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Colonization of vines by Petri disease fungi, susceptibility of rootstocks to *Phaeomoniella chlamydospora* and their disinfection

Colonização de videiras pelos fungos da doença de Petri, suscetibilidade de porta-enxertos ao fungo **Phaeomoniella chlamydospora** *e sua desinfecção*

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ABSTRACT: Petri disease is complex, attacks young vine plants and it is difficult to be controlled. The fungus *Phaeomoniella chlamydospora* (*Phc*) has been identified as the main causative agent of this disease. This study aimed to evaluate the prevalent colonization of the Petri disease fungi in different portions of vine plants; to assess the susceptibility of grapevine rootstocks to the fungus *P. chlamydospora*; to assess the effect of solarization and biofumigation, followed by hot-water treatment (HWT), on the disinfection of cuttings of the rootstock IAC 766 infected with *P. chlamydospora*, and assess the effect of solarization and biofumigation, followed by HWT, on the rooting of cuttings of the rootstock IAC 766. For the prevalent colonization test, the fungus species detected and identified in 'Niagara Rosada' grafted on two rootstocks different were *Phc* and *Phialemoniopsis ocularis*. This is the first report of *P. ocularis* in a young vineyard in Brazil. Both fungi, in particular *Phc*, colonized only the plant's basal part, drawing attention to the rootstock as target for control measures. Measurement of the dark streaks in the vascular system revealed that Golia was the least susceptible rootstock, and IAC 572 was the most susceptible to *Phc*. Moreover, biofumigation or temperature of 37°C applied for 7 and 14 days, both followed by HWT, suppressed *Phc* in cuttings of the rootstock IAC 766 without hampering their rooting. Meanwhile, new studies are needed to validate the efficiency of these disinfection techniques.

KEYWORDS: Petri disease; propagating material; control; resistance; *Vitis* L.

RESUMO: A doença de Petri é complexa, ataca plantas jovens de videira e é difícil de ser controlada. O fungo *Phaeomoniella chlamydospora* é o principal agente causal dessa doença. Os objetivos deste estudo foram: avaliar o local prevalente dos fungos da doença de Petri, em diferentes partes de plantas de videira; avaliar a suscetibilidade de porta-enxertos de videira para o fungo *P. chlamydospora*; avaliar o efeito da solarização e da biofumigação seguido de tratamento com água quente sobre a desinfecção de estacas do porta-enxerto IAC 766 infectadas com o fungo *P. chlamydospora*; avaliar o efeito da solarização e da biofumigação seguido de tratamento com água quente sobre o enraizamento de estacas do porta-enxerto IAC 766. Para o teste de colonização, as espécies de fungos detectadas e identificadas em Niagara Rosada enxertada em dois porta-enxertos diferentes foram *P. chlamydospora* e *Phialemoniopsis ocularis*. Este é o primeiro relato de *P. ocularis* em parreirais jovens de videira no Brasil. Ambos os fungos, em particular *P. chlamydospora*, colonizaram somente a parte basal das plantas, destacando-se os porta-enxertos como foco para medidas de controle. Medidas das estrias escuras no sistema vascular revelaram que Golia foi o porta-enxerto menos suscetível, e o IAC 572 foi o mais suscetíveis para *P. chlamydospora.* Além disso, a biofumigação ou a temperatura de 37ºC aplicadas por 7 e 14 dias seguidas de tratamento com água quente eliminaram *P. chlamydospora* em estacas do porta-enxerto IAC 766 sem afetar o enraizamento. No entanto, novos estudos são necessários ainda para validar a eficiência dessas técnicas de desinfecção.

PALAVRAS-CHAVE: doença de Petri; material de propagação; controle; resistência; *Vitis* L.

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INTRODUCTION

Petri disease causes decline and dieback of grapevines, mainly in young vines. It is a complex disease, and therefore difficult to be controlled. This disease is caused by combination of *Phaeomoniella chlamydospora* (W. Gams, Crous, M. J. Wingf & L. Mugnai Crous & W. Gams) and several species of *Phaeoacremonium,* and also by *Cadophora lutea-olivaceae* (F. H. Beyma) T.C. Harrington & McNew. However, *P. chlamydospora* is more often associated with typical symptoms of Petri disease, causing the largest lesions and being more frequently re-isolated compared to other fungi related to this disease (GRAMAJE et al. 2011; HALLEEN et al., 2007; MOSTERT et al., 2006; MUGNAI et al., 1999). Other genera of fungi that might be associated to the decline in nurseries or in young vineyards are *Acremonium* (*A. charticola* and *A. ochraceum*) and *Phialemoniopsis curvata* (= *Phialemonium curvatum*) (HALLEEN et al., 2007; PERDOMO et al., 2013).

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).

Petri disease has been reported in different parts of the world where grapevine is cultivated (ABREO et al., 2011; CROUS; GAMS, 2000). In Brazil, Petri disease pathogens were found in the state of Rio Grande do Sul (GARRIDO et al., 2004) as well as in the Northeast region of the country (CORREIA et al., 2013).

LORENA et al. (2001) inoculated *P. chlamydospora* in the root of Paulsen 1103 rootstock and observed that the fungus colonization differed between vine plant portions, being greatest at the root collar level and at the base of the stem, becoming less frequent and disappearing above the 7th/8th internode. Thus, the Petri disease pathogens will colonize mostly the rootstocks in grafted vine plants.

Rootstocks and scions of grapevines are susceptible to the Petri disease pathogens (GRAMAJE; ARMENGOL, 2011); however, some grapevine rootstocks inoculated with *C. luteo-olivacea, Phaeoacremonium* spp. and *P. chlamydospora* have shown to be less susceptible in field conditions (GRAMAJE et al., 2010), suggesting the necessity of new studies to verify the susceptibility of different rootstocks to the Petri disease pathogens.

The main sources of inoculum of the Petri disease fungi are infected propagation material, processes for propagation of grapevine plants, infected mother vines, infested soils, and aerial inoculum (AROCA et al., 2010; MOSTERT et al., 2006; MUGNAI et al., 1999). To avoid the dissemination of Petri disease fungi in the field, new studies should be carried out with the purpose of disinfecting rootstock cuttings for the production of healthy mother vines and, consequently, to obtain healthy planting material.

A controversial measure to control Petri disease fungi in grapevine dormant cutting is the hot-water treatment (HWT) (GRAMAJE et al., 2009; ROONEY; GUBLER, 2001). Another measure with potential to disinfect grapevine dormant cutting is biofumigation, a sustainable method for disinfesting the soil. Biofumigation generates anaerobic conditions, toxic volatile compounds (isothiocynates), high temperature (GOPI et al., 2016), and all these factors can weaken or eliminate soilborne phytopathogenic fungi and their resistant structures. Solarization and biofumigation can be simulated in glass flasks kept in a growth chamber, providing an environment that is called microcosm (BUENO et al., 2004). So far, there is no study assessing the effect of biofumigation and solarization in a microcosm, tested solely or complemented with HWT, for the control of Petri disease fungi in rootstock cuttings.

Thus, this study aimed to:

1. evaluate the prevalent colonization of the Petri disease fungi in different portions of vine plant;
2. assess the susceptibility of grapevine rootstocks to the fungus *P. chlamydospora*;
3. assess the effect of solarization and biofumigation in microcosm, followed by hot-water treatment (HWT), on the disinfection of cuttings of the rootstock IAC 766 infected with *P. chlamydospora*, and
4. assess the effect of solarization and biofumigation in a microcosm, followed by HWT, on the rooting of cuttings of the rootstock IAC 766.

MATERIAL AND METHODS

Colonization of vines by Petri disease fungi

This study aimed to assess the portions with the most prevalence for colonization of Petri disease fungi in vine plants: the basal part (rootstock), the stem part (40 cm above the basal part), or the branch part (40 cm far from stem part). To this end, three entire plants of 'Niagara Rosada' grapevine (*Vitis labrusca* L. x *Vitis vinifera* L.) grafted on the rootstock Ripária do Traviú (3 years old) and three plants grafted on the rootstock IAC 766 (2 years old) were collected randomly in a commercial vineyard infected with Petri disease, in the municipality of Jundiaí, São Paulo state (SP), Brazil (23°07'83.0"S and 46°56'85.3"W). Thus, three treatments and three replications were established, represented by the three parts of each plant, and by the three plants of each variety, respectively.

For each plant part, 4 cm-long samples from the vascular system were taken and superficially disinfected by immersing

them for 30 seconds in 70% alcohol and 1 min in a 1.5% solution of sodium hypochlorite, followed by immersion in sterile distilled water. Then, the samples were dried on sterile filter paper and cut into smaller fragments (0.5 cm) with a sterile scalp. The samples were distributed in Petri dishes, using 4 fragments per dish and 15 dishes per each plant part.

The Petri dishes were incubated in a growth chamber under 23°C for a 12-hour photoperiod for 21 days. The incidence of the Petri disease pathogens was expressed as the percentage of infected fragments per each plant part.

Identification of the Petri disease fungi

The isolates of the Petri disease that grew in the medium were processed according to CORREIA et al. (2013) for molecular identification. After this, DNA was extracted by following the CTAB method described by DOYLE; DOYLE (1987). The polymerase chain reaction (PCR) was performed to amplify the ITS-5.8S region of the rDNA with the oligonucleotide primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (WHITE et al., 1990). Fragments of the beta tubulin gene (β Tubulin) were amplified with the pair of primers Bt2a (5'-GGTAACCAAATCGGTGCTGCTTTC-3') and Bt2b (5'-ACCCTCAGTGTAGTGACCCTTGGC-3') of GLASS; DONALDSON (1995) and Btub-F: AAGGGHCAYTAYACYGARGG and Btub-R: CATGTTGGACTCDGCCTC of LAZAROTTO et al. (2014). The reactions were performed in a PTC100 thermocycler (MJ Research) according to the following protocol: initial denaturation at 94°C/2 min, 40 cycles of 94°C/30 s – 54°C/30 s – 72°C/60 s, and final extension at 72°C/4 min. The PCR products were purified by precipitation with polyethylene glycol, according to a protocol described by SCHMITZ; RIESNER (2006). Sequencing was performed by the chain termination method with the reagent BigDye 3.1 (Applied Biosystems) and ABI3500 automatic sequencer (Applied Biosystems).

Nucleotide alignment was carried out by MUSCLE program (EDGAR; MUSCLE, 2004). Phylogenetic trees were built by the Neighbor Joining method using the MEGA 6.0 program, with evaluation of the topology's reproducibility through bootstrap with 1,000 repetitions. The support value obtained for each branch of the tree is shown in Figure 1, not adopting a minimum value to separate the branches.

Susceptibility of rootstocks to the fungus *P. chlamydospora*

This study aimed to assess the susceptibility of nine rootstocks to the fungus *P. chlamydospora*. The rootstocks used were:

- IAC 313 "Tropical" – Golia x *Vitis cinerea*;
- IAC 572 "Jales" –*V. caribaea* x 101-14 Mgt (*V. riparia* x *V. rupestris*);
- IAC 571-6 "Jundiaí" – *V. vinifera* (Pirovano 57) x *V. caribaea*;
- IAC 766 "Campinas" – Ripária do Traviú (106-8 Mgt) x *V. caribaea*;
- Ripária do Traviú (106-8 Mgt) – *V. riparia* x (*V. cordifolia* x *V. rupestris*);
- Ripária Gloire de Montpellier – seedling of *V. riparia*;
- Golia – cross of Castel 156-12 (*V. vinifera* x *V. riparia*) x *V. rupestris*;
- SO4 – *V. berlandieri* x *V. riparia*;
- Paulsen 1103 – *V. berlandieri* x *V. rupestris*.

The experiment was performed in randomized blocks with six replications. Each replication consisted of five pots containing 4.0 kg of soil and a plant inoculated or not inoculated with the fungus *P. chlamydospora* of each rootstock. The sanity of the cuttings of each rootstock was attested before installing the test.

The fungus *P. chlamydospora* (IBVD 01) was isolated from the 'Niagara Rosada' plant grafted on the rootstock Ripária do Traviú from a commercial vineyard in the municipality of Jundiaí, SP (23°07'83.0"S and 46°56'85.3"W). The inoculum of the fungus was produced in Petri dishes containing a PDA medium, and incubated in a growth chamber under 23°C for a 12-hour photoperiod for 30 days.

The methodology to inoculate *P. chlamydospora* in rootstocks was adapted from ESKALEN et al. (2001). The base of the stem (2 cm above soil level) was injured with a metallic cork borer (0.5 cm) and, then, a plug of PDA medium (0.5 cm) colonized by the fungus was inserted into a circular wound and fixed by sealing the wound with gauze soaked in sterile distilled water and parafilm.

Four months after inoculation, the length of dark streaks caused by *P. chlamydospora* in the vascular system of each rootstock was measured.

Effect of technique combinations on disinfection and rooting of rootstock

Disinfection

Eight treatments were established, consisting of the following four techniques (BUENO et al. 2004), complemented or not with hot-water treatment set to 51°C for 30 min (HWT) (GRAMAJE et al., 2009):

1. soil plus kale plants at 37°C (biofumigation);
2. soil without kale plants at 37°C (solarization);
3. without soil and without kale plants at 37°C, and
4. without soil and without kale plants at 23°C (control).

The experiment was performed in randomized blocks with four replications (rootstock IAC 766 infected with

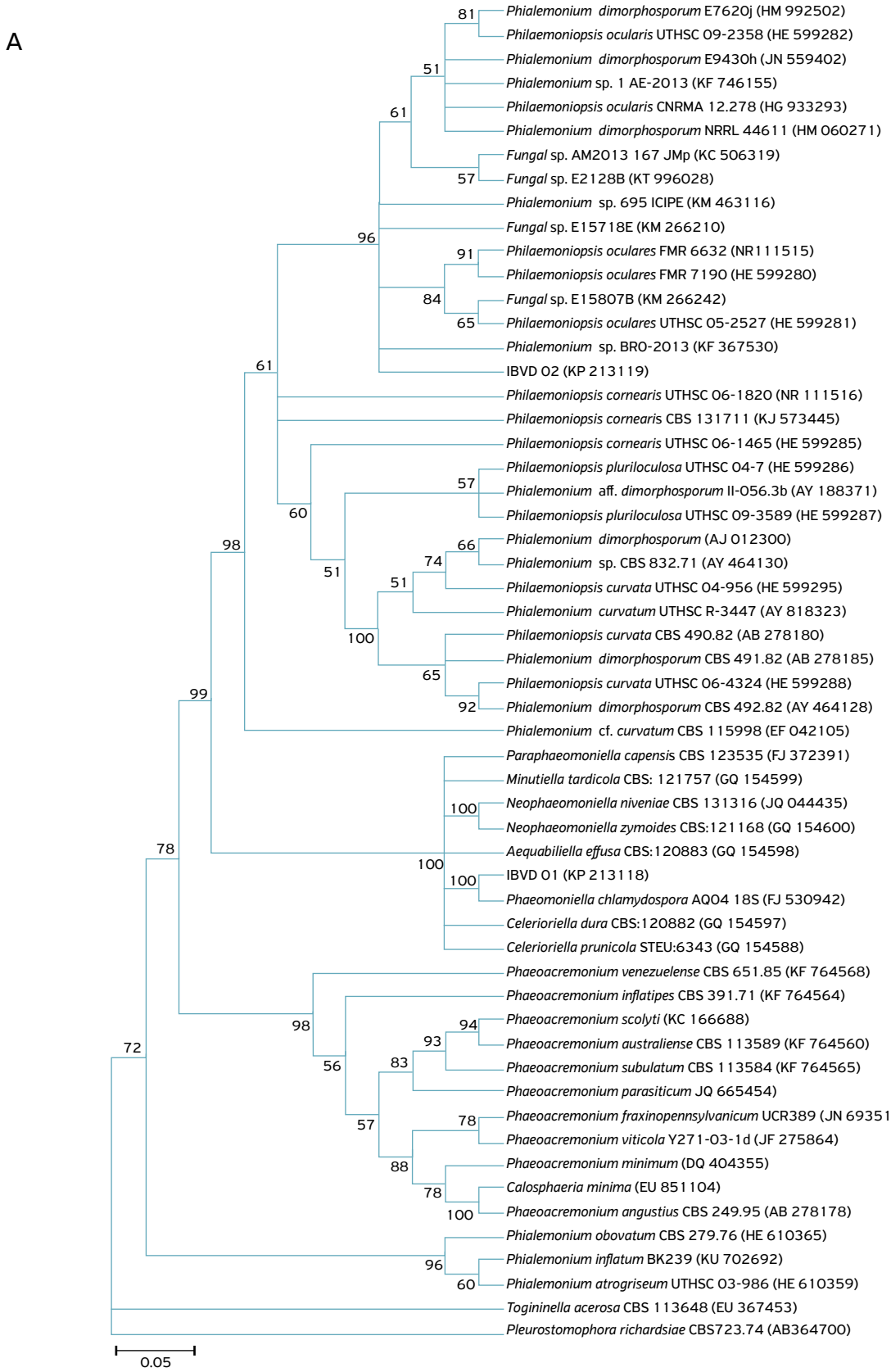

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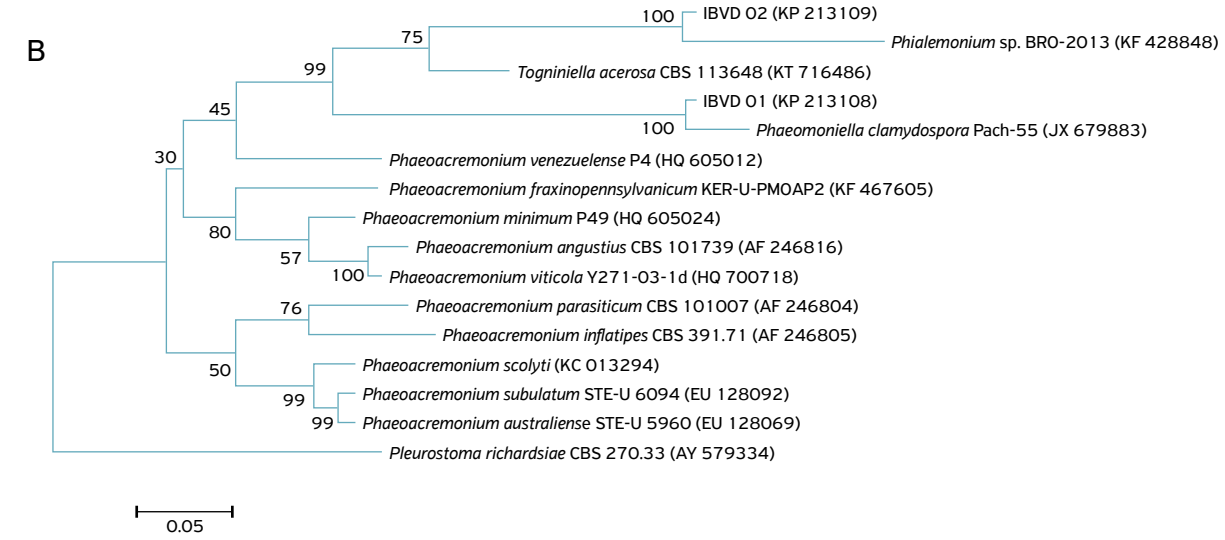
Figure 1. Phylogenetic trees showing the relationship between the isolates of fungi detected in 'Niagara Rosada' vine in comparison with the genera and species of fungi isolated from vines and deposited in GenBank-NCBI. The trees were constructed on the basis of the following sequences: (A) ITS-5.8S region – showing condensed tree with 50% cut-off value; and (B and C) parts of the beta tubulin gene. The isolates *Pleurostoma richardsiae* (= *Pleurostomophora richardsiae*) and *Conioachaeta lignicola* (= *Lecythophora lignicola*) were used as the outgroup. The accession number of the sequences of the isolates in GenBank-NCBI is given parenthetically.



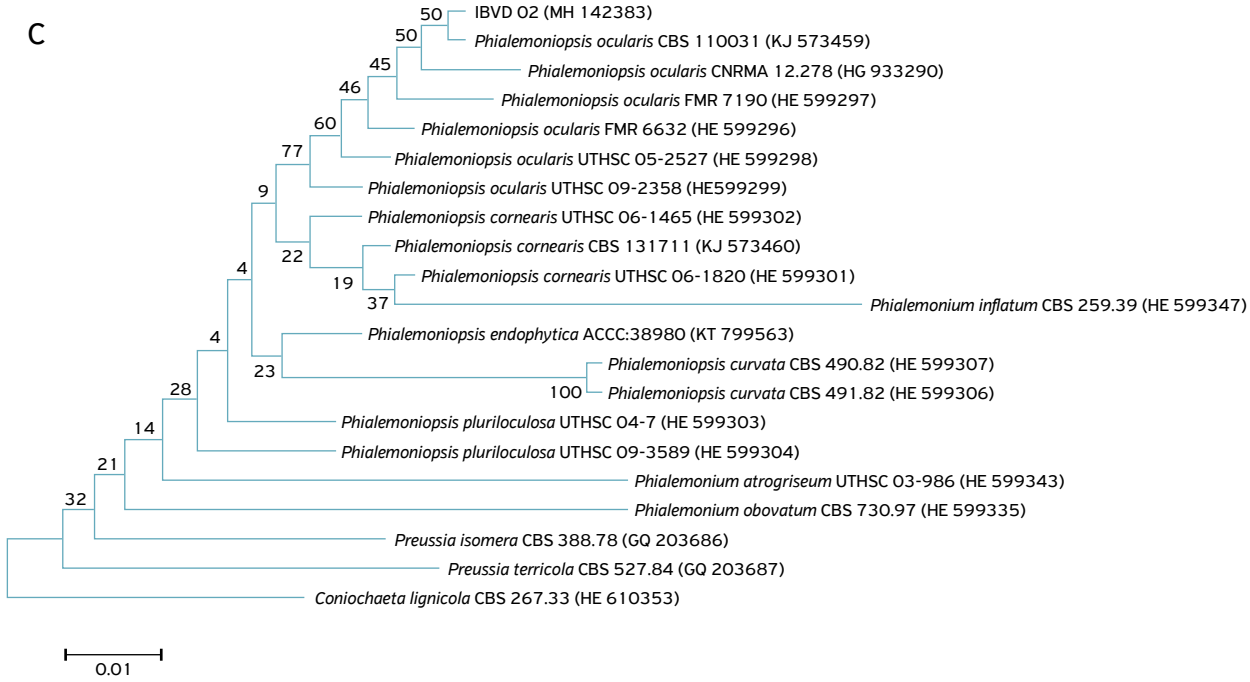


Figure 1. Continuation.

P. chlamydospora) per treatment. Each replication consisted of eight rootstocks (IAC 766) infected with *P. chlamydospora*. To obtain the infected plants, rootstocks (sanity attested) planted in bags with solarized soil were inoculated with the *P. chlamydospora* isolate according to the methodologies of inoculum preparation and inoculation as described for the previous assay (Resistance of rootstocks to the fungus *P. chlamydospora*). Four months after inoculation, 96 plants were removed from the soil, their root system washed, and eight rootstocks were grouped together and then placed inside one of the two glass bottles that comprise the microcosm (BUENO et al., 2004). All treatments were carried out inside the microcosms and incubated within growth chambers under 37±2°C and 23±2°C for a 12-hour photoperiod for 7, 14, and 21 days (BUENO et al., 2004). After each period, eight rootstocks per treatment were removed from each microcosm and four of them were submitted to additional HWT.

Three identical and independent tests were performed, using an independent microcosm for each period mentioned. The soil used in the experiment (Table 1) was moistened with distilled

water at a proportion of 20% (w/v). For biofumigation, 60 g of kale (*Brassica oleracea* var. *acephala* L.) (Table 2) were crushed and mixed into 3 kg of soil, providing a 15-cm thick layer of growing medium in the microcosm (BUENO et al. 2004).

For evaluation, after each period of exposition to the treatments, fragments from vascular system of the basal part of each plant (replication) were removed and disinfested superficially, similarly to the colonization test. The fragments were cut into smaller pieces (0.5 cm) with a sterile scalp, and distributed in a Petri dish containing a PDA medium, with four pieces per dish and 5 dishes per plant. The dishes were incubated in a growth chamber under 23°C for a 12-hour photoperiod for 21 days. Fragments infected with the *P. chlamydospora* fungus were counted and converted into percentages of infected fragments to compare the efficiency of the treatments.

Rooting

To assess the effect of the technique combinations on the rooting of the rootstock, eight healthy cuttings containing 2-3 buds (replications) were grouped together and then placed into one of the two glass bottles that comprised the microcosm. The treatments and the methodology to expose the cuttings to the technique combinations were the same as described before (effect of technique combinations on disinfection of rootstock).

After the different exposition periods, the cuttings were planted in a flowerbed (4×1.5 m), with a spacing of 20 cm between rows and 12 cm between plants. After planting, the soil was moistened, and then a mulching was spread on the soil, around the cuttings, in order to maintain appropriate moisture levels. After germination of the first buds, the mulching was removed.

The formation of the radicular system in the cuttings was evaluated 40 days after planting.

Data Analysis

Colonization data were analyzed by the non-parametric analysis of variance for repeated measurements in independent groups, complemented with the Dunn multiple comparison test (ZAR, 2009), with 5% significance.

Data from the screening test of rootstocks were subjected to analysis of variance for the experiment in randomized blocks with replicates inside the blocks, complemented with the T test at 1% significance (ZAR, 2009).

The data from the disinfection and from the rooting tests were analyzed by the Goodman association test involving contrasts between and within multinomial populations (GOODMAN, 1964; 1965), at 5% significance.

RESULTS

Colonization of vines by Petri disease fungi

'Niagara Rosada' grapevine plants collected from young commercial vineyard were infected by two different fungal species, which were molecularly identified as *Phaeomoniella chlamydospora* (IBVD01) and *Phialemoniopsis ocularis* (IBVD02)(Fig. 1).

'Niagara Rosada' plants grafted on rootstock Ripária do Traviú were infected by two pathogens that colonized only the basal part, with significant more colonization by *P. chlamydospora* than by *P. ocularis*. The plants grafted on rootstock IAC 766 were infected just by *P. chlamydospora,* which also colonized only the plant's basal part (Table 3).

Susceptibility of rootstocks to the fungus *P. chlamydospora*

Control treatment showed no dark streaks; thus, the data on the control were not included in the analyses and in Figure 2.

Golia rootstock was the least susceptible to *P. chlamydospora*, whereas Ripária Glorie, Ripária do Traviú, IAC 766, SO4 and Paulsen 1103 were moderately susceptible, and IAC 572 was the most susceptible (Fig. 2).

Table 1. Mineral composition (macro and micronutrients), pH and organic matter (OM) of the soil used.

pH $CaCl_2$	O.M. g/dm³	P_{resin} mg/dm³	Al^{3+}	H+Al	K	Ca	Mg	SB	CTC	V%	S	B	Cu	Fe	Mn	Zn
			mmol$_c$/dm³								mg/dm³					
4.3	10	12	5	28	1.0	2	2	5	33	15	7	0.14	0.3	9	2.2	0.2

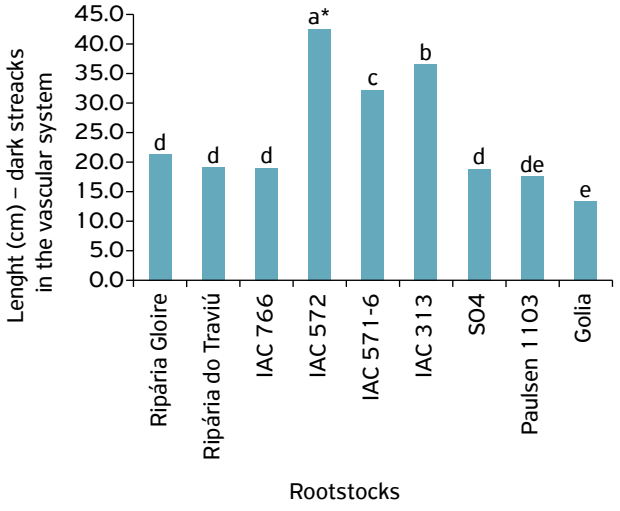
Table 2. Mineral composition (macro and micronutrients) of the kale plants used.

N	P	K	Ca	Mg	S	C	Fe	Mn	Cu	Zn	B	Al	Humidity %
Macronutrients (g/Kg)							Micronutrients (mg/Kg)						
30.6	3.3	34.3	32.5	5.8	2.4	396.3	290.5	218.5	11.3	155.6	40.9	387.3	89.2

Table 3. Median values and quartile semi-amplitude of the percentage of incidence of *Phaeomoniella chlamydospora* and *Phialemoniopsis ocularis* fungi in samples removed from the vascular system of different parts of 'Niagara Rosada' vines grafted on two different rootstocks.

Fungi	Rootstocks	Parts of plants		
		Branch	Stem	Basal
Phaeomoniella chlamydospora	Ripária do Traviú	0.0[1] (±0.0)[1] a[2] A[3]	0.0 (±0.0) a A	25.0 (±25.0) a B
	IAC 766	0.0 (±0.0) a A	0.0 (±0.0) a A	50.0 (±50.0) a B
Phialemoniopsis ocularis	Ripária do Traviú	0.0 (±0.0) a A	0.0 (±0.0) a A	0.0 (±12.50) a A
	IAC 766	0.0 (±0.0) a A	0.0 (±0.0) a A	0.0 (±0.0) a A

[1]Median and quartiles semi-amplitude of 60 fragments analyzed; [2]Lowercase letters compare the rootstocks by fungus in the parts of the plants sampled, according to the Dunn multiple comparison test (ZAR, 2009) at 5% significance; [3]Uppercase letters compare the different parts of the plants of the rootstock by fungus, according to the Dunn multiple comparison test (ZAR, 2009) at 5% significance.



*Letters compare the different rootstocks for their susceptibility, according to the T test at 1% probability (ZAR, 2009).

Figure 2. Susceptibility of vine rootstocks to the fungus *Phaeomoniella chlamydospora*.

Effect of technique combinations on disinfection and rooting of rootstock

The treatments without hot-water treatment (HWT) did not kill the fungus in the vascular system of the cuttings of the rootstock IAC 766 (Table 4).

The only treatments that suppressed the *P. chlamydospora* fungus in the vascular tissues of the cuttings of the rootstock IAC 766, in all exposure times tested, were biofumigation and temperature of 37°C, complemented with HWT. However, the biofumigation and temperature of 37°C applied for 14 days and complemented with HWT suppressed the fungus and allowed rooting on 75% of the rootstock cuttings (Tables 4 and 5). This rate of rootstock rooting is acceptable for later planting and production of healthy rootstock mother plants.

The HWT treatment (51°C for 30 min) tested solely (23°C complemented with HWT) did not affect the rootstock cuttings

Table 4. Percentage of incidence of *Phaeomoniella chlamydospora* in samples of the vascular system of the basal part of the rootstock IAC 766, submitted to different treatments for different periods, with and without added hot-water treatment (HWT).

Treatment	HWT 51°C/ 30 min.	Exposure time to treatment (days)	Incidence (%)
Soil + kale at 37°C "Biofumigation"	Without	7	25.0[1] b B β
		14	6.3 a AB α
		21	7.5 ab A α
Soil - kale at 37°C "Solarization"		7	20.0 a B β
		14	1.3 a A α
		21	45.0 b B β
Without soil and without kale at 37°C		7	2.5 a A α
		14	16.3 a B β
		21	15.0 a A β
Without soil and without kale at 23°C		7	0.0 a A α
		14	13.8 b AB α
		21	2.5 ab A α
Soil + kale at 37°C "Biofumigation"	With	7	0.0 a A α
		14	0.0 a A α
		21	0.0 a A α
Soil - kale at 37°C "Solarization"		7	0.0 a A α
		14	0.0 a A α
		21	1.3 a A α
Without soil and without kale at 37°C		7	0.0 a A α
		14	0.0 a A α
		21	0.0 a A α
Without soil and without kale at 23°C		7	2.5 a A α
		14	2.5 a A α
		21	1.3 a A α

Lowercase letters: comparison of different periods by fixing the thermotherapy and treatment; Uppercase letters: comparison of treatments by fixing the thermotherapy and the period; Greek letters: comparison of thermotherapy by fixing the treatment and the period. The comparisons were done in accordance with the Goodman association test (GOODMAN, 1964; 1965) at 5% significance
[1]Medium of 80 fragments analyzed.

for root formation, but also did not eliminate *P. chlamydospora* from plants (Tables 4 and 5).

The biofumigation for 21 days followed or not by HWT, and the temperature of 37ºC followed by HWT treatments hampered the root formation in the rootstock cuttings, resulting in 0%, 25% and 25% of root formation for these treatments (Table 5). Thus, the application of these treatments for periods over 14 days, complemented with HWT, negatively affected the root formation in the cuttings.

DISCUSSION

This is the first report of the *Phialemoniopsis ocularis* fungus in young vineyards in Brazil. The *P. chlamydospora* fungus that was found infecting 'Niagara Rosada' grafted on two different rootstocks is often the main responsible for Petri disease (HALLEEN et al., 2007; MUGNAI et al., 1999). This fungus had already been found in Brazil, infecting the rootstock SO4 (CORREIA et al., 2013).

HALLEEN et al. (2007) isolated *Phialemoniopsis curvata* (=*Phialemonium curvatum*) from vascular tissues from an asymptomatic vines nursery, and stated that this fungus may cause decline in nurseries or young vineyards. According to the phylogenetic tree of the present study (Fig. 1), the fungus *Phialemonium curvatum* (EF042105) found by HALLEEN et al. (2007) should belong to the *Phialemoniopsis cornearis* species.

The genus *Phialemoniopsis* was created by PERDOMO et al. (2013) to accommodate the *Phialemoniopsis curvata* (=*Phialemonium curvatum*) and *Phialemoniopsis ocularis* (=*Sarcopodium oculorum*) fungi, and two new species, *Phialemoniopsis cornearis* and *Phialemoniopsis pluriloculosa*. Interestingly, the GenBank sequences available for the *P. ocularis* species are related to specimens that cause opportunistic infections in humans and in other animals. A review about *Phaeoacremonium* species involved in Petri disease and Esca shows that some species of *Phaeoacremonium* are able to infect *Vitis vinifera* and humans (MOSTERT et al., 2006). According to HALLEEN et al. (2007), the relative importance of *P. cornearis* and *P. ocularis* to the decline in grapevines should be confirmed by assessing the frequency of incidence of these fungi on diseased grapevines with different ages, which grew in different places.

P. chlamydospora was found predominantly in the basal part of 'Niagara Rosada' vines (rootstocks Ripária do Traviú and IAC 766), confirming results obtained by ABREO et al. (2011) and LORENA et al. (2001). According to ABREO et al. (2011), the *P. chlamydospora* fungus may also be found in other parts of grapevine such as in the apical part. However, the rootstock is the main target for Petri disease fungi. Thus, studies are still necessary to find rootstocks with high level of resistance to Petri disease or techniques that ensure the total disinfection of the rootstock cuttings.

Reviewing on *Phaeoacremonium* species and on plant susceptibility to the Petri and Esca diseases, MOSTERT et al. (2006) emphasized the absence of rootstocks or scions with immunity or with a high level of resistance. In agreement with MOSTERT et al. (2006), none of the rootstocks tested in our study were immune or showed high level of resistance to *P. chlamydospora.*

The rootstocks Paulsen 1103 and Richter 110, artificially inoculated with *P. chlamydospora*, were more susceptible to the fungus compared to the *V. vinifera* cultivars, Chardonnay and Aglianico (ZANZOTTO et al., 2008). Similar results were obtained in Australia, where seven rootstocks (Ramsey, 99 Richter, Schwarzmann, Kober 5BB, P 1103, 101-14 Millardet and SO4) were more susceptible to *P. chlamydospora* than five cultivars of *V. vinifera* (Merlot, Cabernet Sauvignon,

Table 5. Percentage of root formation in the rootstock IAC 766 cuttings submitted to disinfection in different periods, with and without added hot-water treatment (HWT).

Treatment	HWT 51°C/ 30 min.	Exposure time (days)		
		7	14	21
Soil + kale at 37°C "Biofumigation"	Without	100.0[1] a A β	100.0 a A β	25.0 a A α
	With	75.0 a A β	75.0 a A β	0.0 a A α
Soil – kale at 37°C "Solarization"	Without	75.0 a A α	100.0 a A α	50.0 a AB α
	With	50.0 a A α	75.0 a A α	50.0 a AB α
Without soil and without kale at 37°C	Without	75.0 a A α	50.0 a A α	100.0 b B α
	With	100.0 a A β	75.0 a A αβ	25.0 a A α
Without soil and without kale at 23°C	Without	100.0 a A α	100.0 a A α	100.0 a B α
	With	75.0 a A α	100.0 a A α	100.0 a B α

[1]Medium of four cuttings analyzed. Lowercase letters: comparison of thermotherapy (with and without) by fixing the treatment and the period; uppercase letters: comparison of treatments by fixing the thermotherapy and the period; Greek letters: comparison of different periods (7, 14 and 21 days) by fixing treatment and thermotherapy. The comparisons were done in accordance with the Goodman association test (GOODMAN, 1964; 1965) at 5% significance.

Pinot Noir, Shiraz PT10 and Shiraz PT23) (WALLACE et al., 2004). These studies show that *V. vinifera* materials are indeed less susceptible to the *P. chlamydospora* fungus compared to the rootstocks. Furthermore, GRAMAJE et al. (2010) suggested that grapevine rootstock crosses of *V. riparia* x *V. berlandieri* could be less susceptible to the patogens *C. luteo-olivacea*, *Phaeoacremonium* spp. and *P. chlamydospora*, which are involved with Petri disease in young vines.

In the present study, the rootstocks with low and moderate susceptibility come from *Vitis riparia*, *V. rupestris, V. berlandieri* and *V. vinifera*, which suggests that their genetic backgrounds from species of *Vitis* can be responsible for the least susceptibility to *P. chlamydospora*. New breeding studies are needed to obtain a rootstock with a satisfactory resistance level to Petri disease pathogens.

In agreement with ROONEY; GLUBER (2001), the application of HWT at 51°C for 30 min solely as a curative measure is not sufficient to control *P. chlamydospora* and *P. inflatipes* from dormant material. On the other hand, GRAMAJE et al. (2009), when applying HWT with temperatures above 50°C for different periods, managed to eliminate *P. chlamydospora* from vine materials, but the process affected the sprouting and the weight of branches of the combination Temp – scion and 161-49 C – rootstock. In the present study, HWT (51°C for 30 min) tested as sole treatment (23°C followed by HWT) did not affect the rootstock cuttings for root formation, but also did not eliminate *P. chlamydospora* from the plants.

The biofumigation or temperature of 37°C treatments should be applied for 14 days, followed by the complementary treatment of HWT at 51°C for 30 min in order to eliminate *P. chlamydospora* from rootstock cuttings without affect the rooting. The interaction among different treatments such as biofumigation plus HWT can be more efficient to control the Petri disease fungi in rootstock cuttings compared to HWT tested as a sole technique.

Once the efficiency of these new techniques is validated, the healthy and normal cuttings can be used by growers to obtain healthy rootstock mother plants and, consequently, produce healthy nursery plants, eliminating the following problems reported by other authors:

- transmission of the Petri disease fungi in nurseries through the use of infected mother plants (MOSTERT et al., 2006), and
- use of HWT with temperatures above 50°C for long periods, which eradicates the fungus *P. chlamydospora*, but that can damage the plants (GRAMAJE et al., 2009).

CONCLUSION

In conclusion, *P. chlamydospora* and *P. ocularis* colonized prevalently the basal part of 'Niagara Rosada' plants, denoting colonization of the rootstock. *P. ocularis* was detected by the first time in young vineyards in Brazil. Golia was the least susceptible rootstock to *P. chlamydospora*, and IAC 572 was the most susceptible. Biofumigation or temperature of 37°C applied for 7-14 days and followed by HWT at 51°C for 30 min, when used as treatments, suppressed *P. chlamydospora* in the rootstock cuttings without hampering the rooting. Meanwhile, new studies are necessary to validate the efficiency these disinfection techniques.

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