Crop Protection 100 (2017) 203–210

Contents lists available at ScienceDirect

Crop Protection

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

Investigating the durable effect of the hot water treatment used in nurseries on pathogenic fungi inhabiting grapevine wood and involved in Grapevine Trunk Diseases

Emilie Bruez [a, b, *], Philippe Larignon [c], Stéphane Compant [d], Patrice Rey [a, b]

[a] Université de Bordeaux, ISVV, UMR1065 Santé et Agroécologie du Vignoble (SAVE), Bordeaux Sciences Agro, F-33140, Villenave d'Ornon, France
[b] INRA, ISVV, UMR1065 SAVE, F-33140, Villenave d'Ornon, France
[c] Institut Français de la Vigne et du Vin (ENTAV-ITV France), Pôle Rhône-Méditerranée, 7 Avenue Cazeaux, 30230 Rodilhan, France
[d] AIT Austrian Institute of Technology GmbH, Bioresources Unit, Health & Environment Department, Konrad Lorenz Strasse 24, 3430 Tulln, Austria

ARTICLE INFO

Article history:
Received 26 May 2016
Received in revised form
20 March 2017
Accepted 1 July 2017

Keywords:
Hot water treatment
Fungal microflora
Vine
Grapevine Trunk Diseases

ABSTRACT

Hot water treatment (HWT) is used in nurseries to control pathogenic fungi involved in Grapevine Trunk Diseases (GTDs), as well as other pathogens, such as phytoplasmas. The long-term impact of this treatment on the entire microflora, especially on the general fungal microbiota living inside plants, still remains however unknown. In this study, the fungal microflora of vineyard plants, treated or not by HWT 14 and 15 years earlier were compared at different plant part levels. The fungal microflora was relatively abundant in the different types of wood tissues. Certain fungal genera were first isolated and then identified on the basis of their treatment or not by HWT. A significant change between 2010 and 2011, the two sampling years, was detected. Although the HWT may have affected the cuttings microflora at the nursery stage, this had not persisted after several years of HWT treatment for the fungi, especially the pathogenic ones. As HWT does not have a significant long-term control effect on GTD pathogens in mature plants in the vineyards, applying other sanitary methods as soon as HWT-young vines are planted in the vineyards is recommended.

1. Introduction

Grapevine Trunk Diseases (GTDs), the most predominant vine diseases, are found throughout many of the world's vine-growing countries, such as France, Spain, Italy, Portugal, South Africa, Australia, Chile, USA (California). The causal agents are various fungi, principally *Phaeomoniella chlamydospora*, *Phaeoacremonium minimum*, *Fomitiporia mediterranea, Diaporthe ampelina, Cylindrocarpon* spp. and *Botryosphaeria* spp. (Scheck et al., 1998; Mugnai et al., 1999; Armengol et al., 2001; Rumbos and Rumbou, 2001; Edwards and Pascoe, 2004; Gimenez-Jaime et al., 2006). In France, 13% of vineyards are currently unproductive because of these diseases (Grosman and Doublet, 2012). In certain wine regions, such as Cognac, the percentage of unproductive grapevines was estimated between 2003 and 2008 at 32.6% and, in the Jura, at 18.4% (Bruez et al., 2013). The use of sodium arsenite, the only chemical product capable of reducing such diseases in the vineyards, was banned in 2003 in Europe, due to its toxicity for Human health and the environment. Various treatments have since been tested, with variable success, to control such diseases in the vineyards. Additionally, sanitary measures have to be applied in nurseries to prevent initial plant contamination by pathogenic fungi. Gramaje and Armengol (2011) have suggested that an integrated management programme for grapevine propagation material should be developed. In that programme, Hot Water Treatment (HWT) has been used to prevent pathogen infections in nursery cuttings. Initially, HWT was principally used against Flavescence Dorée and Bois Noir, two Phytoplasma-related diseases, but it also has an impact on GTDs (Chalak et al., 2013). Crocker et al. have equally pointed out that some *Vitis vinifera* L. cultivars, such as Pinot Noir, Chardonnay, Merlot, Riesling and Petit Verdot, and certain rootstock varieties, Ramsey and Ruggeri 140, are naturally sensitive to HWT (Crocker et al., 1999). Laukart et al. (2001) have shown, too, that when HWT is used on Pinot Noir, it influences the dark pith coloration (Waite and May, 2005). HWT can be a practical and

* Corresponding author. INRA, ISVV, UMR1065 SAVE, F-33140, Villenave d'Ornon, France.
E-mail address: emilie.bruez@inra.fr (E. Bruez).

http://dx.doi.org/10.1016/j.cropro.2017.07.001
0261-2194/

relatively inexpensive means of controlling fungal pathogens in dormant wood (Caudwell et al., 1997). Nonetheless, negative effects, including the delayed callusing and rooting of HWT cuttings, have been observed by growers (Halleen et al., 2007). Even if HWT does control a number of grapevine pests and diseases in dormant grapevine cuttings and young rooted grapevines (Mannini, 2007), it needs to be carefully applied because it may interfere with the vitality of wood propagation (Bleach et al., 2013). Caudwell et al. (1997) have described that HWT eliminates microorganisms without damaging the grapevines when it is applied at 50 °C for 45 min. In New Zealand and Australia, HWT is usually used during the production of cuttings. HWT is also used to control black-foot disease, caused by *Cylindrocarpon* spp., and Petri disease, associated with *P. chlamydospora* and *P. minimum*. In South Africa, Fourie and Halleen (2004) have shown that infected propagation material is responsible for the dispersal of pathogens that cause young vine decline and that HWT (50 °C for 30 min) could control such infection. In Spain, Gramaje et al. (2009) have shown that, when applied for at least 30 min at 54 °C, HWT had an effect on Petri disease pathogens. In France, Larignon et al. (2008) have shown that, immediately after applying HWT to the cuttings for 50 °C during 45 min, the pathogenic fungi involved in GTDs, such as *P. chlamydospora*, *Diplodia seriata, Diaporthe ampelina,* were not isolated from the wood, but that *P. minimum* and *Neofusicoccum parvum* were isolated (Larignon and Dubos, 1997).

Although HWT can protect nursery cuttings from infection by certain phytopathogens involved in GTDs, there is currently no data regarding its effect on fungal microflora as a whole and over a long period. As growing vines contain a wide range of fungal microflora, HWT might also impact some members of this microbiota.

The aim of this study was to determine whether HWT could have a long-term impact on the pathogenic fungal microflora involved in GTDs 14 and 15 years after the treatment. The specific objectives were to: *(i)* isolate and characterize the fungi colonizing the HWT and non-HWT vines (HWT/Control); *(ii)* compare their respective fungal communities; *(iii)* observe any differences between the microflora colonizing the wood tissues of vines sampled in 2010 and 2011.

2. Materials and methods

2.1. Plant material and sampling

Experimentation was carried out by uprooting grafted Pinot noir grapevines planted in sandy soil in 1996 from a vineyard located in Marsannay-la-Côte in Burgundy (France). The rootstock was 3309 Couderc clone 114, and the scion cultivar a Pinot noir clone 115. Before the grapevines were planted, half of the 2508 plants plotted in 26 ranks had been subjected to HWT (50 °C for 45 min). The plants, which were collected in September 2010 and 2011 were, consequently, either 14 or 15 years old.

2.2. Fungal isolation and identification

Eight HWT and eight control grapevines were sampled. Each plant had expressed leaf stripes symptoms and an apoplectic form, for the first time in 2010 or in 2011. In order to study the fungal microflora colonizing the wood tissues, the plants were cut horizontally and transversally, and the rootstock part, graft union and trunk were separated. Different types of necrosis were then determined as white-rot, black punctuation (xylem vessels becoming black), black dots (zone with a lot of black punctuation), sectorial necrosis and central necrosis. All these tissues were sampled, and distinguished according to whether HWT or Control plants were concerned. Chips of each type of wood tissue were sampled.

2.3. Isolation and identification on agar media

For each type of necrosis, located in the rootstock, graft union or trunk, ten chips (5 × 5 × 1 mm) of wood were aseptically dissected, before being immerged for 15 s in 3% calcium hypochlorite. The 10 chips were then deposited on two Malt Agar Petri plates amended with chloramphenicol (0.025% w/v) against the bacterial development.

Fungal strain development was monitored for three weeks. Whenever possible, the taxonomic identification of the endophytic fungi was based on morphological and cultural features, combined with an examination of fruiting structures and fungal conidia under the microscope (Leitz LaborLux D, Germany). All isolated fungi were kept in Malt Agar Petri Plates in a collection room chamber at 4 °C.

2.4. Data analysis

2.4.1. Species diversity

Specific diversity indexes, Shannon H, and Simpson D (Shannon and Weaver, 1963; Buckland et al., 2005) were calculated and used to separate the different parts and types of grapevines (HWT and Control vines). Such indexes were estimated using the package Vegan of R version 3.0.2 software.

Comparison of the pathogenic fungal species between the HWT and Control were made using the Shapiro-Wilk test. As the data did not follow the normality, the Wilcoxon test was applied.

2.4.2. Rarefaction curves, diversity estimates

Species accumulation curves were estimated, using the number of species, for all the grapevines studied. The data of the eight HWT and the eight control plants were pooled. Curves were defined using Estimates software.

2.4.3. Canonical correspondence analysis

Canonical correspondence analysis (CCA) was used to determine the effect of the HWT on the fungal communities of the grapevines studied (Bruez et al., 2015). CCA was performed using R software package ade4. Analyses were based on the relative abundance of all the species found in the samples. The sampling year was treated as an independent variable, the species relative abundance as a dependent variable, and HWT or control vines as the co-variable. Centroids for independent variables (sampling dates and HWT or Control), as well as species scores for the fungi, were presented in a biplot plan. Proximity of a species at the centroid signifies that the species has a high relative abundance in the samples.

2.4.4. Non-metric multidimensional scaling (NMDS)

Non-metric multidimensional scaling (NMDS) was used to visualize the similarity level of individual cases in a dataset (Holland, 2008) and to refer to a set of related ordination techniques for information visualization, especially concerning the information contained in a distance matrix. The NMDS were defined using the package Vegan of R version 3.0.1 software. To validate those results, the statistical test ANOSIM was verified using package Vegan of R software.

3. Results

3.1. Status of the wood

When the plants were cut, different types of necrosis were observed, depending on the specific parts (Fig. 1). Necrotic wood

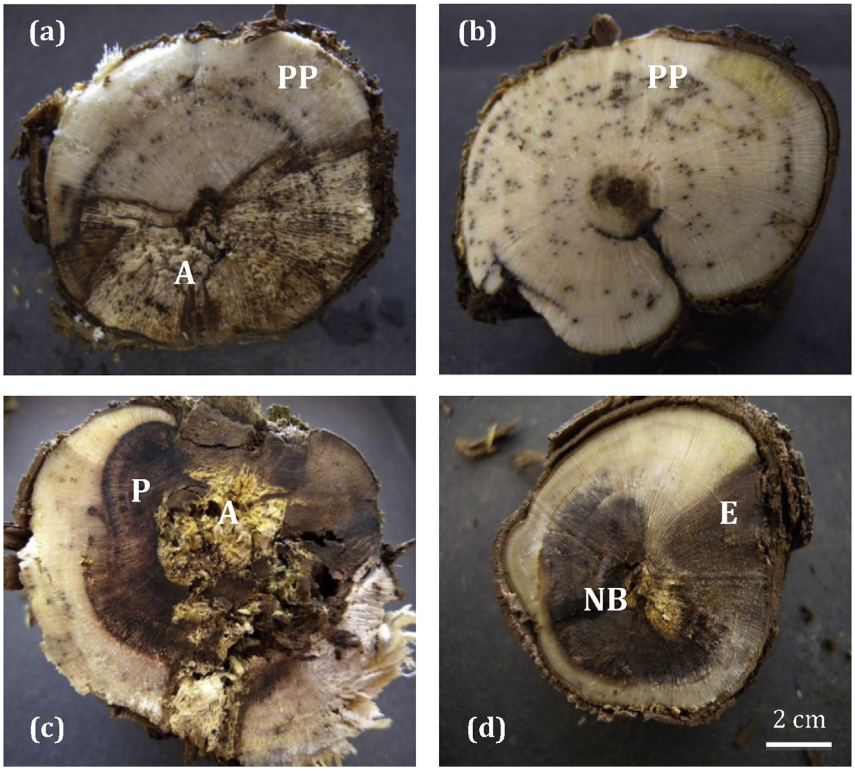

Fig. 1. Photographs of cross-sections of grapevine trunks showing necrosis symptoms (a), (b), (c) and (d). A = white-rot; E = sectorial necrosis; NB = central necrosis; P = black dot; PP = black punctuation.

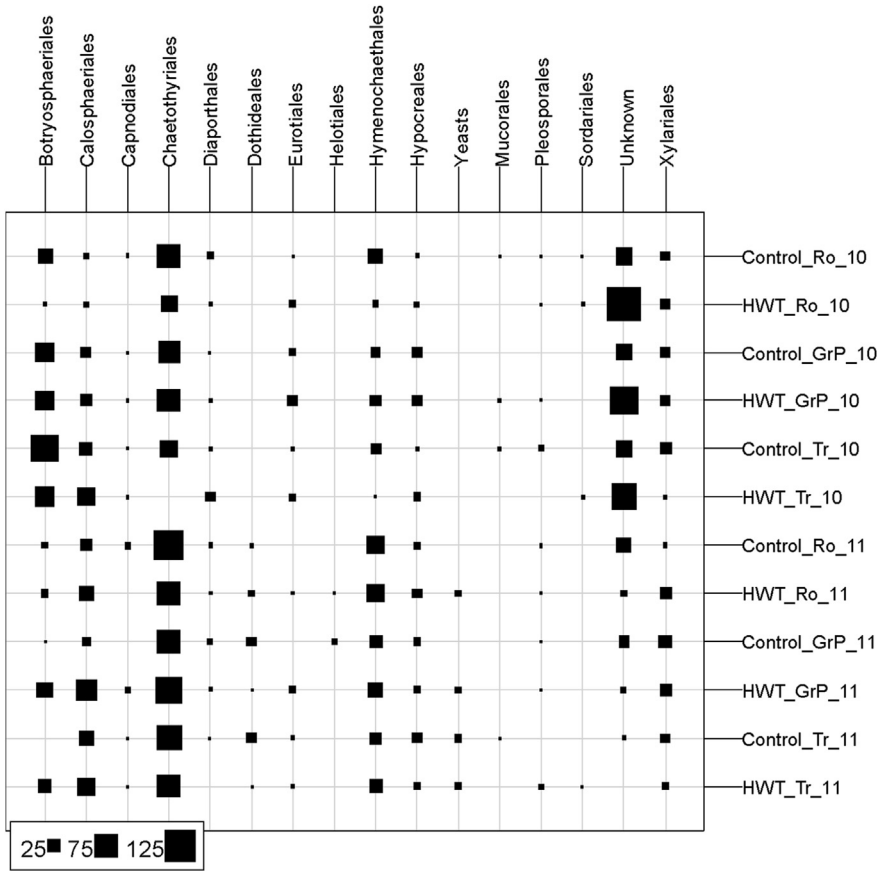

Fig. 2. Distribution of fungal orders in the different HWT/Control plant parts. Each square represents the number of isolates per type of plant and part according to the sampling dates. HWT = hot-water treated plants; Control = plants without HWT; Ro = rootstock; Tr = trunk; GrP = graft union.

tissues were predominant and white-rot, black punctuation (xylem vessels becoming black), black dots (zone with a lot of black punctuation), sectorial necrosis and central necrosis were observed.

3.2. Colonization rate

In total, 2250 fungal cultures were isolated from the 5760 chips taken from the thirty-two vines during 2010 and 2011. In 2010, 512 fungi were isolated from the HWT plants and 525 from the Control plants. In 2011, 583 fungi were isolated from the HWT plants and 630 from the Control ones.

3.3. Distribution of the fungal communities

Ascomycota, the predominant division (88.7%), contained 14 orders, followed by Basidiomycota (10%) with only 1 order. Fig. 2 shows the distribution of the fungal orders according to whether the grapevine parts were HWT/Control plants. The most representative order was Chaetothyriales (34% of Ascomycota), followed by Botryosphaeriales (27% of Ascomycota). Yeasts and Dothideales were isolated, but only in 2011, with more unidentified fungi being isolated in 2010.

Twenty-six genera and species were identified from the different parts and tissue types. Fig. 3 shows the distribution of the fungal genera and species according to sampling year and HWT/Control. These results showed that *Phaeomoniella chlamydospora* was isolated mainly in the black line and black dot parts. *Fomitiporia mediterranea* was principally isolated from white-rot, black line and sectorial necrosis from both HWT/Control plants, in both sampling years.

Overall, the most abundant species isolated were *P. chlamydospora* (30%), *Diplodia seriata* (12%), *F. mediterranea* (10%) and *P. minimum* (7.5%). For HWT/Control plants, *P. chlamydospora* was the most abundant species for both sampling dates. Fig. 3 shows the species according to the sampling date and the status of vines. In 2011, for *Botryosphaeria* sp. and *Neofusicoccum parvum,* differences were observed between the HWT and Control grapevines (respectively $p = 0.0152$ and $p = 0.0192$).

The correspondence analysis in Fig. 4 represents the species according to the sampling year and HWT/Control status. Two groups were separated by the vertical axis. The species identified in 2010 and 2011 tended to differ. In 2011, *D. seriata* was isolated more in Control plants, whereas *F. mediterranea* and *P. chlamydospora* were isolated more in HWT grapevines in the same year. In 2010, *Chaetomium* sp., *Aspergillus* sp., and *Fusarium* sp. were more isolated from HWT plants.

3.4. Fungal biodiversity

Shannon and Simpson indexes were calculated for each HWT/Control plant part for both sampling dates (Fig. 5). The Shannon indexes were higher in plants sampled in 2011, particularly for the rootstock (Fig. 5a). The values obtained for the Simpson index were higher in 2011 than in 2010 (Fig. 5b).

Species accumulation was plotted in order to study the relationship between the number of isolated fungi and the plants uprooted for the study (Fig. 6). To calculate the species accumulation curves (Sobs), the Mao-Tao estimator was used to compare species richness, with a confidence interval of up to 95%. Species

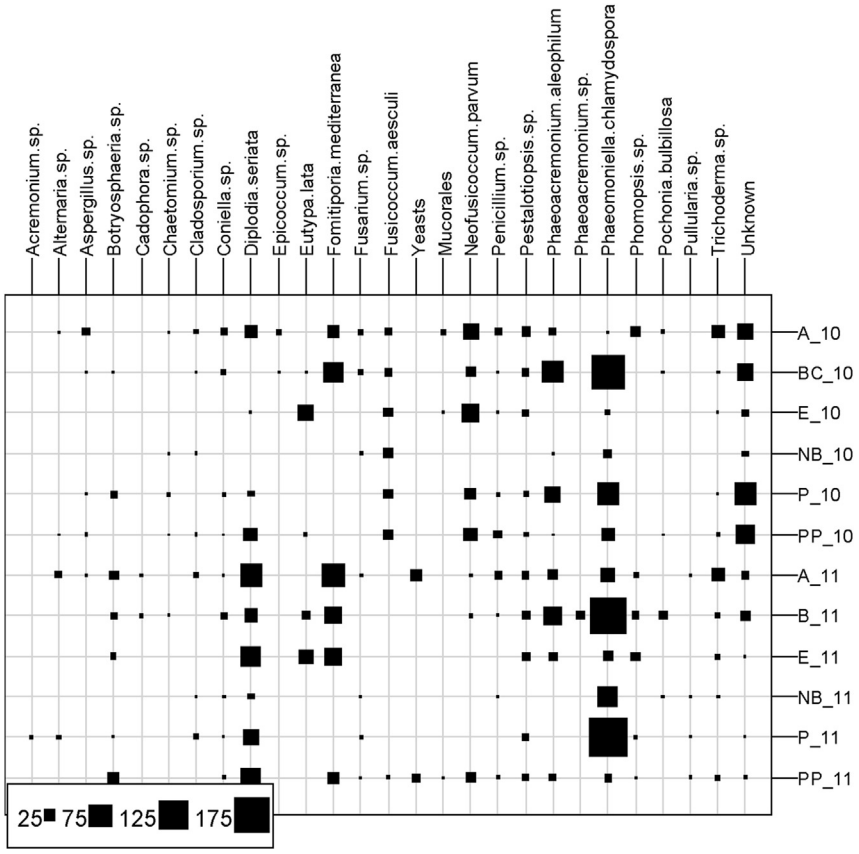

Fig. 3. Distribution of fungal species according to the different parts of all HWT/Control plants, sampled in 2010 and 2011. Each square represents the number of isolates per type of plant and part according to the year. A = white-rot; BC = black line; E = sectorial necrosis; NB = central necrosis; P = black dot; PP = black punctuation.

Fig. 4. Correspondence Analysis representing fungal species according to the sampling date (2010 or 2011) and the HWT and Control vine status. TEC = hot-water treated vines; TEM = control vines.

accumulation did not reach the asymptote, but the curves of the species richness of plants uprooted in 2010 were closer to the asymptote.

3.5. Fungal communities

The NMDS represented all OTUs, without the singletons of the thirty-two samples. Two NMDS, based on Bray-Curtis dissimilarity, were performed (Fig. 7): *(i)* The fungal communities of HWT/Control plants were similar (Fig. 7a); *(ii)* The fungal communities of the plants uprooted either in 2010 or in 2011 were different (Fig. 7b). ANOSIM results indicated data similarities or differences within the group (Fig. 7a: $R = -0.004$ $p < 0.564$; Fig. 7b: $R = 0.181$ $p = 0.001$). Both NMDS and ANOSIM showed that, although there were few differences in the composition of fungal communities of HWT/Control plants, there were significant differences according to sampling date.

4. Discussion

Fourteen and 15 years later, after HWT had been applied to young plants for 45 min at 50 °C, we analyzed in both the HWT and Control vines the principal microflora inhabiting the different types of necrotic tissue observed. For the two sampling dates, all the vines showed the typical leaf stripes and apoplectic form of wood grapevine disease, independently of the treatment. When both the HWT and Control vines were longitudinally cut, it was observed that the frequency of necrosis was the same in the trunk of HWT and Control vines. As regards the different types of necrotic woody tissues, *(i)* they were colonized by fungal species involved in GTDs; *(ii)* in the HWT and Control vines, these species were similar.

In the present experiment, fungi frequently colonized wood chips, since they were isolated from 40% of them (5760 wood chips were analyzed). Several authors, such as Casieri et al. (2009), Gonzalez and Tello (2011), Hofstetter et al. (2012) and Bruez et al. (2014) have also isolated a high percentage of fungi from the woody tissues of vines, but with a higher fungal diversity in the non-necrotic tissues than in the necrotic ones.

In our study, twenty-six species, in both 2010 and 2011, were isolated from the six types of necrotic wood tissues. Necroses were colonised by specific pathogenic fungal species, such as *F. mediterranea*, found in white-rot tissues. In central necrosis, the most abundant fungus, *P. chlamydospora*, was also isolated in the five other types of tissue. Our results are consistent with those in the literature, which indicate that certain fungal species predominate in vine necroses: *F. mediterranea* predominates in white-rot (Fisher, 2006), *P. chlamydospora* in central necrosis, and *Botryosphaeria* spp. in sectorial necrosis (White et al., 2011; Urbez-Torres et al., 2006; Armengol et al., 2001).

Although pathogenic GTDs fungi were isolated in both sampling years, the communities did not have the same percentage. For example, in 2011, *D. seriata* represented 18% of the isolates, but only 5% in 2010. *N. parvum* was isolated in 12% of the total isolates in 2010, but only 1.4% in 2011. Depending on the sampling date, the composition and quantity of the fungi inhabiting the different wood tissues are different, as shown by Bruez et al. (2014) when they analyzed the fungal community colonizing the wood of grapevines over a period of one year. In a recent study, Bruez et al.

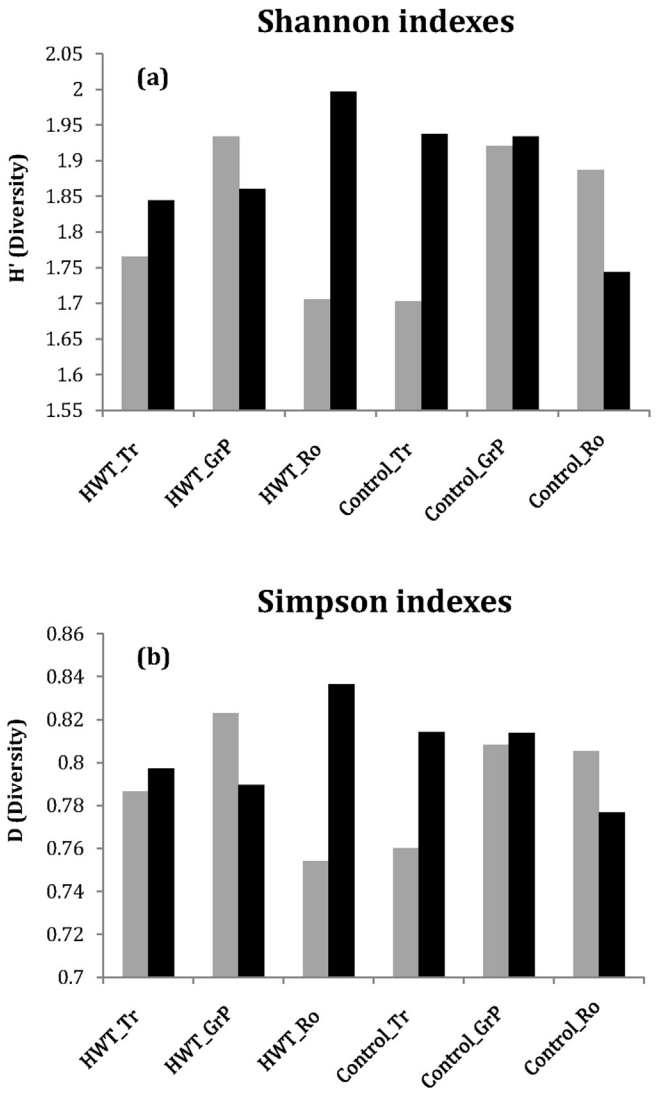

Fig. 5. Diversity indexes of the different parts of the HWT/Control plants, sampled in 2010 and 2011. (a): Shannon indexes; (b): Simpson indexes. HTW = hot-water treated plants; Control = plants without hot-water treatment; Ro = rootstock; Tr = trunk; GrP = graft union.

have also shown that, for the same cultivar, the age of the plant has a significant effect on the wood microbiota (Bruez et al., 2015). Comparison between pathogenic fungal communities of HWT and Control grapevines in 2010 and 2011 showed that, differences were only observed for *Botryosphaeria* sp. and *N. parvum* in 2011. Isolates of the two fungi were detected in higher numbers in the HWT grapevines. So, HWT does not seem to induce significant changes on the pathogenic microflora over a long-time period.

Larignon et al. (2008) have shown that, although HWT has a potential effect on the eradication of the pathogenic fungi, it has a negative effect on the other fungi. However, 14 or 15 years after HWT, fungi known to be either potentially protective or saprophyte were isolated, even if the pathogenic ones predominated. *Trichoderma* sp. was isolated in white-rot, and *Pestalotiopsis* sp. in all types of tissue except in the central necrosis. *Trichoderma* spp., frequently isolated from young vines in nurseries or in vines from vineyards, are known to protect many plants and to have antagonistic abilities for parasiting pathogenic fungi (Cobos et al., 2015; Casieri et al., 2009). However, their potential to protect grapevines in the present study seems to be very limited. Laukart et al. (2001) have shown that HWT is not a curative treatment, but is useful as a disinfectant during the propagation process. In cuttings, the percentages of *P. chlamydospora* or *P. minimum* decrease after being subjected to the treatment. Gramaje et al. (2009) have demonstrated that, at the end of the growing season, a strong decrease in the pathogenic fungi could be observed in the shoots, even when different water temperatures were used. Their study shows globally that, depending on the rootstock, cultivar and treatment temperature, the treated cuttings were re-colonized by the pathogenic fungi involved in Esca disease. In our study comparing the fungal microflora of HWT and control vines, the 3 species involved in Esca, *P. chlamydospora, P. minimum* and *F. mediterranea*, were isolated in 2010 and 2011 from the 6 types of grapevine woody tissue. No impact of HWT treatment 14 or 15 years after being applied to young plants could be discerned in any of the fungal communities, including those associated with Esca. This observation is not really surprising since, once young vines are removed to the vineyards, they become subjected to many sources of fungal infection, sources which are not present at the nursery stage.

The most important source of fungal infection could well be the annual pruning of vines. After a 14- or 15-year period, various wounds were created on the vine cordon, enabling the fungi to penetrate and colonize the woody tissues. In a very recent experiment on vines, Travadon et al. (2016), have shown that the specific

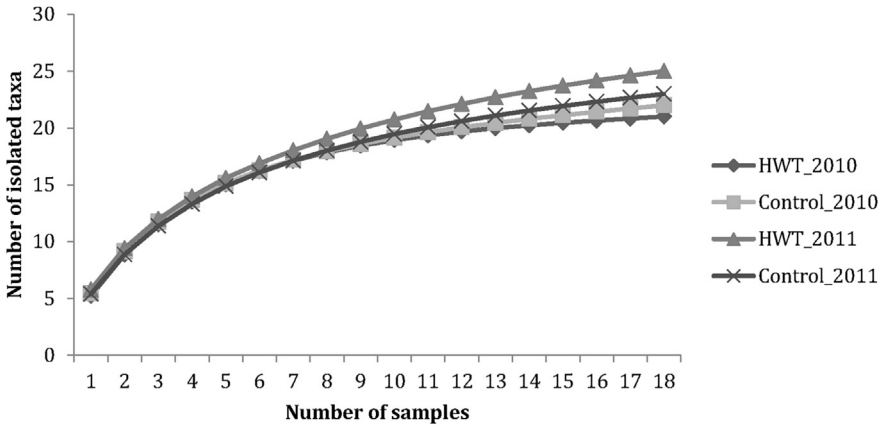

Fig. 6. Species accumulation curves showing the relationship between the number of plants analyzed and the number of fungal taxa isolated from HWT and Control plants, sampled in 2010 and 2011.

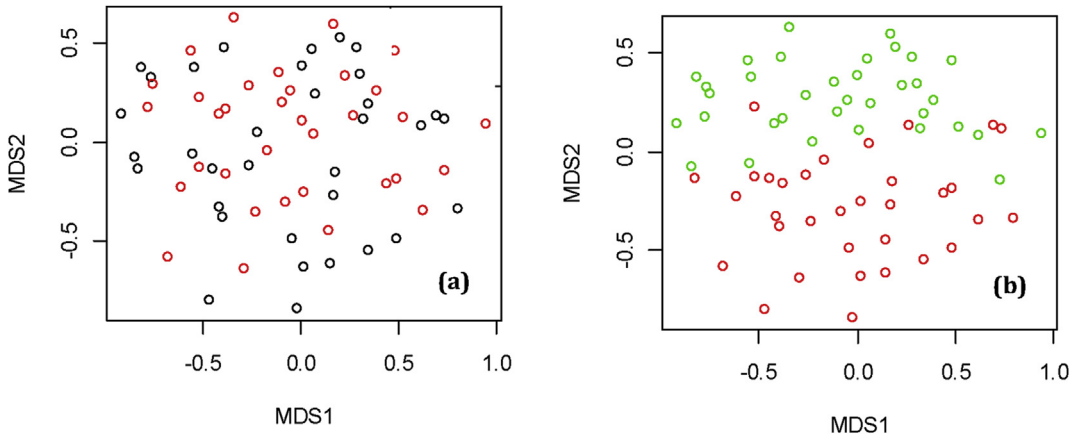

Fig. 7. nMDS analysis of the fungal communities for the different data sets generated, based on Bray-Curtis dissimilarity. Each data point represents data from a single sample. The data used for this analysis contain all the OTUs defined. (a): nMDS represents all vines sampled in 2010 and 2011, either HWT vines (black) or Control vines (red). (b): nMDS represents all HWT/Control plants sampled in 2010 (green) and 2011 (red).

type of pruning system, minimal vs spur-pruning, influences woody fungal diversity, with the size of woody necrosis being due to the ensuing composition of the fungal community, particularly that of its pathogens. In their experiment, minimal pruning, the system that caused the fewer wounds, was the one associated with reduced necrosis.

Another experiment by Cobos et al. (2015) demonstrated that pathogens, such as *Diplodia seriata* and *P. chlamydospora*, could penetrate via wounds. These two reports, which might explain why HWT-vines are colonized by Esca-pathogenic fungi, also strongly indicate the need for sanitary methods to be continuously applied to plants.

In conclusion, the pathogens involved in GTDs still colonized grapevine wood tissues, even though the plants had received HWT in the nursery, and no essential difference was found between either HWT or Control fungal microflora. In order to ensure that the initially beneficial effect of HWT on the young vines is prolonged, sanitary measures should be applied once the vines have been planted in the vineyards, and subsequently, throughout their entire lifespan.

Acknowledgments

This work was financed by CASDAR (Compte d'Affectation Spéciale au Développement Agricole et Rural, Ministère de l'Agriculture, V1302). The authors would like to thank Cost action FA1303, the Fondation Poupelain and the LabEx COTE.

References

Armengol, J., Vicent, A., Torne, L., Garcia-Figueres, F., Garcia-Jimenez, J., 2001. Fungi associated with decline and infections of grapevine wood in various Spanish zones. Bol. Sanid. Vegetal. Plagas 27, 137–153.

Bleach, C., Jones, E., Ridgway, H., Jaspers, M., 2013. Hot water treatment to reduce incidence of black foot pathogens in young grapevines grown in cool climates. Phytopathol. Mediterr. 52, 347–358.

Bruez, E., Lecomte, P., Grosman, J., Doublet, B., Bertsch, C., Fontaine, F., Rey, P., 2013. Overview of grapevine trunk diseases in France in the 2000s. Phytopathol. Mediterr. 52, 262–275.

Bruez, E., Vallance, J., Gerbore, J., Lecomte, P., Da Costa, J.P., 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. http://dx.doi.org/10.1371/journal.pone.009592.

Bruez, E., Baumgartner, K., Bastien, S., Travadon, R., Guérin-Dubrana, L., Rey, P., 2015. Various fungal communities colonize the functional wood tissues of old grapevines externally free from Grapevine Trunk Disease symptoms. Aus J. Grape Wine Res. 22 (2), 288–295.

Buckland, S., Magurran, A.E., Green, R.E., Fewster, R.M., 2005. Monitoring change in biodiversity through composite indices. Philos. Trans. R. Soc. B-Biol Sci. 360, 243–254.

Casieri, L., Hofstetter, V., Viret, O., Gindro, K., 2009. Fungal communities living in the wood of different cultivars of young Vitis vinifera plants. Phytopathol. Mediterr. 48, 73–83.

Caudwell, A., Larrue, J., Boudon-Padieu, E., McLean, G.D., 1997. Flavescence doree elimination from dormant wood of grapevines by hot-water treatment. Aus J. Grape Wine Res. 3, 21–25.

Chalak, L., Elbitar, A., Mourad, N., Mortada, C., Choueiri, E., 2013. Elimination of grapevine bois noir phytoplasma by tissue culture coupled or not with heat therapy or hot water treatment. Adv. Crop Sci. Tech. 1 (2).

Cobos, R., Mateos, R.M., Álvarez-Pérez, J.M., Olego, M.A., Sevillano, S., González-García, S., Garzón-Jimeno, E.R., Coque, J.J., 2015. Effectiveness of natural antifungal compounds in controlling infection by grapevine trunk disease pathogens through pruning wounds. App Env. Microb. 18 (81), 6474–6483.

Crocker, J., Waite, H., Fletcher, G., 1999. Development of Effective, Efficient and Reliable Hot Water Treatments. Grape and Wine Research and Development Corporation project SAR 99/4.

Edwards, J., Pascoe, I.G., 2004. Occurrence of *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum* associated with Petri disease and esca in Australian grapevines. Aus Plant Pathol. 33, 273–279.

Fisher, M., 2006. Biodiversity and geographic distribution of basidiomycetes causing esca-associated white rot in grapevine: a worldwide perspective. Phytopathol. Mediterr. 45, S30–S42.

Fourie, P.H., Halleen, F., 2004. Occurrence of grapevine trunk disease pathogens in rootstock mother plants in South Africa. Aus Plant Pathol. 33, 313–315.

Gimenez-Jaime, A., Aroca, A., Raposo, R., Garcia-Jimenez, J., Armengol, J., 2006. Occurrence of fungal pathogens associated with grapevine nurseries and the decline of young vines in Spain. J. Phytopathol. 154, 598–602.

Gonzalez, V., Tello, M.L., 2011. The endophytic mycota associated with Vitis vinifera in central Spain. Fungal Div. 47, 29–42.

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.

Gramaje, D., Armengol, J., Salazar, D., Lopez-Cortes, I., Garcia-Jimenez, J., 2009. Effect of hot-water treatments above 50 degrees C on grapevine viability and survival of Petri disease pathogens. Crop Prot. 28, 280–285.

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 65, 31–35.

Halleen, F., Fourie, P.H., Crous, P.W., 2007. Control of black foot disease in grapevine nurseries. Plant Pathol. 56, 637–645.

Hofstetter, V., Buyck, B., Croll, D., Viret, O., Couloux, A., Gindro, K., 2012. What if esca disease of grapevine were not a fungal disease? Fungal Div. 54, 51–67.

Holland, S., 2008. Non-metric Multidimensional Scaling (MDS) R Software. Department of Geology, University of Georgia, Athens.

Larignon, P., Dubos, B., 1997. Fungi associated with esca disease in grapevine. Eur. J. Plant Pathol. 103, 17–157.

Larignon, P., Giansetto, K., Salancon, E., Berud, F., Girardon, K., Jacquet, O., 2008. Effet de divers traitements à l'égard des champignons associés aux maladies du bois en pépinière. Le. pépiniériste 179, 10–14.

Laukart, N., Edwards, J., Pascoe, I.G., Nguyen, N.K., 2001. Curative treatments trialed on young grapevines infected with *Phaeomoniella chlamydospora*. Phytopathol. Mediterr. 40, S459–S463.

Mannini, F., 2007. Hot water treatment and field coverage of mother plant vineyards

to prevent propagation material from phytoplasma infections. Bul. Insect 60, 311–312.
Mugnai, L., Graniti, A., Surico, G., 1999. Esca (Black measles) and brown wood-streaking: two old and elusive diseases of grapevines. Plant Dis. 83, 404–418.
Rumbos, I., Rumbou, A., 2001. Fungi associated with esca and young grapevine decline in Greece. Phytopathol. Mediter 40, S330–S335.
Scheck, H., Vasquez, S., Fogle, D., Gubler, W.D., 1998. Grape growers report losses to black-foot and grapevine decline. Calif. Agri 52, 19–23.
Shannon, C., Weaver, W., 1963. The Mathematical Theory of Communication. University Illinois Press.
Travadon, R., Lecomte, P., Diarra, B., Lawrence, D.P., Renault, D., Ojeda, H., Rey, P., Baumgartner, K., 2016. Grapevine pruning systems and cultivars influence the diversity of wood-colonizing fungi. Fungal Ecol. 24, 82–93.
Urbez-Torres, J.R., Leavitt, G.M., Voegel, T.M., Gubler, W.D., 2006. Identification and distribution of *Botryosphaeria* spp. associated with grapevine cankers in California. Plant Dis. 90, 1490–1503.
Waite, H., May, P., 2005. The effects of hot water treatment, hydration and order of nursery operations on cuttings of *Vitis vinifera* cultivars. Phytopathol. Mediterr. 44, 144–152.
White, C., Hallen, F., Mostert, L., 2011. Symptoms and fungi associated with esca in South African vineyards. Phytopathol. Mediterr. 50, 236–246.