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SCIENTIFIC ARTICLE
Phytopathology

New treatments to disinfect vine cuttings with *Phaeomoniella chlamydospora* and the reason for the control

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ABSTRACT

Petri disease is a problem for vineyard caused mainly by the fungus *Phaeomoniella chlamydospora*. Contaminated seedlings are source of inoculum for the disease. Treatment to disinfect vine rootstock cuttings for seedling production is hot water treatment (HWT) by 50 °C for 30 min, but the efficiency is contested. To improve its efficacy, the study aimed to assess the combination of the following methods and the reason for the control: i) exposition of the fungus to five different temperatures in HWT bath for 30 min; ii and iii) exposition of the fungus and also plants infected with *P. chlamydospora* to different disinfection treatments (biofumigation = soil + cabbage at 40 °C; temperatures of 40 and 23 °C, all in microcosm), in different periods (7, 14 and 21 days), with and without additional HWT (51 °C for 30 min). The results showed that HWT with high temperatures (55–70 °C) for 30 min inactivated the fungus. Biofumigation technique at 40 °C and the temperature solely of 40 °C applied for up to 21 days and combined with HWT (51 °C for 30 min) inhibited mycelial growth and inactivated the fungus in vine plant tissues without compromising the rooting.

Keywords: Petri disease; soilborne fungus, seedlings; alternative control; grapevine.

INTRODUCTION

Petri disease causes decline and dieback of grapevines. It is a complex disease and therefore difficult to be controlled. This disease is caused by *Phaeomoniella chlamydospora* (W. Gams, P.W. Crous, M.J. Wingf. et L. Mugnai) Crous et W. Gams combined or not with other species of the genus *Phaeoacremonium*, or *Cadophora luteo-olivacea* (F. H. Beyma) Harrington & McNew, which was reported as another fungus that might be responsible for the Petri disease in young vineyards. In inoculated plants, the fungus *P. chlamydospora* causes the largest lesions, being more frequently reisolated (GRAMAJE et al., 2009; GRAMAJE; ARMENGOL, 2011; HALLEEN et al., 2007; MOSTERT et al., 2006; MUGNAI et al., 1999).

External symptoms of the Petri disease show late budbreak, stunted shoot growth, reduced vegetative vigor, shortened internodes, lower stem diameter, interveinal chlorosis foliage with necrotic margins, premature defoliation, wilting and dieback. Internal symptoms (xylem vessel) of the trunk show black spots and black streaking, tyloses and black gums (AROCA; RAPOSO, 2009; GRAMAJE; ARMENGOL, 2011; MOSTERT et al., 2006; MUGNAI et al., 1999).

The main sources of Petri disease inoculum are infected vegetative propagation materials (seedlings), propagation processes of grapevine plants, infested soil, and aerial inoculum (AROCA et al., 2010, MOSTERT et al., 2006). The mother-plants for rootstocks production, rootstock cuttings and bench-grafts are usually infected (AROCA et al., 2010, OLIVEIRA et al., 2004), highlighting the need of studies for disinfection the rootstocks infected with the Petri disease pathogens. One of the measures to disinfect these rootstock cuttings is the hot water treatment (HWT) (GRAMAJE et al., 2011) which has also shown some failures to control Petri disease (ROONEY-LATHAM et al., 2005, WHITING et al., 2001).

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Another measure with potential to control Petri disease pathogens in rootstocks cuttings is the biofumigation that generates an anaerobic condition, toxic volatile compounds (isothiocyanates) and high temperature (GOPI et al., 2016), which can weaken or eliminate soilborne phytopathogenic fungi and their structures resistance. Solarization and biofumigation can be simulated in glass flasks kept in growth chamber, providing an environment that is called microcosms (BUENO et al., 2004). So far, there is just one study assessing the effect of biofumigation and solarization in microcosm, tested solely or complemented with HWT, concerning their efficacy to control the Petri disease in the vascular system of rootstock cuttings, as well as their effect on the rooting (FERREIRA et al., 2018).

Searching for more efficient techniques, the objectives of the present study were to assess the combination of the following methods and the reason for the control: i) exposition of fungus to five different temperatures in a HWT bath for 30 min; ii) exposition of fungus to different disinfection treatments (biofumigation = soil + cabbage at 40 °C; temperatures of 40 and 23 °C, all in microcosm), in different periods (7, 14 and 21 days), with and without additional HWT (51 °C for 30 min); and iii) exposition of plants infected with the fungus to disinfection treatments of the item 2, with and without additional HWT (51 °C for 30 min), aiming to eliminate the fungus in the plant tissues ('IAC 766' grape) without affecting the rooting.

MATERIAL AND METHODS

Effect of HWT with different temperatures tested during 30 min on the fungus *P. chlamydospora*

The study assessed temperatures above 50 °C in HWT conditions, for 30 min, regarding their efficiency to inactivate the fungus *P. chlamydospora*. Inoculum of the fungus *P. chlamydospora* (IBVD01) (FERREIRA et al., 2018) was grown on potato dextrose agar (PDA) medium for 30 days at 23 °C under a photoperiod of 12 h. Fragments of the fungus colony were obtained in rectangular shapes (1 cm wide and 2 cm long) and transferred individually to inside sterile glass tubes. The tubes were immersed in a water bath set to 50, 55, 60, 65, and 70 °C for 30 min. Each temperature had three replications, with each replication represented by a tube. The methodology used was adapted from study of WHITING et al. (2001).

After their exposition to different temperatures, the fragments of the fungus were cut in five smaller pieces (5 mm) and plated individually in a Petri dish containing the PDA medium. For control, the fungus was not exposed to different temperatures. All plates were incubated in growth chamber for 30 days at 23 °C and 12-h photoperiod. Evaluation consisted of measuring the colony diameter of the fungus *P. chlamydospora* grows on the medium with the aid of a millimeter ruler. Two perpendicular measurements were made and the average was used as diameter of the colony.

The experiment was conducted twice.

Effect of disinfection techniques on the fungus *P. chlamydospora*

The study assessed the disinfection techniques with or without HWT, regarding their efficiency to inactivate the fungus *P. chlamydospora*. Colonies of the fungus were cut in rectangular shapes (1 cm wide by 2 cm long) and transferred individually to a sterile glass tubes.

The experimental design adopted in this trial was entirely random, with three treatments tested in microcosms involving two bottles connected by a silicone hose that allow air to pass from one flask to another (BASSETO et al., 2011): Treatment 1 = one bottle containing soil + cabbage, and other with six test tubes containing the fungus, all kept in a growth chamber at 40 °C (biofumigation) and 12 h of photoperiod; Treatment 2 = one bottle with distilled water, and other containing six test tubes with the fungus, all kept in a growth chamber at 40 °C and 12 h of photoperiod; and Treatment 3 = one bottle with distilled water, and other containing six test tubes with the fungus, all kept in a growth chamber at 23 °C and 12 h of photoperiod. The treatments were maintained in growth chamber for different periods: 7, 14 and 21 days.

After each period, the six test tubes containing the fungus were removed from each microcosm and half of them, from each treatment, were subjected to additional HWT in a water bath set at 51 °C for 30 min (FERREIRA et al., 2018; GRAMAJE et al., 2009). The fragments with *P. chlamydospora* were plated on PDA medium. For each tube, there were five PDA plates containing a 5-mm fungus fragment in the center of the plate. The plates were incubated in growth chamber for 30 days at 23 °C with photoperiod of 12 h. After this period, the diameter of the fungus colony was assessed with the aid of a millimeter rule. Two perpendicular measurements were made and the average was used as diameter of the colony.

The experiment was conducted twice.

Efficiency of procedures to disinfect of vine rootstock cuttings infected with *P. chlamydospora*

The study assessed the disinfection techniques with or without HWT, regarding their efficiency to inactivate the fungus *P. chlamydospora* in vine rootstock cuttings. Vine rootstock cuttings ('IAC 766') in vases were inoculated with the fungus isolate. Following the method described by DÍAZ et al. (2009) and ESKALEN et al. (2001), the base of the rootstock cutting was wounded with a metallic hole punch (diameter: 7 mm), 4 cm above the ground, inoculated with a disk of PDA medium colonized by the fungus and sealed with sterile gauze soaked in autoclaved distilled water plus parafilm.

The rootstock cuttings were incubated in a greenhouse under room temperature for 4 months, in order to allow the development of the fungus in the vascular system. The plants were irrigated every 2 days.

After 4 months, the rootstock cuttings were removed from the substrate and the root system was washed in running water. Then, roots and stem were pruned above the third bud, and 18 cuttings were tied together and placed in one of the flasks of the microcosm (BASSETO et al., 2011).

The experimental design adopted was entirely random, with three treatments tested in microcosms (BASSETO et al., 2011): Treatment 1 = one flask containing soil + cabbage, and other with 18 rootstock cuttings infected with *P. chlamydospora*, all kept in a growth chamber at 40 °C (biofumigation) and 12 h of photoperiod; Treatment 2 = one flask with distilled water, and other containing 18 rootstock cuttings infected with the fungus, all kept in a growth chamber at 40 °C and 12 h of photoperiod; and Treatment 3 = one flask with distilled water, and other containing 18 rootstock cuttings infected with the fungus, all kept in a growth chamber at 23 °C and 12 h of photoperiod.

The soil used in the microcosms was previously moistened with sterile distilled water, in the proportion of 20% (Table 1). The soil used was classified as a typical dystrophic red latosol, with a clay texture (OLIVEIRA et al. 2004). The mineral composition of the soil used (Fazenda Santa Eliza – IAC – coordinates 22°52'16.64" S; 47°05'04.70" W) contain pH ($CaCl_2$) 5.1, organic matter of 14.0 g·dm^{-3}, P_{resin} of 17.0 mg·dm^{-3}, macronutrients (H+Al/K/Ca/Mg and SB of 16.0, 1.6, 21.0, 4.0, and 27 mmolc.dm^{-3}, respectively), CTC of 43, V(%) of 62, and micronutrients (B/Cu/Fe/Mn and Zn of 0.1, 3.1, 7.0, 7.6 and 0.4 mg·dm^{-3}, respectively).

Table 1. Mineral composition (macro- and micronutrients, pH and organic matter [OM]) of the soil used (Fazenda Santa Eliza – IAC – 22°52'16.64"S; 47°05'04.70"W).

pH	O. M.	P_{resin}	Al^{3+}	H+Al	K	Ca	Mg	SB	CTC	V%	S	B	Cu	Fe	Mn	Zn
$CaCl_2$	g/dm³	mg/dm³	---------- mmol$_c$/dm³ ----------								---------- mg/dm³ ----------					
5.1	14	17	-	16	1.6	21	4	27	43	62	-	0.1	3.1	7	7.6	0.4

- : Analysis not performed

The cabbage (*Brassica oleracea* var. *acephala* L.) used (Table 2) was first cut into small pieces with a knife and distributed in flasks at the proportion 75 g to 3 kg of soil (2.5 L) (BASSETO et al., 2011). The mineral composition of the cabbage used in the test contain the following macronutrients (N/P/K/Ca/Mg and S of 44.4, 3.6, 53.7, 34.4, 4.6 and 17.7 g·kg^{-1}, respectively) and micronutrients (Fe/Mn/Cu and B of 330, 227.7, 0.6, and 37.7 mg·kg^{-1}, respectively).

Table 2. Mineral composition (macro- and micronutrients) of the cabbage used in the test.

N	P	K	Ca	Mg	S	C	Fe	Mn	Cu	Zn	B	Al	Humidity %
Macronutrients (g/Kg)							Micronutrients (mg/Kg)						
44.4	3.6	53.7	34.4	4.6	17.7	-	330	227.7	0.6	-	37.7	-	-

- : Analysis not performed

Cuttings were kept in the microcosms for three different periods: 7, 14 and 21 days. After each period, the 18 rootstock cuttings were removed from each microcosm, and nine of them, for each treatment, were subjected to additional HWT bath set at 51 °C for 30 min (FERREIRA et al., 2018; GRAMAJE et al., 2009).

The parameter assessed was the incidence of the fungus *P. chlamydospora* in tissues of the vascular system at the base of cuttings. For this, fragments of this region were removed and disinfected superficially by immersion for 30 s in a 70% alcohol solution; 1 min in a 1.5% sodium hypochlorite solution, followed by washing in autoclaved distilled water. The disinfected fragments were dried on sterile filter paper, cut into smaller pieces (5 mm), and plated on PDA medium. There were five plates per rootstock cutting, with each plate containing four pieces.

The plates were incubated in growth chamber for 21 days at 23 °C and photoperiod of 12 h. After this period, fragments were assessed by the presence or absence of the *P. chlamydospora* colony on the medium.

The experiment was conducted twice.

Effect of disinfection techniques on the rooting of vine rootstock cuttings

The study assessed disinfection techniques with or without HWT, regarding their effects on the rooting of vine rootstock cuttings.

The experiment was carried out in an identical manner to the previous trial, except that the grapevine rootstock cuttings ('IAC 766') were not inoculated with the fungus *P. chlamydospora*.

After each period, the 20 rootstock cuttings placed in one of the microcosm glasses of each treatment were removed and half of them were subjected to additional HWT bath set to 51 °C for 30 min (FERREIRA et al., 2018; GRAMAJE et al., 2009). All the rootstock cuttings were planted in a cement box containing sand, inside a greenhouse. The treated cuttings were buried up to 1/3 of their length in the sand, leaving 2/3 out (2 buds). The cuttings were spaced 5 cm between plants and 7 cm between rows. Treatments were spaced 8 cm apart.

After 30 days, cuttings were removed and assessed concerning root formation or not.

The experiment was conducted twice.

Statistical analysis

Diameters of colony were transformed into $\sqrt{x} + 0.5$ and subjected to the Tukey's test with 5% significance.

The percentages of fungus incidence in the cuttings fragments of each treatment as well as percentage of rootstock cuttings with root formation per treatment were analyzed by the Goodman association test, for contrasts between binomial proportions (GOODMAN, 1964) considering the 5% level of significance.

The statistical program used was Sisvar 5.4 from DEX/Federal University of Lavras.

RESULTS

Effect of HWT with different temperatures tested during 30 min on the fungus *P. chlamydospora*

Temperatures of 55, 60, 65, and 70 °C applied in a water bath for 30 min affected the structures and inactivated the fungus. The temperature of 50 °C for 30 min did not totally inactivate the fungus but the mycelial growth was significatively lower in relation to the control treatment (Table 3). Interesting, older structures of the fungus (center of the colony) were more susceptible to the high temperatures, while younger structures (edge of the colony) were more resistant. Thus, new studies will be needed to confirm this finding.

Table 3. Effect of different temperatures in a water bath on *P. chlamydospora* and the reflection in the growth on PDA medium.

Temperatures (°C) / Mycelial growth (cm)					
50	55	60	65	70	Control
1.0B[1]	0.0A	0.0A	0.0A	0.0A	3.7C

[1]Average of the tests. Equal capital letters in the line mean that there is no significative difference between averages, according to the Tukey's test with 5% significance.

Effect of disinfection techniques on the fungus *P. chlamydospora*

The treatments biofumigation at 40 °C and the temperature of 40 °C applied for 7 and 14 days inactivated the fungus. The interaction of these treatments with HWT also inactivated the fungus. The temperature of 23 °C applied for 7 and 14 days did not inactivate the fungus, but it grew shorted after 14 days. The temperature of 23 °C applied for 7 and 14 days with HWT also did not inactivate the fungus and did not differ from each other. The temperature of 23 °C applied for 7 days with and without HWT differed in respect to the growth of the fungus in PDA medium (Table 4).

Disinfection treatments applied for 21 days and, with and without HWT, caused severe dryness of the culture medium containing the fungus.

Table 4. Effect of different disinfection treatments, in different periods, with and without additional HWT, on *P. chlamydospora* and the reflection in its growth on PDA medium.

Treatments	HWT 51 °C·30 min^{-1}	Periods (days)	
		7	14
Soil + cabbage at 40 °C "Biofumigation"	Without	0.0aA[1]	0.0aA
	With	0.0aA	0.0aA
Without soil and cabbage at 40 °C	Without	0.3aA	0.0aA
	With	0.0aA	0.0aA
Without soil and cabbage at 23 °C	Without	3.2cB	2.9bA
	With	1.5bA	2.6bA

[1]Average of tests. Same lowercase letters in the column means that the fungus did not grow different when submitted to the different treatments, according to Tukey's test with 5% significance; and Equal capital letters on the line means that the fungus did not grow different when incubated in different periods, according to Tukey's test with 5% significance.

Efficiency of procedures to disinfect of vine rootstock cuttings infected with *P. chlamydospora*

The treatments with temperature of 23 °C applied for 7, 14 and 21 days, without HWT, differed from other treatments in respect to the percentage of incidence of the fungus *P. chlamydospora* in the plant tissues (Table 5).

There were no differences among the temperature of 40 °C and biofumigation at 40 °C, applied for 7, 14 and 21 days, with and without HWT, and the temperature of 23 °C applied for 7, 14 and 21 days with HWT. However, treatments that showed complete inactivation of the *P. chlamydospora* fungus in plant fragments were temperature solely of 40 °C and the biofumigation at 40 °C applied for 7, 14 and 21 days followed by additional HWT and treatment with the temperature of 23 °C applied for 14 and 21 days plus additional HWT (Table 5).

Table 5. Percentage of incidence of *P. chlamydospora* in fragments taken from the vascular system of the basal part of rootstock cutting (cv. IAC 766) submitted to different disinfection treatments for different periods, and with additional HWT or not, and plated on PDA medium.

Treatments	HWT 51 °C·30 min^{-1}	Treatments periods (days)	*P. chlamydospora* incidence (%)
Soil + cabbage at 40 °C "Biofumigation"	Without	7	0.0[1]aAα
		14	2.5aAα
		21	0.0aAα
Without soil and cabbage at 40 °C		7	1.3aAα
		14	2.5aBα
		21	3.8aAα
Without soil and cabbage at 23 °C		7	16.3bBα
		14	47.5bBγ
		21	32.5bBβ
Soil + cabbage at 40 °C "Biofumigation"	With	7	0.0aAα
		14	0.0aAα
		21	0.0aAα
Without soil and cabbage at 40 °C		7	0.0aAα
		14	0.0aAα
		21	0.0aAα
Without soil and cabbage at 23 °C		7	2.5aAα
		14	0.0aAα
		21	0.0aAα

Lower case: comparison of treatments fixed periods and HWT; Capital letters: comparison of HWT fixed treatments and periods; Greek letters: comparison of periods fixed treatments and HWT. The comparisons were made based on the Goodman Association Test, for contrasts between binomial proportions (GOODMAN, 1964) with a 5% significance level. [1]Average of tests.

Effect of disinfection techniques on the rooting of vine rootstock cuttings

There was not difference between the techniques with additional HWT or not and among the disinfection techniques in respect to the percentage of rooting of the vine rootstock cuttings. Differences occurred only along the periods for the techniques soil + cabbage at 40 °C without HWT and temperature at 40 °C without HWT (Table 6). Temperature of 40 °C and biofumigation at 40 °C applied for up 21 days, followed by additional HWT, inactivated the fungus in the plant tissues without compromising the rooting (Tables 5 and 6).

Table 6. Percentage of grapevine rootstock cutting (cv. IAC 766) with fully developed roots, after treatment with different disinfection techniques, in different periods, with and without additional HWT.

Treatments	HWT 51 °C·30 min^{-1}	Periods (days)		
		7	14	21
Soil + cabbage at 40 °C "Biofumigation"	Without	90.0[1]aAβ	100.0aBβ	40.0aAα
	With	80.0aAα	60.0aAα	80.0aAα
Without soil and cabbage at 40 °C	Without	90.0aAβ	30.0aAα	60.0aAαβ
	With	90.0aAα	60.0aAα	70.0aAα
Without soil and cabbage at 23 °C	Without	80.0aAα	60.0aAα	70.0aAα
	With	100.0aAα	80.0aABα	80.0aAα

[1]Average of tests. Lower case: Without HWT x With HWT fixed treatment and period; Capital letters: 23 °C x 40 °C x Biofumigation fixed HWT and period; Greek letters: 7; 14 and 21 days (periods) fixed treatments and HWT. The comparisons were made according to the Goodman association test, for contrasts between binomial proportions (GOODMAN, 1964) with a 5% significance level.

DISCUSSION

The fungus *P. chlamydospora* was inactivated by HWT in temperature ranging from 55 to 70 °C for 30 min. The fungus developed on PDA medium HWT at 50 °C for 30 min but with smaller diameter of colony compared to the fungus without HWT.

WHITING et al. (2001) studied the effects of temperature and water potential on survival and mycelial growth of *P. chlamydospora* and *Phaeoacremonium* spp. A suspension of *P. chlamydospora* conidia did not germinate after HWT at 51 °C for 15 min. The fungus *P. chlamydospora* was inactivated by HWT at 51 °C for 120 min. *Phaeoacremonium inflatipes* developed on the PDA medium after HWT at 51 °C for 120 min. Thus, adjustment of time and temperature of HWT is crucial for inactivation of the phytopathogen.

In the current study, treatment in microcosm with the temperature of 23 °C applied for up to 14 days, with HWT at 51 °C for 30 min, did not inactivate the fungus *P. chlamydospora*. This treatment is similar to the classic HWT applied directly on the fungus. According to WHITING et al. (2001) and ROONEY; GLUBER (2001), the application of HWT at 51 °C for 30 min as solely curative measure is not sufficient to inactive or even to reduce *P. chlamydospora* and *P. inflatipes* from vine material.

The HWT technique at 50 or 51 °C for 30 min eliminate phytopathogens from the stem of vines? Yes, it does if HWT interact with other techniques. In the present study, the biofumigation technique at 40 °C and the temperature of 40 °C for up to 14 days, with HWT at 51 °C for 30 min, inactivated the fungus, which is not possible only with HWT (ROONEY; GLUBER, 2001; WHITING et al., 2001).

The solarization technique used alone does not effectively control some soilborne phytopathogenic fungi, such as *Macrophomina phaseolina*, *Fusarium oxysporum* and *Plasmodiophora brassicae* (SOUZA; BUENO, 2003). One of the measures that have been used and that improves solarization and allows reduction of treatment time is the biofumigation that consists in the prior incorporation of plant parts of specific crops to the soil followed by solarization (AMBRÓSIO et al.; 2008; GAMLIEL; STAPLETON, 1993; SOUZA; BUENO, 2003). According to AMBRÓSIO et al. (2008), the production of volatile fungitoxic compounds in biofumigation reduce the number of resistant structures of phytopathogens living in the soil. The combination of biofumigation with HWT may have caused the death of the fungus *P. chlamydospora* in the current study.

In the present study, the techniques biofumigation at 40 °C and the temperature of 40 °C acting for up to 21 days and combined with HWT (51 °C for 30 min) inactivated the fungus *P. chlamydospora* in vine plant tissues ('IAC 766') without compromising the rooting. In other study, HWT with temperatures above 50 °C for different periods inactivated *P. chlamydospora* from vine materials but the process affected the sprouting and the weight of branches of some grapevines (GRAMAJE et al., 2009). FERREIRA et al. (2018) also found suppression of *P. chlamydospora* in the tissues of rootstock cutting ('AC 766') when treated with biofumigation at 37 °C and the temperature of 37 °C applied for up 21 days, followed by HWT at 51 °C for 30 min. As for the rooting, FERREIRA et al. (2018) found that biofumigation at 37 °C and the temperature

of 37 °C applied for up to 14 days of time plus HWT allowed rooting of the rootstock cuttings. After 21 days of treatment, only biofumigation at 37 °C plus HWT affected the rooting of the cuttings (FERREIRA et al., 2018). The difference in the percentage of rooting of the cuttings comparing the study by FERREIRA et al. (2018) with the current study, to the 21 days of treatment, is due to the following factor: the cuttings treated in the study of FERREIRA et al. (2018) were pruned at both ends with a pruning knife, while in the present study the treated rootstock cuttings were planted directly in the sandbox, inside the greenhouse, without going through any kind of pruning.

In the present study, treatment with the temperature of 23 °C applied for 14 and 21 days, followed by additional HWT (51 °C for 30 min), inactivated the fungus *P. chlamydospora* in the vascular tissues of rootstock cuttings. In the study of FERREIRA et al. (2018), on the other hand, the fungus was not totally inactivated in the tissues of rootstock cuttings treated with the temperature of 23 °C applied for 7, 14 and 21 days followed by HWT (51 °C for 30 min). This treatment is similar to the HWT treatment of GRAMAJE et al. (2009), differing only in the fact that the vine cuttings remained for up to 21 days without soil in the microcosm and then subjected to additional HWT. For further study, infected vine cuttings can be collected, to store them for a certain period without soil and then to treat them with HWT, verifying if there will be inactivation or not of the phytopathogens of Petri disease.

According to CROCKER; WAITE (2004), injuries caused by HWT are less common in vineyards of regions with hot climate, suggesting a higher thermotolerance compared to those in cold regions. For vines adapted to hot weather, proteins synthesized during thermal shock induce plants to a dormant state, protecting them during HWT.

In the present study, the purpose was not to obtain 100% of cuttings with root callus or root formation. The intended objective was to inactivate the fungus *P. chlamydospora* in the infected vine cuttings, so they can grow healthily to generate new healthy matrix materials to produce seedlings free of phytopathogens. According to MOSTERT et al. (2006), the transmission of fungi from Petri disease occurs through propagating materials generated from sick mother plants.

CONCLUSION

The biofumigation technique at 40 °C and the temperature solely of 40 °C can be applied for up to 21 days and must always be combined with HWT at 51 °C for 30 min to inactivate the fungus *P. chlamydospora* inside the vine plant tissues without compromising the rooting.

AUTHORS' CONTRIBUTIONS

Conceptualization: Bueno, C.J.; Brambatti, F. **Data curation:** Brambatti, F.; Bueno, C.J. **Formal analysis:** Padovani, C.R. **Funding acquisition:** Bueno, C.J. **Investigation:** Brambatti, F. **Methodology:** Brambatti, F.; Leite, L.G.; Bueno, C.J. **Project administration:** Bueno, C.J. **Resources:** Bueno, C.J. **Supervision:** Bueno, C.J. **Validation:** Brambatti, F.; Bueno, C.J. **Visualization:** Brambatti, F.; Leite, L.G.; Bueno, C.J. **Writing – original draft:** Brambatti, F.; Leite, L.G.; Bueno, C.J. **Writing – review & editing:** Brambatti, F.; Leite, L.G.; Bueno, C.J.

AVAILABILITY OF DATA AND MATERIAL

The datasets generated and/or analyzed during the current study are not publicly available because these data will be analyzed in new study, but are available from the corresponding author on reasonable request.

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CONFLICTS OF INTEREST

All authors declare that they have no conflict of interest.

ETHICAL APPROVAL

Not applicable.

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