk	*P. chlamydospora*

2. Materials and methods

2.1. Microorganisms and media/bacterial and fungal preparations

2.1.1. Pythium oligandrum

P. oligandrum strain “Sto 7” inoculum was prepared through a specific fermentation process and provided by BIOVITIS (Gerbore et al., 2014b). The concentration of the final product was adjusted to 6×10^3 oospores/ml.

2.1.2. Bacterial strains

Two bacterial strains used as BCAs were obtained from the collection of UMR SAVE, INRA, Bordeaux (Table 1). They were previously isolated from French vineyards and screened on various grapevine host organs (Haidar et al., 2016a). In this study, they were used specifically for controlling a GTD pathogen, either *N. parvum* or *P. chlamydospora*. The bacterial strains were grown on TSA (trypticase soy agar) in Petri dishes at 28 °C.

2.1.3. Pathogen strains

The *N. parvum* isolate “Cou 02” was selected from the INRA-UMR 1065 SAVE collection, Bordeaux. This strain was originally obtained in 2008 from a Cabernet Sauvignon grapevine in an experimental INRA vineyard near Bordeaux and was characterized as highly aggressive in previous preliminary studies at INRA (Haidar et al., 2016a, 2016b). *P. chlamydospora* strain “SO44” was selected from the INRA-UMR 1065 SAVE collection, Bordeaux. This strain was originally obtained in 1996 from a Cabernet Franc grapevine in Moncaup, France. It was characterized as highly aggressive in previous studies at INRA (Laveau et al., 2009). The strains were subcultured on Malt Agar (MA) medium and incubated at 22 °C for one month (12 h light/12 h dark). They were retrieved as 4 mm plugs the day before artificial inoculation in bioassays.

2.2. Plant material

For each experiment, grapevine plantlets (*Vitis vinifera* L., cv. Cabernet Sauvignon) were propagated from 2-node woodcuttings in a greenhouse. Plants were grafted on rootstock 101-14 (1st and 2nd year) and SO4 (2nd year), selected because of their potential difference in susceptibility to esca (Liminana et al., 2009). The cuttings were rooted for 30–45 days before infection and grown under controlled conditions (Table 2). The temperature was maintained between 22 and 28 °C. The plants were watered for 2 min per day via a drip system (2 L/h) and fertilized twice a week (nutrient solution N/P/K 20/20/20). Plantlets were drilled a week before the first application. The hole was made on the scion trunk 1 cm below the second node. It was then covered by a layer of protective film (Cellofrais®) to prevent external contamination.

2.3. Experimental design

2.3.1. 1st year bioassay

In the 1st year bioassay, all plants were grafted on rootstock 101-14. Treatments carried out in the 1st year bioassay, as shown in Table 3, included a non-inoculated control: “Y1.Drilled”. Only in the first year assay, another supplementary negative control was “Y1.Non-drilled”, which was not inoculated or drilled. The controls “Y1.Np” and “Y1.Pch”

Table 2
Chronology of bioassays.

Bioassay	Grafting	Drilling	Application of *P. oligandrum*	Application of *P. oligandrum* and/or bacterial strains	Pathogen inoculation	Assessment of necrosis*
1st year	- 30 days	- 14 days	- 7 days	- 1 h 30 min	0	90 dpi (Np) 103 dpi (Pch)
2nd year	- 45 days	- 7 days	- 7 days	- 1 h 30 min	0	150 dpi

* dpi: days post-inoculation with the pathogen.

Table 3
Experimental design of the 1st year bioassay (Y1 = 1st year; bold = treatments in which roots were used for *P. oligandrum* quantification).

	Pathogen	Biotization agent			Treatments
		Po	S32	S1	
Phaeomoniella chlamydospora (*Pch*)	−	+	−	−	**Y1.Po**
	+	+	−	−	**Y1.Po + Pch**
	+	−	−	−	**Y1.Pch**
	−	−	+	−	Y1.S32
	−	+	+	−	**Y1.Po + S32**
	+	+	+	−	**Y1.Po + S32 + Pch**
Neofusicoccum parvum (*Np*)	+	−	−	−	Y1.Np
	−	−	−	+	Y1.S1
	+	−	−	+	Y1.S1 + Np
	+	+	−	−	Y1.Po + Np
	+	+	−	+	Y1.Po + S1 + Np
Non-inoculated controls	−	−	−	−	Y1.Non-drilled
	−	−	−	−	Y1.Drilled

were inoculated only with the pathogen *N. parvum* or *P. chlamydospora*, respectively. All treatments and controls were drilled beforehand (except for the "Y1.Non-drilled" treatment) in order to compare strictly all the treatments because the drilling step is required for successful artificial inoculations by the pathogens.

2.3.2. 2nd year bioassay

All treatments for the 2nd year bioassay, shown in Table 4, were applied either on cuttings grafted on rootstock 101-14 (Y2.R1) or on rootstock SO4 (Y2.R2). Treatments "Y2.R1" and "Y2.R2" were non-inoculated controls. Treatments "Y2.R1.Np" and "Y2.R2.Np" were controls inoculated with *N. parvum* only for each rootstock. "Y2.R1.Pch" and "Y2.R2.Pch" were controls inoculated with *P. chlamydospora* only for each rootstock. All treatments and controls were drilled beforehand.

2.4. BCAs applications and pathogens inoculations

BCA application processes are displayed in Table 2. For *P. oligandrum*, there were two series of applications and a pathogen inoculation. The second BCA application took place a week (seven days) after the first one, and approximately 1 h 30 min before pathogen inoculation. For the bacterial strains, the application took place approximately 1 h 30 min before pathogen inoculation.

2.4.1. Application of Pythium oligandrum

First, 50 ml of BIOVITIS suspension (*P. oligandrum*) was applied to the soil by drenching right under the surface of the grapevine cutting. The input was estimated at $3x10^5$ oospores for each pot. A second application was carried a week after the first one under the same conditions. The inoculum amounts were the same for both years.

2.4.2. Application of bacterial strains

Twenty-four hours before bacterial application, the bacterial inoculum was cultured on TSB at 27 °C to achieve a high concentration of approximately 10^7 UFC/ml. For each treatment, 30 µl of bacterial suspension was inoculated in the drilled hole in the scion stem. Two layers of protective film (Cellofrais®) were used to close the application site. The estimated amounts of inoculums were the same for both years.

2.4.3. Inoculation of pathogens

The pathogens were inoculated at a single time point, referred to as 0 days post-inoculation (dpi). A scalpel was used to retrieve plugs from *P. chlamydospora* or *N. parvum* on Petri dishes and the plug was inoculated in the trunk's drilled hole. Two layers of protective film (Cellofrais®) were used to close the site.

2.5. Assessments

2.5.1. BCAs efficacy by evaluation of stem necrosis

To assess the control efficacy of the BCAs against the two GTD pathogens, the length of stem necrosis in the grapevine wood was

Table 4
Experimental design of the 2nd year bioassay (Y2 = 2nd year; R1 = rootstock 101-14 and R2 = rootstock SO4; bold = treatments in which roots were used for *P. oligandrum* quantification).

	Pathogen	Biotization agent			Treatments on rootstock 101-14	Treatments on rootstock SO4
		Po	S32	S1		
Phaeomoniella chlamydospora (*Pch*)	−	+	−	−	**Y2.R1.Po**	Y2.R2.Po
	−	−	+	−	Y2.R1.S32	Y2.R2.S32
	−	+	+	−	**Y2.R1.Po + S32**	Y2.R2.Po + S32
	+	−	−	−	**Y2.R1.Pch**	Y2.R2.Pch
	+	+	−	−	**Y2.R1.Po + Pch**	Y2.R2.Po + Pch
	+	−	+	−	Y2.R1.S32 + Pch	Y2.R2.S32 + Pch
	+	+	+	−	**Y2.R1.Po + S32 + Pch**	Y2.R2.Po + S32 + Pch
Neofusicoccum parvum (*Np*)	−	−	−	+	Y2.R1.S1	Y2.R2.S1
	−	+	−	+	Y2.R1.Po + S1	Y2.R2.Po + S1
	+	−	−	−	Y2.R1.Np	Y2.R2.Np
	+	+	−	−	Y2.R1.Po + Np	Y2.R2.Po + Np
	+	−	−	+	Y2.R1.S1 + Np	Y2.R2.S1 + Np
	+	+	−	+	Y2.R1.Po + S1 + Np	Y2.R2.Po + S1 + Np
Non-inoculated controls	−	−	−	−	**Y2.R1.Control**	Y2.R2.Control

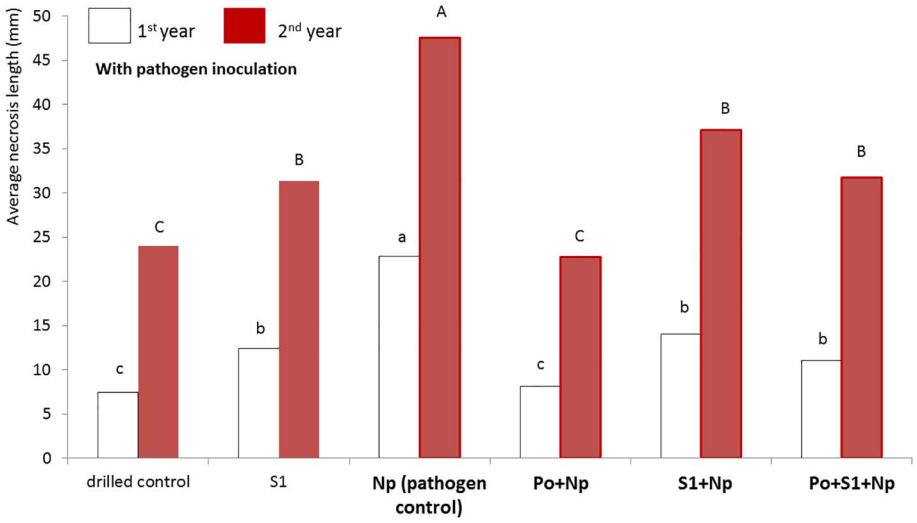

Fig. 1. Effects of BCAs (Po: *P. oligandrum* and S1: *P. agglomerans*) on necrosis length in wood in bioassays with *Neofusicoccum parvum* (Np) on grapevine plants of cv Cabernet Sauvignon grafted on rootstock 101-14 grown in a nursery greenhouse. BCAs were applied on the cuttings twice in 7 days and the pathogen was artificially inoculated 1 h 30 min after the second BCA application. The assessment was carried out after a pathogen inoculation at 90 days (dpi) and 150 days (dpi) in the 1st and 2nd year, respectively. Significantly different mean values are marked with different letters in accordance with the ANOVA ($P < 0.001$) and the Newman-Keuls test ($P > 0.05$) on transformed data. Lowercases are for the 1st year and uppercases for the 2nd year. The drilled control was used to assess wood decay caused by natural inoculums and stress following drilling.

measured (Bruez et al., 2014; Haidar et al., 2016a; Yacoub et al., 2016a). In the 1st year bioassay, wood samples were collected from the scion stem at two sampling times: 90 days post-inoculation (dpi) for plantlets inoculated with *N. parvum* and 103 dpi for plantlets inoculated with *P. chlamydospora*. In the 2nd year bioassay, for both pathogens, wood samples were collected at three consecutive sampling times, corresponding to 123, 150 and 183 dpi, respectively. Thus, the average incubation time in the 2nd year bioassay was estimated at approximately 150 dpi. Each plantlet scion stem was cut in two longitudinally, through the center of the inoculation hole site. In the case of occurrence of typical longitudinal necrosis, its total size in mm was reported, as well its length below the inoculation site and above, and the total size of the scion. At each sampling time, three wood samples per treatment of 4 cm in length (2 cm downstream and 2 cm upstream from the inoculation site) were cut and kept at -20 °C until molecular analysis.

2.5.2. Assessment of grapevine root colonization by *P. oligandrum*

In the first year bioassay, root colonization by *P. oligandrum* was monitored by quantitative polymerase chain reaction (qPCR) during the experiments at three time points: 0 dpi (second application of *P. oligandrum* and pathogen inoculation), 14 and 80 dpi. Root samples were collected from selected treatments (Table 3). Plants were uprooted gently from each pot, and the closest roots from the trunk (where *P. oligandrum* should be) were kept. To collect a sufficient yield of raw material, samples were not washed, but the roots were shaken in a plastic bag to remove the clumps of soil around them. Collected samples were ground in liquid nitrogen as described above for wood samples. DNA extraction from roots was carried with a STRATEC Invisorb® Spin Plant Mini Kit on 100 mg of the powder previously obtained according to the manufacturer's instructions with some modifications at the first step. These modifications were that lysis buffer was added to 3% PVP 40 and 0.1% v/v β-mercaptoethanol to reduce the lysis incubation time to one minute and reduce the presence of inhibitors.

The primers used for quantitative PCR were specific to *P. oligandrum* ITS regions: up_F1 and lo_146, and used with the probe 142_LNA (Vallance et al., 2009). The reaction volume was 25 µl and consisted of 12.5 µl of QuantiTect® PCR Master Mix (Qiagen), 0.4 µM of each primer and probe, 2 µl of DNA and 9 µl of RNase free water. The cycling parameters were 95 °C for 15 min (initial denaturation), followed by 40 cycles at 95 °C for 30 s (denaturation), 60 °C for 30 s (annealing), and 72 °C for 30 s (extension). A Stratagene thermocycler (Agilent) was used for the qPCR and fluorescence was monitored after the extension step for every PCR cycle with the software Mx3005Pro (Agilent). Standard curves were generated by plotting the "Cycle Threshold" (CT) values for a dilution series of a known amount of genomic DNA from a pure strain of *P. oligandrum* ($R^2 = 0.99$). Quantitative PCR was conducted at least two times for each sample.

2.6. Statistical analysis

Statistical tests were performed to analyze and show the main effects and their interaction(s) associated with the year effect (bioassay effect), the BCA application effect (bacterial strains, *P. oligandrum*) and/or the rootstock effect. The main variable analyzed was the average length of internal necrosis measured in the scion wood. The timing of when the observations were made (measured in days post-inoculation, dpi) was also included in the analysis. The software package used for analysis of variance (ANOVAs) was Statbox (version 6.6, Grimmer© Logiciels, Paris) and the data were analyzed using the Newman and Keuls test ($P > 0.05$). When necessary, the data were normalized in log10 for analysis.

3. Results

3.1. Two years of bioassays on BCAs efficacy against *Neofusicoccum parvum*

The average necrosis lengths caused by *N. parvum* in cuttings grafted on rootstock 101-14 in the two years are shown in Fig. 1. There was a significant difference in necrosis length between the two years (ANOVA, $P < 0.001$) and a significant main effect of BCA treatment ($P < 0.01$). In the 2nd year, necrosis lengths were approximately twice as long as those in the 1st year. Moreover, there was no significant interaction between the two main effects on the ANOVA: BCA treatments and years ($P = 0.78$). Therefore, the resulting order of BCA treatments was similar in both years. Necrosis lengths in the pathogen control (Np) were significantly greater than in all other treatments (i.e., 22.8 mm in the 1st year and 47.6 mm in the 2nd year), showing that the artificial inoculation of the pathogen was successful. The most efficient BCA treatment against *N. parvum* was the application of *P. oligandrum* (Po + Np), with an efficacy of 62.4% and 52.3% in the 1st and 2nd year, respectively. Interestingly, in the two years, necrosis lengths measured in this treatment (Po + Np) were not significantly different than in the drilled control, where there was no artificial pathogen inoculation. Biotization with the bacterial strain *P. agglomerans* (S1 + Np) was efficient against *N. parvum* at the rates of 38.4% and 22.0% in the 1st and 2nd year, respectively. The double biotization using both *P. oligandrum* and *P. agglomerans* (Po + S1 + Np) was efficient at the rates of 51.5% and 33.4% for the two years, respectively, which was not significantly different from the application of *P. agglomerans* alone.

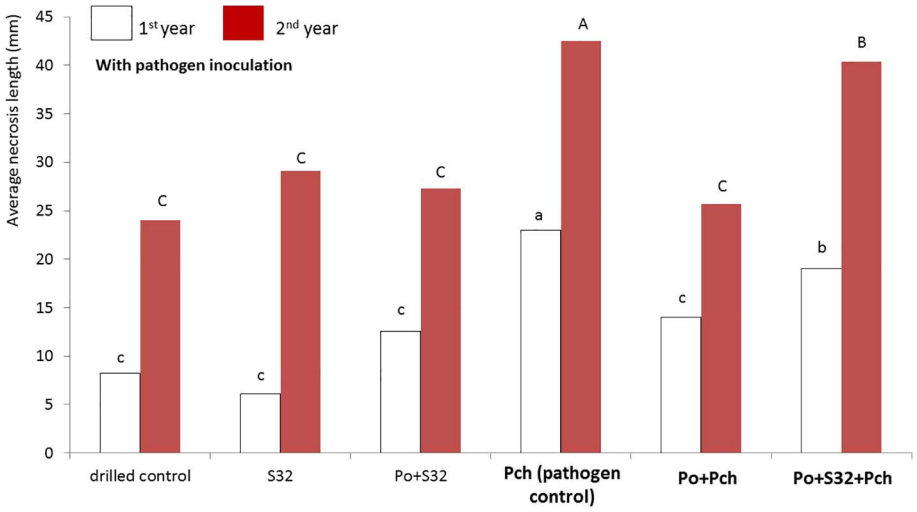

Fig. 2. Effects of BCAs (Po: *P. oligandrum* and S32: *B. pumilis*) on necrosis length in wood in bioassays with *Phaemoniella chlamydospora* (Pch) on grapevine plants of cv Cabernet Sauvignon grafted on rootstock 101-14 grown in a nursery greenhouse. BCAs were applied on the cuttings twice in 7 days and the pathogen was artificially inoculated 1 h 30 min after the second BCA application. The assessment was carried out after a pathogen inoculation at 103 days (dpi) and 150 days (dpi) in the 1st and 2nd year, respectively. Significantly different mean values are marked with different letters in accordance with ANOVA ($P < 0.001$) and Newman-Keuls test ($P > 0.05$) on raw data in mm. Lowercases are for the 1st year and uppercases for the 2nd year. The drilled control was used to assess wood decay caused by natural inoculums and stress following drilling.

3.2. Two years of bioassays on BCAs efficacy against Phaeomoniella chlamydospora

The average necrosis lengths caused by *P. chlamydospora* in cuttings grafted on rootstock 101-14 measured in the two years are shown in Fig. 2. There was a significant difference in necrosis length between the two years (ANOVA, $P < 0.001$) and a significant main effect of BCA treatments ($P < 0.01$). In the 2nd year, necrosis lengths were approximately twice as long as those in the 1st year. Moreover, there was no significant interaction between the two main effects on the ANOVA: BCA treatments and years ($P = 0.08$). Therefore, the resulting order of BCA treatments was similar in both years. Necrosis lengths in the pathogen control (Pch) were significantly greater than in all other treatments (i.e., 23 mm in the 1st year and 42.5 mm in the 2nd year), showing that the artificial inoculation of the pathogen was successful. The most efficient BCA treatment against *P. chlamydospora* was the application of *P. oligandrum* (Po + Pch), with an efficacy of 39.1% and 39.5% in the 1st and 2nd year, respectively. The drilled control and the treatment with *P. oligandrum* both showed the smallest necrosis lengths of all the treatments. Interestingly, in the two years, necrosis lengths measured in this treatment were not significantly different than in the drilled control, where there was no artificial pathogen inoculation. Biotization with the bacterial strain *B. pumilus* (S32 + Pch) was not assessed in this bioassay. The double biotization using both *P. oligandrum* and *B. pumilus* (Po + S32 + Pch) was efficient at the rates of 17.4% and 4.9% for the two years, respectively.

3.3. Compared effects of rootstocks on BCAs efficacy against N. parvum

The average necrosis lengths due to *N. parvum* treatment of cuttings grafted on rootstocks 101-14 and SO4 measured in the 2nd year bioassay are shown in Fig. 3. There was a significant difference in necrosis lengths measured between the two rootstocks (ANOVA, $P = 0.002$). Indeed, average necrosis lengths measured in cuttings grafted on rootstock SO4 were longer than the ones measured in cuttings grafted on rootstock 101-14. Moreover, there was no significant interaction between the two main effects by ANOVA: BCA treatments and rootstock ($P = 0.36$). Therefore, the resulting order of BCA treatments was similar in both rootstocks. Necrosis lengths in the pathogen control (Np) were significantly greater than in all other treatments, showing that the artificial inoculation of the pathogen was a success. For both rootstocks, the most efficient treatment was the biotization with *P. oligandrum* against *N. parvum* (Po + Np). The necrosis length was shorter by 52.3% in cuttings grafted on 101-14 and by 41.4% in cuttings grafted on SO4. Additionally, the necrosis lengths observed in this treatment were not significantly different than the ones measured in the drilled control, where there was no artificial inoculation, and they both had the smallest necrosis length. The necrosis lengths following biotization with the bacteria *P. agglomerans* only (S1 + Np) and in the double biotization treatment (S1 + Po + Np) were not significantly different from those observed in the pathogen control (less than 10% efficacy). Furthermore, the average necrosis length measured in cuttings was significantly decreased when *P. oligandrum* alone was applied (Po, i.e., without pathogen inoculation) compared to the necrosis measured in drilled controls. Therefore, by considering the cuttings grafted on 101-14, *P. oligandrum* could have an effect on the natural necrosis caused by unidentified pathogens or mechanisms.

3.4. Compared effects of rootstocks in BCAs efficacy against P. chlamydospora

The average necrosis lengths caused by *P. chlamydospora* in cuttings grafted on rootstock 101-14 and SO4 measured in the 2nd year bioassay are shown in Fig. 4. There was a significant difference in necrosis lengths measured between the two rootstocks (ANOVA, $P = 0.017$). Indeed, average necrosis lengths measured in cuttings grafted on rootstock SO4 were longer than the ones measured in cuttings grafted on rootstock 101-14. Moreover, there was no significant interaction between the two main effects on the ANOVA: BCA treatments and rootstock ($P = 0.11$). Therefore, the resulting order of BCA treatments was similar in both rootstocks. Necrosis lengths in the pathogen control (Pch) were significantly higher than in all other treatments, showing that the artificial inoculation of the pathogen was a success. For both rootstocks, the most efficient treatment was the biotization with *P. oligandrum* against *P. chlamydospora* (Po + Pch). There was a shorter necrosis length by 39.5% in cuttings grafted on 101-14 and by 51.9% in cuttings grafted on SO4. Additionally, necrosis lengths observed in this treatment were not significantly different than the ones measured in the drilled control, where there was no artificial inoculation, and they both had the smallest necrosis length. The double biotization treatment with *B. pumilus* and Po (S32 + Po + Pch) was efficient against Pch but less than biotization with *P. oligandrum* alone (Po + Pch). The necrosis lengths were greater in plantlets grafted on rootstock SO4, and in addition, the double biotization appeared to be more efficient on this rootstock than on rootstock 101-14 (respectively, 17.6% and 4.9%). Biotization with the bacterial strain *B. pumilus* only (S32 + Pch) was the least efficient treatment, as it was not efficient in plantlets grafted on rootstock 101-14, and its efficacy was less than 10% in the second rootstock.

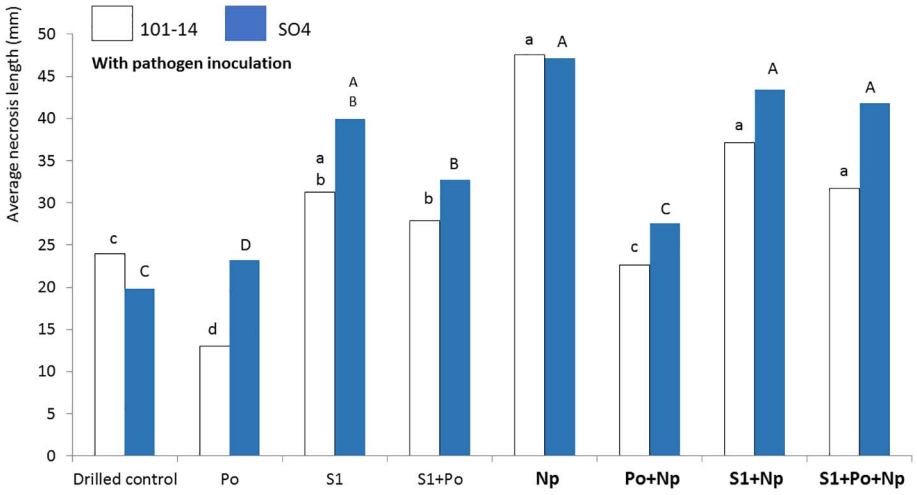

Fig. 3. Effects of BCAs (Po: *P. oligandrum* and S1: *P. agglomerans)* on necrosis length in wood affected by *Neofusicoccum parvum* (Np) on grapevine plants of cv Cabernet Sauvignon and rootstocks 101-14 and SO4 grown in a nursery greenhouse. BCAs were applied on the cuttings twice in 7 days and the pathogen was artificially inoculated 1 h 30 min after the second BCA application. The assessment was carried out 150 days post-inoculation (dpi). Significantly different values are marked with different letters in accordance with the ANOVA ($P = 0.002$) and the Newman-Keuls test ($P > 0.05$) on data transformed in log10. Lowercases are for rootstock 101-14 and uppercases for rootstock SO4. The drilled control was used to assess wood decay caused by natural inoculums and stress due to drilling.

3.5. Root colonization by *P. oligandrum*

P. oligandrum colonization of the grapevine root system was assessed by qPCR in the 1st year bioassay on selected treatments grafted on rootstock 101-14 (Table 3, Fig. 5). The results from the different treatments obtained at each sampling time (0 dpi, 14 dpi and 80 dpi) were pooled to detect the quantity *P. oligandrum* over time (Fig. 6). *P. oligandrum* colonized the root systems of grafted plantlets over the entire experimental period. *P. oligandrum* DNA quantities clearly decreased over time. Higher values were obtained at the first sampling time, 0 dpi, following the second *P. oligandrum* application and pathogen inoculation (1.5×10^3 fg.µl^{-1}). At 14 dpi, the quantity of *P. oligandrum* was approximately 50% of the one at 0 dpi (0.6×10^3 fg.µl^{-1}). At 80 dpi, root colonization by *P. oligandrum* was the lowest, just above the limit of detection ($< 2 \times 10^2$ fg.µl^{-1}).

In the 2nd year bioassay, *P. oligandrum* colonization of the grapevine root system was assessed at two sampling times for selected treatments only (Tables 4 and 5). *P. oligandrum* was detected at the first sampling time, 0 dpi, in low quantities (2.9×10^2 or 2.2×10^2 fg.µl^{-1}) compared with the first year assay, but *P. oligandrum* was not detected 14 days later (14 dpi).

4. Discussion

The first major conclusion of this study is that the efficacy of *P. oligandrum* as a BCA was significantly demonstrated on grafted young grapevines, which confirms and is totally consistent with previous studies on ungrafted plant material (Yacoub et al., 2016a). This result is of the utmost importance because the grafted system is more widely used in the wine industry. An equally important conclusion was that treatment with *P. oligandrum* was always the most efficient in reducing necrosis lengths caused by the two fungal pathogens tested, *N. parvum* and *P. chlamydospora*, in the Cabernet Sauvignon scion. This conclusion results from comparing (i) the average necrosis lengths measured in the two experimental years and (ii) the cuttings grafted on two widely used rootstocks, 101-14 and SO4. This suggests the ability of *P. oligandrum* to induce a protective effect in Cabernet Sauvignon scions against *P. chlamydospora*, as shown in previously published studies (Yacoub et al., 2016b). More importantly, these data allowed us to demonstrate significantly and for the first time the *P. oligandrum* efficacy against *N. parvum*, which is a major plant pathogen associated with GTDs worldwide. Furthermore, since the bioassays were carried out over two different years, another key conclusion is the marked stability of *P. oligandrum* efficacy. Average necrosis length following its application was reduced by approximately one-half when compared to pathogen controls. On rootstock 101-14, the efficacy levels reached 62.4% and 52.3% against *N. parvum* and 39.1% and 39.5% against *P. chlamydospora* in the 1st and 2nd year, respectively. These results are consistent with previous studies that assessed the protection against *P. chlamydospora* following *P. oligandrum* application at the root level of non-grafted Cabernet Sauvignon cuttings (Yacoub et al., 2016a).

The second major conclusion of this study is the stability of *P. oligandrum* efficacy on scions grafted on the two tested rootstocks. The application of *P. oligandrum* induced a significant reduction in average

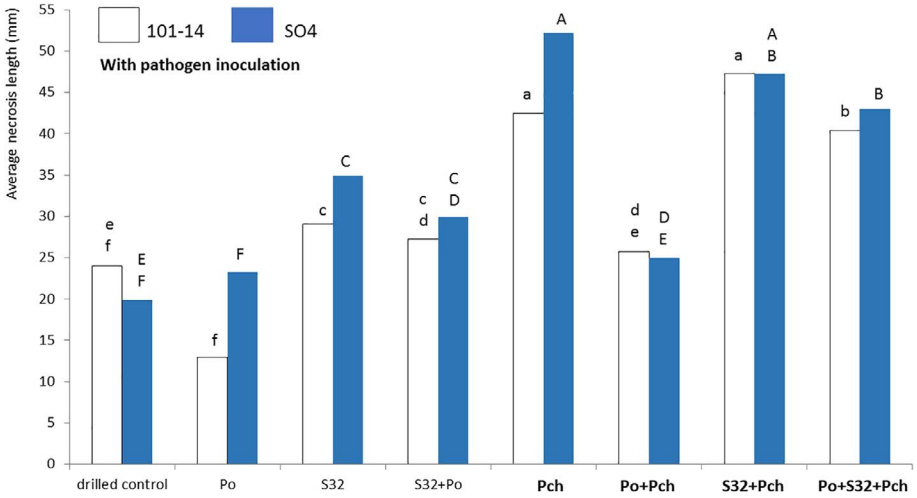

Fig. 4. Effects of BCAs (Po: *P. oligandrum* and S32: *B. pumilis*) on necrosis length in wood affected by *Phaemoniella chlamydospora* (Pch) on grapevine plants of cv Cabernet Sauvignon and rootstocks 101-14 and SO4 grown in a nursery greenhouse. BCAs were applied on the cuttings twice in 7 days and the pathogen was artificially inoculated 1 h 30 min after the second BCA application. The assessment was carried out 150 days post-inoculation (dpi). Significantly different values are marked with different letters in accordance with the ANOVA ($P = 0.017$) and the Newman-Keuls test ($P > 0.05$) on raw data in mm. Lowercases are for rootstock 101-14 and uppercases for rootstock SO4. The drilled control was used to assess wood decay caused by natural inoculums and stress due to drilling.

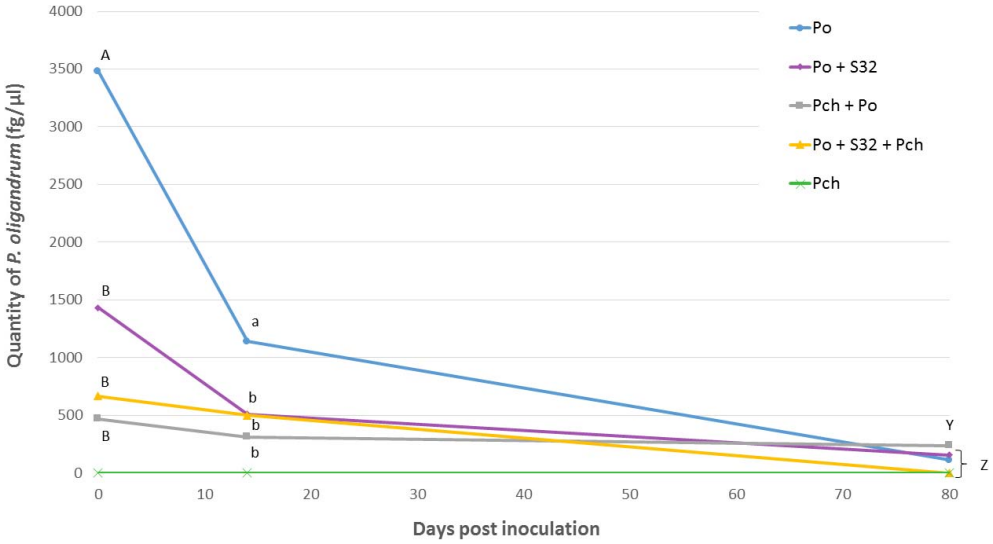

Fig. 5. Decrease in root colonization by *P. oligandrum* in grapevines grafted on rootstock 101-14 in the 1st year bioassay as assessed by qPCR. The values reported are for samples collected from each different treatment at each sample point: 0, 14 and 80 days post-inoculation of *P. chlamydospora*. *P. oligandrum* was applied on the roots 7 days (−7 dpi) and 1 h 30 min before pathogen inoculation (0 dpi). Statistical analyses were carried on data from the qPCR on the quantity of *P. oligandrum* in fg/µl on a per date basis. Treatments with identical letters are not significantly different at P = 0.05, according to the Newman-Keuls test following ANOVA.

necrosis length within the Cabernet Sauvignon scions grafted on 101-14 or SO4. Following its application, there was a reduction by approximately one-half in the wood symptom expression when compared to the necrosis lengths in pathogen controls. On rootstock 101-14 and SO4, respectively, the efficacy levels reached 52.3% and 41.4% against *N. parvum* and 39.5% and 51.9% against *P. chlamydospora*. Another observation was that average necrosis lengths were generally greater, to some extent, in scions grafted on SO4 than those on 101-14. Therefore, the combination between the Cabernet Sauvignon scion and a rootstock material may affect the scion susceptibility to GTD pathogens. Indeed, by considering the rootstock susceptibility by itself in non-grafted material, the susceptibility order may be different from previous studies (Liminana et al., 2009; Billones-Baaijens et al., 2013). In adult grapevine rootstock mother plants, they demonstrated that rootstock 101-14 was more susceptible to GTDs than SO4 in field conditions.

To the best of our knowledge, on grafted grapevine plantlets, the persistence of *P. oligandrum* in the grapevine root system associated with a significant reduction in necrosis length has been demonstrated for the first time in this study. The observed efficacy of *P. oligandrum* against the fungal infections was associated with its persistence in the rhizosphere during the three-month experimental period, as demonstrated in the first year bioassay. Similarly, Yacoub et al. (2016a, 2017) detected the oomycete in and/or the roots of non-grafted Cabernet Sauvignon cuttings by comparing its level of colonization in different treatments. The progressive decrease in *P. oligandrum* DNA quantity detected in the days following its application is consistent with the observations of Le Floch et al. (2007), when they assessed the oomycete relationship with plants and fungi. In the 2nd year bioassay, *P. oligandrum* was also detected following its application in the root systems, but

Table 5
P. oligandrum DNA quantified by qPCR in the 2nd year bioassay on the root system of grapevine cuttings grafted on rootstock 101-14 (in selected treatments). The values reported are from samples collected from each different treatment at two sample points: 0 and 14 days post-inoculation of *P. chlamydospora* (Pch). *P. oligandrum* was applied on the roots 7 days (−7 dpi) and 1 h 30 min before inoculation by the pathogen (0 dpi). No significantly different mean values are marked with the same letter "a" in accordance with the ANOVA (P > 0.5). NA: not assessed. ND: not detected.

	P. oligandrum DNA ($fg.\mu l^{-1}$)	
	0 dpi	14 dpi
Y2.R1.Control	ND	ND
Y2.R1.Pch	NA	ND
Y2.R1.Po	291.8[a]	ND
Y2.R1.Po + S32	223.9[a]	ND
Y2.R1.Po.Pch	NA	ND
Y2.R1.Po + S32 + Pch	NA	ND

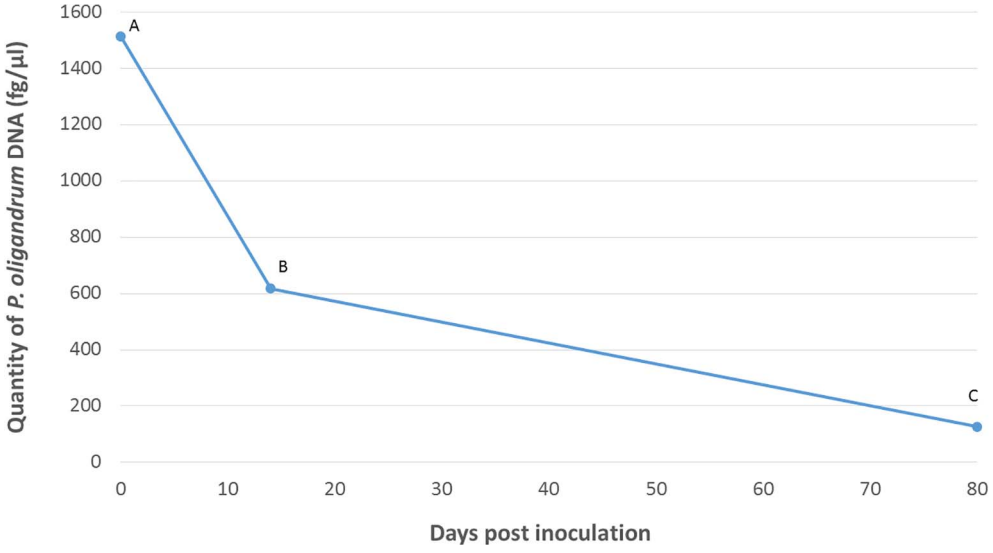

Fig. 6. Decrease in root colonization by *P. oligandrum* in grapevines grafted on rootstock 101-14 in the 1st year bioassay as assessed by qPCR. The values reported are means of samples collected in the different treatments at each sampling point: 0, 14 and 80 days post-inoculation with *P. chlamydospora*. *P. oligandrum* was applied 7 days before pathogen inoculation the first time (−7 dpi) and 1 h 30 min before pathogen inoculation the second time (0 dpi).

to a much lesser extent.

Despite the difference in the root colonization level detected by qPCR between the two years, *P. oligandrum* treatment efficacies were similar in both years. This finding is important because, in microbial biocontrol strategies, the scientific literature notes the need to have good colonization of the host plant rhizosphere as a key ecological fitness feature for such BCAs. The success of biological control depends not only on plant–microbial interactions, but also on the ecological fitness of the biological control agents to ensure a significant protection efficacy (Alabouvette et al., 2009; Gerbore et al., 2014a).

However, to date we do not know the real relationship between the *P. oligandrum* biocontrol agent population level and the associated protection effect, which may be dependent on the pathosystem considered (grapevine, other host plant). In our case, from this study, a low rhizosphere colonization level in nursery grapevines by *P. oligandrum* seems enough to protect against some major esca fungal pathogens.

As for the *P. oligandrum* mode of action, in this study, the oomycete was applied at the soil surface of plantlets at root level. Its efficacy in reducing the necrosis lengths in the cutting scions where the pathogen was inoculated suggested that *P. oligandrum* indirectly induced the plant defense mechanisms rather than directly interfering with pathogen development. These results are supported by several experiments showing that treatments with *P. oligandrum* hyphae or elicitor (oligandrin and cell wall proteins) induced the plant resistance mechanisms (Benhamou et al., 1999; Le Floch et al., 2009). Thus, this biocontrol efficacy may be partially due to the priming effect by *P. oligandrum* that may trigger plant defense mechanisms when stressed by pathogen(s) (Le Floch et al., 2009; Rey et al., 2008;Yacoub et al., 2016a).

Another interesting complementary observation following *P. oligandrum* application was the significant reductions of necrosis lengths in the drilled controls, which were not artificially inoculated by any of the pathogens tested. In previous studies, the occurrence of a natural risk of contamination by GTDs pathogens during the grafting process in the nursery could originate partly from the plant material and/or an abiotic stress such as a drilling process. (Gramaje and Armengol, 2011; Fourie and Halleen, 2004). These results, which should be further investigated in various nursery conditions, may be an important feature showing the ability of *P. oligandrum* to protect plantlets against natural infections.

Treatments with the bacterial strains *Pantoea agglomerans* or *Bacillus pumilus* applied separately were less effective than treatments with *P. oligandrum* alone, but they did decrease necrosis lengths when applied with the pathogen. To the best of our knowledge, this study demonstrates for the first time the respective efficacies of these two bacterial strains against two major GTDs pathogens in grafted plantlets. In the two years, on rootstock 101-14, the efficacy level of *P. agglomerans* against *N. parvum* was approximately 30%. Such a BCA efficacy against this aggressive pathogen is non-negligible and consistent with the previous study carried out on ungrafted plants by Haidar et al. (2016a).

Regarding the BCA combination efficacy, the results against *N. parvum* and *P. chlamydospora* suggested that there was no synergetic effect between the oomycete *P. oligandrum* and each bacterial strain, *P. agglomerans* and *B. pumilus*. In combination with *P. oligandrum*, *P. agglomerans* efficacy against *N. parvum* was not significantly different from when the bacterial strain was applied separately, i.e., approximately 57% for the two years on rootstock 101-14. Similar results were observed as a trend when assessing the average necrosis lengths in treatments of both rootstocks. Further experimentations on these bacterial strains in terms of mode of action, active concentration and/or formulation before application, could help in better understanding their role and efficacy as biocontrol agents, since Haidar et al. (2016b) have obtained promising results against these two GTD pathogens. Lastly, it is important to note that *P. oligandrum*, much more previously investigated, was applied in the present experiment using an adapted formulation provided by BIOVITIS, whereas the bacterial strains used were suspended in a general bacterial medium. Thus, it should be further investigated how to improve an appropriate formulation for such bacterial application and its potential effect on BCA efficacy.

In conclusion, our results demonstrate that *P. oligandrum* has great potential as a biocontrol agent against *N. parvum* and *P. chlamydospora*, two major aggressive fungal pathogens associated with grapevine trunk diseases. For the first time, its high level of efficacy has been demonstrated in grafted plantlets cultivated in a nursery greenhouse, on two model rootstocks widely used in vineyards, 101-14 and SO4. To use *P. oligandrum* as a BCA in the field, further studies should investigate other key parameters regarding its application (e.g., concentration, treatment frequency, method of application). This study has also confirmed the efficacy, although to a lesser extent, of two bacterial strains of *P. agglomerans* and *B. pumilus* against these two pathogens. Furthermore, an important conclusion was the non-synergetic effect between the oomycete *P. oligandrum* and each of these bacterial strains tested. Lastly, the significant effect of rootstock on the scion (Cabernet Sauvignon cv) susceptibility to the GTD fungal pathogens tested would lead to modulating the field grapevine susceptibility rankings for the disease.

Acknowledgments

The authors are grateful to the French Agricultural, Agrifood and Forestry Ministry for its financial support (“PSPE2-Ecophyto” research program). The authors would like to thank Jessica Vallance (INRA UMR 1065, BSA, Bordeaux, France) for her scientific and technical help with molecular techniques. The authors are grateful to the two French companies MERCIER Frères and BIOVITIS and to the Bordeaux Wine Council (CIVB) for another research project for biocontrol development in grapevine and to the UMR 1065 SAVE technical staff: J. Olivet, G. Taris and F. Marsan.

References

Alabouvette, C., Olivain, C., Steinberg, C., 2006. Biological control of plant diseases: the european situation. Eur. J. Plant Pathol. 114 (3), 329–341.

Alabouvette, C., Olivain, C., Migheli, Q., Steinberg, C., 2009. Microbiological control of soil-borne phytopathogenic fungi with special emphasis on wilt-inducing Fusarium oxysporum. New Phytol. 184 (3), 529–544.

Benhamou, N., Rey, P., Picard, K., Tirilly, Y., 1999. Ultrastructural and cytochemical aspects of the interaction between the mycoparasite pythium oligandrum and soil-borne plant pathogens. Phytopathology 89 (6), 506–517.

Benhamou, N., le Floch, G., Vallance, J., Gerbore, J., Grizard, D., Rey, P., 2012. Pythium oligandrum: an example of opportunistic success. Microbiology 158 (Pt_11), 2679–2694.

Bertsch, C., Ramírez-Suero, M., Magnin-Robert, M., Larignon, P., Chong, J., Abou-Mansour, E., Spagnolo, A., Clément, C., Fontaine, F., 2013. Grapevine trunk diseases: complex and still poorly understood. Plant Pathol. 62 (2), 243–265.

Billones-Baaijens, R., Jones, E., Ridgway, H., Jaspers, M., 2013. Susceptiblity of common rootstock and scion varieties of grapevines to Botryosphaeriaceae species. Australas. Plant Pathol. 43 (1), 25–31.

Bruez, E., Vallance, J., Gerbore, J., Lecomte, P., Da Costa, J., Guerin-Dubrana, L., Rey, P., 2014. Analyses of the temporal dynamics of fungal communities colonizing the healthy wood tissues of esca leaf-symptomatic and asymptomatic vines. PLoS One 9 (5), e95928.

Compant, S., Brader, G., Muzammil, S., Sessitsch, A., Lebrihi, A., Mathieu, F., 2012. Use of beneficial bacteria and their secondary metabolites to control grapevine pathogen diseases. BioControl 58 (4), 435–455.

Ec.europa.eu., n.d. EU Pesticides database – European Commission. [online] Available at: http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/ (Accessed 16 Jan. 2017).

Fourie, P., Halleen, F., 2004. Proactive control of Petri disease of grapevine through treatment of propagation material. Plant Dis. 88, 1241–1245.

Gerbore, J., Benhamou, N., Vallance, J., Le Floch, G., Grizard, D., Regnault-Roger, C., Rey, P., 2014a. Biological control of plant pathogens: advantages and limitations seen through the case study of Pythium oligandrum. Environ. Sci. Pollut. Res. 21 (7), 4847–4860.

Gerbore, J., Vallance, J., Yacoub, A., Delmotte, F., Grizard, D., Regnault-Roger, C., Rey, P., 2014b. Characterization of Pythium oligandrum populations that colonize the rhizosphere of vines from the Bordeaux region. FEMS Microbiol. Ecol. 90 (1), 153–167.

Gramaje, D., Armengol, J., 2011. Fungal trunk pathogens in the grapevine propagation process: potential inoculum sources, detection, identification, and management strategies. Plant Dis. 95 (9), 1040–1055.

Grosman, J., Doublet, B., 2012. Maladies du bois de la vigne: synthèse des dispositifs

d'observation au vignoble, de l'observatoire 2003–2008 au réseau d'épidémiosurveillance actuel. Phytoma 651, 31–35.
Haidar, R., Deschamps, A., Roudet, J., Calvo-Garrido, C., Bruez, E., Rey, P., Fermaud, M., 2016a. Multi-organ screening of efficient bacterial control agents against two major pathogens of grapevine. Biol. Control 92, 55–65.
Haidar, R., Roudet, J., Bonnard, O., Dufour, M., Corio-Costet, M., Fert, M., Gautier, T., Deschamps, A., Fermaud, M., 2016b. Screening and modes of action of antagonistic bacteria to control the fungal pathogen Phaeomoniella chlamydospora involved in grapevine trunk diseases. Microbiol. Res. 192, 172–184.
Larignon, P., 2012. Maladies cryptogamiques du bois de la vigne: symptomatologie et agents pathogènes. Vigne vin, 74.
Laveau, C., Letouze, A., Louvet, G., Bastien, S., Guerin-Dubrana, L., 2009. Differential aggressiveness of fungi implicated in esca and associated diseases of grapevine in France. Phytopathol. Mediterr. 48 (1), 32–46.
Le Floch, G., Tambong, J., Vallance, J., Tirilly, Y., Lévesque, A., Rey, P., 2007. Rhizosphere persistence of three Pythium oligandrum strains in tomato soilless culture assessed by DNA macroarray and real-time PCR. FEMS Microbiol. Ecol. 61 (2), 317–326.
Le Floch, G., Vallance, J., Benhamou, N., Rey, P., 2009. Combining the oomycete Pythium oligandrum with two other antagonistic fungi: Root relationships and tomato grey mold biocontrol. Biol. Control 50 (3), 288–298.
Lecomte, P., Darrieutort, G., Liminana, J.-M., Comont, G., Muruamendiaraz, A., Legorburu, F.-J., Choueiri, E., Jreijiri, F., El Amil, R., Fermaud, M., 2012. New insights into esca of grapevine: the development of foliar symptoms and their association with xylem discoloration. Plant Dis. 96 (7), 924–934.
Liminana, J., Pacreau, G., Boureau, F., Menard, E., David, S., Himonnet, C., Fermaud, M., Goutouly, J., Lecomte, P., Dumot, V., 2009. Inner necrosis in a grapevine rootstock vineyard in the Cognac area (Charentes, France). Phytopathol. Mediterr. 48 (1).
Maher, N., Piot, J., Bastien, S., Vallance, J., Rey, P., 2012. Wood necrosis in esca-affected vines: types, relationships and possible links with foliar symptom expression. J. Int. Sci. Vigne Vin 46 (1), 15–27.
Pouzoulet, J., Mailhac, N., Couderc, C., Besson, X., Daydé, J., Lummerzheim, M., Jacques, A., 2013. A method to detect and quantify Phaeomoniella chlamydospora and Phaeoacremonium aleophilum DNA in grapevine-wood samples. Appl. Microbiol. Biotechnol. 97 (23), 10163–10175.
Rey, P., Le Floch, G., Benhamou, N., Tirilly, Y., 2008. Pythium Oligandrum Biocontrol: Its Relationships with Fungi and Plants, Plant-microbe Interactions, first ed. Research Signpost, Kerala, pp. 43–67.
Vallance, J., Le Floch, G., Deniel, F., Barbier, G., Levesque, C., Rey, P., 2009. Influence of pythium oligandrum biocontrol on fungal and oomycete population dynamics in the rhizosphere. Appl. Environ. Microbiol. 75 (14), 4790–4800.
Yacoub, A., Gerbore, J., Magnin, N., Haidar, R., Compant, S., Rey, P., 2017. Transcriptional analysis of the interaction between the oomycete biocontrol agent, Pythium oligandrum, and the roots of Vitis vinifera L. Biol. Control. http://dx.doi.org/10.1016/j.biocontrol.2017.02.007.
Yacoub, A., Gerbore, J., Magnin, N., Chambon, P., Dufour, M., Corio-Costet, M., Guyoneaud, R., Rey, P., 2016a. Ability of Pythium oligandrum strains to protect Vitis vinifera L., by inducing plant resistance against Phaeomoniella chlamydospora, a pathogen involved in Esca, a grapevine trunk disease. Biol. Control 92, 7–16.
Yacoub, A., Gerbore, J., Renault, D., Candido da costa, D., Guyoneaud, R., Rey, P., 2016b. Pythium oligandrum root colonization of grafted vines and protection against the Grapevine Trunk Disease pathogen Phaeomoniella chlamydospora. IOBC-WPRS Bull. 117, 122–127.