Effects of hot-water treatment on grapevine viability, yield components and quality of must

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Abstract

Background and Aims: Hot-water treatment **(**HWT) has been shown to be effective for the control of a number of endogenous and exogenous grapevine pests and diseases in dormant grapevine cuttings and young rooted vines. However, little is still known about the long-time effects of HWT on plant viability under field conditions. The effects of HWT on the performance of dormant plants in a 4-growing seasons study were investigated.

Methods and Results: The effects of HWT at 53ºC for 30 min on shoot weight, yield parameters and quality of must in dormant grafted plants (Tempranillo cultivar grafted onto 110 Richter rootstock) were evaluated. Eight bundles of 20 grafted plants were assigned to HWT, and eight additional bundles of 20 untreated grafted plants were prepared as controls (non-HWT). Dormant grafted plants were immediately planted in two field sites in April 2007. Shoot fresh weight was evaluated during winter in four consecutive growing seasons.

Yield parameters and must quality indicators were evaluated in the 3^{rd} and 4^{th} growing seasons. In general, there were no significant differences in shoot weight at pruning, yield parameters and must components between treatments, with the exception of some must quality indicators in the 4^{th} growing season.

Conclusions: The findings obtained in this study indicate that HWT at 53ºC for 30 min did not affect plant viability, yield parameters and the main indicators of must quality, and could be used successfully in a commercial situation.

Significance of the Study: This study represents the first approach to investigate the long-term effect of HWT on plant development, yield and the quality of must under field conditions. It suggests that the success of HWT not only depends on the most adequate protocol applied by nurseries, but management practices before, during and after the propagation process could affect the viability of HWT grapevine propagating material.

Keywords: grape, must analysis, nursery, *Vitis vinifera*

Introduction

Sporadic and costly failures of large batches of vines due to hot-water treatments (HWTs) are an ongoing problem for both grape growers and nurseries worldwide (Waite and May, 2005, Waite and Morton, 2007). HWTs have been shown to be effective for the control of a number of endogenous and exogenous grapevine pests and diseases in dormant grapevine cuttings and young rooted vines, including *Agrobacterium vitis* (Burr et al. 1989, 1996, Ophel et al. 1990), the mealy bug *Planococcus ficus* (Haviland et al. 2005), mites (Szendrey et al. 1995), nematodes (Lear and Lider 1959, Meagher 1960, Nicholas et al. 2001), phylloxera (Buchanan and Whiting 1991, Stonerod and Strik 1996), *Phytophthora cinnamomi* (Von

Broembsen and Marais 1978), the phytoplasma Flavescence dorée (Caudwell et al. 1997), Pierce's Disease (Goheen et al. 1973) and *Xylophilus ampelinus* (Psallidas and Argyropoulou 1994).

However, questions about its efficacy arose after the wine industry planting boom in the 1990s, when many planted vines were found to be infected with fungal trunk pathogens (Mugnai et al. 1999). However, HWT is currently the most promising and relatively inexpensive method for the control of endogenous diseases caused by these pathogens in grapevine propagating material (Fourie and Halleen 2004, Gramaje and Armengol 2011). Since then, some anecdotal reports of unacceptably high losses when long duration HWT (50°C for 30 or 45 min) is applied to commercial batches of cuttings and rootlings have been published. The transfer of HWT from small batch research laboratory treatments to commercial practice has met with mixed success and significant losses have been attributed to HWT in Australia (Waite and Morton, 2007). In Italy, Habib et al. (2009) reported negative side-effects on shoot development and growth of graftlings, rootstocks and grafted rootstocks treated (140 Ruggeri and 1103 Paulsen grafted with 'Negroamaro' cultivar) at 50º C for 45 min after one-growing season. Bleach et al. (2009) indicated that, although HWT of young grapevine plants reduces incidence of black-foot disease in New Zealand, the standard HWT protocols (50ºC for 30 min) sometimes damage young plants, possibly due to poor heat acclimatization in the cool climate of New Zealand. Conversely, Waite and May (2005) investigated the effects of different hydration times and HWT protocols on cuttings of Chardonnay and Cabernet Sauvignon at callusing phase under glasshouse conditions and, despite the variable responses of the 2 varieties to HWT when measuring callus, shoot and root development, no cuttings of either variety died and all the cuttings were of good commercial quality and considered saleable by the host nursery.

Although HWT of rootstock cuttings prior to grafting (Edwards et al. 2004, Fourie and Halleen 2004, Eskalen et al. 2007) or HWT of dormant nursery plants after uprooting (Fourie and Halleen 2002, 2004, Halleen et al. 2007, Gramaje et al. 2009, Vigues et al. 2010) has been strongly recommended as a means of reducing fungal infection levels in nursery plants, there is still confusion in the wine industry about the efficacy and safety of HWT. Concerns expressed by nurseries and growers resulted in a significant body of research into the effects of HWT on cuttings and rootlings; however, these investigations have been performed under controlled conditions within a short period of time (Laukart et al. 2001, Waite and May 2005, Serra et al. 2011), or under field conditions within one-growing season (Crous et al. 2001, Edwards et al. 2004, Fourie and Halleen 2004, Gramaje et al. 2009, Serra et al, 2011, Gramaje and Armengol 2012). In addition, the criteria used to determine the effects of HWT have focused only on plant development (Goussard 1977, Orffer and Goussard 1980, Burr et al. 1989, 1996, Bazzi et al. 1991, Wample 1993, 1997, Caudwell et al. 1997, Waite and May 2005, Gramaje et al. 2009, Gramaje and Armengol 2012).

In Spain, Gramaje et al. (2008, 2010) fixed 53°C for 30 min as the most effective treatment to reduce conidial germination and mycelial growth of black-foot and Petri disease pathogens. The effect of this treatment was further evaluated in dormant rootstock cuttings and grafted plants after one growing season (Gramaje et al. 2009, Gramaje and Armengol, 2012), and results demonstrated that it is possible to hot-water treat grapevine planting material in Spanish nurseries using protocols with temperatures of up to 53ºC. However, little is still known about the long-term effects of HWT on plant viability once they are planted in the vineyard; therefore, the objective of this research was to investigate the impact of HWT at 53ºC for 30 min on grapevine development, yield parameters and quality of must in a 4-growing seasons study (2008-2011).

Methods and materials

Planting material and treatment

A total of 320 grafted plants ready to be sold to producers of the scion/rootstock combination Tempranillo/110 Richter were obtained from a commercial nursery in Valencia (Spain). This planting material was allocated at random to 16 bundles of 20 plants. Eight bundles (160 grafted plants) were assigned to non-HWT (control) and the remaining 8 bundles were assigned to HWT. For HWT, planting material was placed in a hydrating bath for 1 h in order to presoak material before treatment. Following hydration, plants were placed in mesh polyethylene bags and immersed in a temperature-controlled bath at 53°C for 30 min (Gramaje et al. 2009). On removal from the HWT bath, grafted plants were immediately plunged into a cool bath of clean potable water at ambient temperature for 30 min in order to stop the heating process. Plants were then removed from the bath and allowed to drain until there was no free moisture on the surface of the plants.

Hot-water treated and noHWTed plants were immediately planted in April 2007 in two field sites (four bundles of 20 plants per treatment, 160 grafted plants per field site) where grapevines had not been grown, at "Las Tiesas" experimental farm. The farm is located at the city limits between Barrax and Albacete (Spain) and its average geographical coordinates are latitude 39°14′north, longitude 2°5′west and the altitude is 695 m above sea level. Each bundle (20 grafted plants) was planted in one single row, with grafted plants 1.4 m apart from centre to centre and an inter-row spacing of 2.8 m. The vines were trained to a standard T-trellis system. Each field plot was 30 m long and included eight rows. In both sites, the experimental design consisted of four randomized blocks, each containing 2 rows of grafted vines (one bundle each of HWT or non-HWT in each row) (40 plants per block). Standard cultural practices were used at both sites during the grapevine growing season.

Assessment of plant growth, fruit sampling and must analyses

Plants were pruned in four consecutive growing seasons to two buds per spur and eight spurs along the cordon, during conventional winter pruning time. Shoots of all treatments were immediately wrapped and taken back to the laboratory for weight assessment. In the 3^{rd} and 4^{th} growing seasons the fruits of each plan were weighed (yield) at harvest, and the berry sample weight and Ravaz index (yield/pruning weight) were calculated per plant. In addition, samples of 500 berries per bundle were taken at random to analyse the must. The fruit was gently macerated by hand, coarsely sieved and the must analysed for total acidity, tartaric acid, malic acid, anthocyanins, reducing sugars, colour and intensity, total soluble acids, total phenol content, volumetric mass, yeast assimilable nitrogen (YAN), pH and potassium. Analyses of must were performed by LIEC Agoalimentaria S. L. (Ciudad Real, Spain) using the FT-IR spectroscopy technique (Foss WineScan™ FT120).

Statistical methods

Statistical analysis of the results within each growing season was done using one-way analysis of variance with treatment as independent variable and the following dependant variables: shoot weight (g/plant), yield (g/plant), Ravaz index (g fruit/g pruning), berry sample weight (g), titratable acidity (g H_2SO_4/L), tartaric acid (g/L), malic acid (g/L), anthocyanins (g/L), reducing sugars (g/L), colour and intensity (Absorbance Units), total soluble acids (ºBaumé), total phenol content, volumetric mass (g/mL), YAN (mg/L), pH and potassium (g/L). The Student's Least Significant Difference test was used to compare the overall means of each treatment at $P < 0.05$. Statistical analyses were performed using SAS (version 9.0, SAS Institute, Cary, NC, USA).

Results

Shoot weight and yield parameters

There were no significant differences in shoot weight and yield parameters between the two field sites in each of the four growing seasons evaluated, so the data were combined for analyses ($P > 0.1$). There were no significant differences in shoot weight at pruning between treatments (HWT and non-HWT) within each growing season ($P > 0.05$) (Figure 1). Shoot weight was similar among treatments throughout the 4-growing seasons of the study, with slight, but not significant ($P > 0.05$) reductions of shoot weight in hot-water treated (HWTed) plants in 2010 and 2011 (834.8 and 967.3 g/plant, respectively) compared to plants that were non-HWTed (946.2 and 1,080.5 g/plant, respectively).

There were no significant differences in yield and Ravaz index between treatments (HWT and non-HWT) in the 3rd and 4th growing seasons ($P > 0.05$) (Table 1). In the 3rd growing season the yield was 4.9 kg/plant and 5.4 kg/plant in HWTed and non-HWTed plants, respectively. The Ravaz index was 6.6 g fruit/g pruning and 5.8 g fruit/g pruning in HWTed and non-HWTed plants in this season. In the 4th growing season, yield was 8.0 kg/plant in the HWTed plants and the Ravaz index was 8.6 g fruit/g pruning, while the yield was 7.8 kg/plant and the Ravaz index was 7.6 g fruit/g pruning in plants that were non-HWTed.

Must analyses

Must quality indicators of Tempranillo/110 R combination in the 3rd and 4th growing seasons are shown in Table 1. There were no significant differences in must quality indicators between field sites in each growing season, so the data were combined for analyses ($P > 0.1$). Significant differences between HWTed and non-HWTed plants in must quality indicators were only observed for total soluble solids (*P* = 0.0440) and volumetric mass (*P* = 0.0454) in the 4th growing season.

Discussion

This study analyses the effects of HWT on grapevine viability, yield parameters and quality of must under field conditions over the first 4 growing seasons after planting in the vineyard. Although shoot weight at pruning was similar among treatments throughout the study, there was a slight reduction of shoot weight in HWTed plants in the 3rd and 4th growing seasons. This phenomenon has already been observed in trials performed over one-growing season in Australia and Spain. Waite and May (2005) indicated that HTWTed cuttings, particularly sensitive varieties, are generally slower to establish, than cuttings that have not been treated with hot water, and suffer delayed early growth. Waite (2002) argued that HWTed plants begin to recover from mid summer and make up the difference in growth and are indistinguishable from untreated cuttings by the end of the first growing season. Most recently, Gramaje et al. (2009) and Gramaje and Armengol (2012) observed that, although planting material in Spain is able to tolerate HWT at 53ºC for 30 min, sprouting was delayed and, as a consequence, shoot weight was significantly reduced in HWTed plants with respect to the untreated controls. On the basis of our results, we did not find statistically significant differences among treatments when measuring the shoot weight, but HWTs seemed to still produce an effect on other aspects of plant development after 4 growing seasons. However, the retarded growth of HWTed plants did not result in a decrease in yield parameters, and the levels obtained for yield, ravaz index and berry sample weight were not significantly different among treatments through the study.

We evaluated the effects of HWT on viability, yield components and quality of must of Tempranillo/110 R combination, Tempranillo is the most planted red wine variety in Spain and rootstock 110 R is the most widely used rootstock, accounting for 33.7% of the rootstock mother-field planted area and one of the rootstock most often demanded by Spanish grapegrowers (Hidalgo 2002). Little variability in the tolerance among grapevine cultivars (Bobal, Merlot and Tempranillo) to temperatures in Spain has been reported previously (Gramaje et al. 2009). However, Waite et al. (2001) studied the sensitivity of different grapevine varieties to HWT in Australia and concluded that Pinot Noir was the most sensitive variety, Chardonnay, Reisling and Merlot were moderately sensitive and Cabernet Sauvignon the least sensitive. Further studies with additional grapevine cultivars are needed to evaluate the long term effects of HWT post the initial years of vineyard establishment.This is the first study to investigate the effect of HWT on the quality of must. Previous research has focused only on the assessment of bud, shoot, callus and sometimes root development, over time (Goussard 1977, Orffer and Goussard 1980, Burr *et al.* 1989, 1996, Bazzi *et al.* 1991, Wample 1993, 1997, Caudwell *et al*. 1997, Waite and May 2005, Gramaje et al. 2009, Gramaje and Armengol 2012). In general, our results showed that HWT at 53ºC for 30 min did not affect the main indicators of must quality, and could be used successfully in a commercial situation. Slight reductions were observed in total acidity and tartaric acid in both growing seasons, and statistically significant reductions in the total soluble solids and volumetric mass in the 4th growing season for HWTed plants with respect to the control (not hot-water-treated plants).

Must acidity usually varies depending on the cultivar, the climate and grape maturity (Ribéreau-Gayon et al. 2000a). These authors reported that in musts from northerly vineyards, concentrations of tartaric acids are often over 6 g/L whereas, in the south, they may be as low as 2–3 g/L since respiration is more effective when the grape bunches are maintained at high temperatures. In our study, the differences observed in total acidity and tartaric acid among treatments ($\leq$ 0.5 g/L) are not sufficiently important to cause a significant variation in wine quality, since a maximum dose of tartaric acid of 1.5 g/L is permitted to further correct the acidity and effect a positive impact on wine stability and flavor (Ribéreau-Gayon et al. 2000b).

Total soluble solids and the volumetric mass are used to evaluate the sugar concentration of must by refractometric and densimetric analyses, respectively (Iland et al. 2004). Crippen and Morrison (1986) reported that ºBrix is a good indicator of berry sugar content at levels above 18 (>10 ºBaumé), when sugars become the predominant soluble solids in grapes. The small but significant differences observed in total soluble solids ($\leq$ 0.6 ºBaumé) and volumetric mass ($\leq$ 0.7 g/mL) in the 4^{th} growing season could be due to different aspects of sampling (different ºBaumé among berries within the same fruit, sampling moment), yield storage, measurement error or even slight differences in types of sugars that might give different readings for different methods.

The results of recent research, which has identified HWT as the most important tool to limit and/or reduce fungal pathogen infection in grapevine nurseries (Edwards et al., 2004, Fourie and Halleen 2004, Eskalen et al. 2007, Halleen et al. 2007, Gramaje et al. 2009, Gramaje and Armengol, 2012), have not resulted in increased acceptance of HWT as a reliable technique that can be used with confidence by nurseries. In Australia, many nurseries have experienced costly failures of HWTed cuttings and vines and are reluctant to use HWT unless it is required to move vines between quarantine jurisdictions (Waite 2010). The sporadic nature of cutting and vine failure after HWT has made it difficult to determine the reasons for the problems. Recent research indicated that many management practices before, during and after the propagation process could affect the viability of HWT grapevine propagating material. These include management of mother vines (level of fertilization, crop load, pests and diseases), pre HWT processes (harvesting and transporting cuttings and rootlings, hydration of propagation material, water quality, anaerobic conditions) and post

HWT nursery processes (cool down tanks, cold storage, callusing and growing on conditions), nursery hygiene and storage and environmental conditions in the nursery. Among them, cold storage conditions are key to the survival of HWTed cuttings and vines (Waite and Morton 2007). Gramaje and Armengol (2012) evaluated the effect of HWT, cooling and cold storage on plant viability in dormant grafted grapevines and concluded that long-time cold storage could be detrimental to planting material especially when plants have not been soaked following HWT. Ventilation of plastic wrapping on cuttings during cold storage is strongly recommended in order to prevent oxygen deprivation and damaging fermentation (Waite et al. 2001).

Our findings demonstrate that although there is some slight long term effect of HWT on vines, this is not statistically significant. This suggests that the success of HWT not only depends on the most adequate temperature and time combination applied by nurseries. The consensus in other literature is that the operations pre and post HWT are important; however, these nursery practices that are used in the propagation process are often viewed and assessed separately rather than as part of a continuum, with HWT frequently singled out by the industry as the cause of cutting and vine failure. The possibility that each operation may have a slight, but incremental negative effect on the material is not normally considered. Investigations have recently revealed that cutting and vine failure is the result of many, seemingly minor, but poor decisions during the propagating and planting process, each of which has had a small but cumulative impact on the quality of the vine.

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References

Bazzi C., Stefani, E., Gozzi, R., Burr, T.J., Moore, C.L. and Anaclerio, F. (1991) Hot-water treatment of dormant grape cuttings; its effects on *Agrobacterium tumefaciens* and on grafting and growth of vine. Vitis **30**, 177–187.

Bleach, C. M., Jones, E. E. and Jaspers, M. V. (2009) Hot water treatment for elimination of *Cylindrocarpon* species from infected grapevines. Phytopathologia Mediterranea **48,** 183.

Buchanan, G.A. and Whiting, J.R. (1991) Phylloxera management: prevention is better than cure. Australian and New Zealand Wine Industry Journal **6**, 223–230.

Burr, T.J., Ophel, K., Katz, B.H. and Kerr, A. (1989) Effect of hot water treatment on systemic *Agrobacterium tumefaciens* Biovar 3 in dormant grape cutting. Plant Disease **73**, 242–245.

Burr, T.J., Reid, C.L., Splittstoesser, D.F. and Yoshimura, M. (1996) Effect of heat treatments on grape bud mortality and survival of *Agrobacterium vitis in vitro* and in dormant grape cuttings. American Journal of Enology and Viticulture **47**, 119–123.

Caudwell, A., Larrue, J., Boudon-Padieu, E. and McLean, G.D. (1997) Flavescence dorée elimination from dormant wood of grapevines by hot-water treatment. Australian Journal of Grape and Wine Research **3**, 21–25.

Crippen, D.D. and Morrison, J.C. (1986) The effects of sun exposure on the compositional development of ‘Cabernet Sauvignon’ berries. American Journal of Enology and Viticulture **37**, 235–242

Crous, P.W., Swart, L., Coertze, S. (2001) The effect of hot-water treatment on fungi occurring in apparently healthy cuttings. Phytopathologia Mediterranea **40**, S464–S466.

Edwards, J., Pascoe, I.G., Salib, S. and Laukart, N. (2004) Hot treatment of grapevine cuttings reduces incidence of *Phaeomoniella chlamydospora* in young vines. Phytopathologia Mediterranea **43**, 158–159.

Eskalen, A., Rooney-Latham, S. and Gubler, W.D. (2007) Identifying effective management strategies for esca and Petri disease. Phytopathologia Mediterranea **46**, 125–126.

Fourie, P.H. and Halleen, F. (2002) Investigation on the occurrence of *Phaeomoniella chlamydospora* in canes of rootstock mother vines. Australasian Plant Pathology **31**, 425–426.

Fourie, P.H. and Halleen, F. (2004) Proactive control of Petri disease of grapevine through treatment of propagation material. Plant Disease **88**, 1241–1245.

Goheen, A.C., Nyland, G. and Lowe, S.K. (1973) Association of a rickettsia-like organism with Pierce’s disease of grapevines and alfalfa dwarf and heat therapy of the disease in grapevines. Phytopathology **63**, 341–345.

Goussard, P.G. (1977) Effect of hot-water treatments on vine cuttings and one-year-old grafts. Vitis **16**, 272–278.

Gramaje, D., García-Jiménez, J. and Armengol, J. (2008) Sensitivity of Petri disease pathogens to hot-water treatments *in vitro*. Annals of Applied Biology **153**, 95–103.

Gramaje, D., Armengol, J., Salazar, D., López-Cortés, I. and García-Jiménez, J. (2009) Effect of hot-water treatments above 50ºC on grapevine viability and survival of Petri disease pathogens. Crop Protection **28**, 280–285.

Gramaje, D., Alaniz, S., Abad-Campos, P., García-Jiménez, J. and Armengol, J. (2010) Effect of hot-water treatments *in vitro* on conidial germination and mycelial growth of grapevine trunk pathogens. Annals of Applied Biology **156**, 231–241.

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

Gramaje, D. and Armengol, J. (2012) Effects of hot-water treatment, post-hot-water-treatment cooling and cold storage on the viability of dormant grafted grapevines under field conditions. Australian Journal of Grape and Wine research **18**, 158–163.

Habib, W., Pichierri, A., Masiello, N., Pollastro, S. and Faretra, F. (2009) Application of hot water treatment to control *Phaeomoniella chlamydospora* in grapevine plant propagation materials. Phytopathologia Mediterranea **48**, 186.

Halleen, F., Fourie, P. H. and Crous, P.W. (2007) Control of black foot disease in grapevine nurseries. Plant Pathology **56**, 637–645.

Haviland, D.R., Bentley, W.J. and Daane, K.M. (2005) Hot-water treatments for control of *Planococcus ficus* (Homoptera: Pseudococcidae) on dormant grape cuttings. Journal of Economic Entomology **98**, 1109–1115.

Hidalgo, L. (2002) Tratado de Viticultura General. Ed. Mundi-Prensa, Madrid.

Iland, P.G., Bruer, N. and Wilkes, E. (2004) Chemical Analysis of Grapes and Wine: Techniques and Concepts, Patrick Iland Wine Promotions, South Australia.

Laukart, N., Edwards, J., Pascoe, I.G. and Nguyen, N.K. (2001) Curative treatments trialled on young grapevines infected with *Phaeomoniella chlamydospora*. Phytopathologia Mediterranea **40**, S459–S463.

Lear, B. and Lider, L.A. (1959) Eradication of root-knot nematodes from grapevine rootings by hot water. Plant Disease Reporter **14**, 314–317.

Meagher, J.W. (1960) Root-knot nematode of the grapevine. The Journal of Agriculture, Victoria **7**, 419–445.

Mugnai, L., Graniti, A. and Surico, G. (1999) Esca (black measles) and brown wood-streaking: two old and elusive diseases of grapevines. Plant Disease **83**, 404–416.

Nicholas, P.R., Chapman, A.P. and Cirami, R.M. (2001) Grapevine Propagation. Pages 1-22 in: Viticulture, Vol. 2, Practices. B. G. Coombe and P. R. Dry, eds. Winetitles, Adelaide, Australia.

Ophel, K., Nicholas, P.R., Magarey, P.A. and Bass, A.W. (1990) Hot water treatment of dormant grape cuttings reduces crown gall incidence in a field nursery. American Journal of Enology and Viticulture **41**, 325–329.

Orffer, C.J. and Goussard, P.G. (1980) Effect of hot-water treatments on budburst and rooting of grapevine cuttings. Vitis **19**, 1–3.

Psallidas, P.G. and Argyropoulou, A. (1994) Effect of hot water treatment on *Xylophilus ampelinus* in dormant grape cuttings. in: Lemattre, M. et al. (eds.) Plant Pathogenic Bacteria, Symposium, Versailles, France June 9-12, 1992). Colloques de l'INRA **66**, 993–998.

Ribéreau-Gayon, P., Dubourdieu, D., Donéche, B. and Lonvaud, A. (2000a) Handbook of Enology, Volume 1: The microbiology of wines and vinification (John Wiley & Sons Ltd: Chichester, UK) pp. 454.

Ribéreau-Gayon, P., Glories, Y., Maujean, A. and Dubourdieu, D. (2000b) Handbook of Enology Volume 2: The Chemistry of Wine Stabilisation and Treatments. (John Wiley & Sons Ltd: Chichester, UK) pp. 404.

Serra, S., Mannoni, M.A., Ligios, V. and Fiori, P.P. (2011) Occurrence of *Phaeomoniella chlamydospora* on grapevine planting material in Sardinia and its control with combined hot water and cyproconazole treatments. Phytopathologia Mediterranea **50**, S61–S76.

Stonerod, P. and B. Strik. (1996) Hot water dipping eradicates phylloxera from grape nursery stock. HortTechnology **6**, 381–383.

Szendrey, G., Dulinafka, Gy. and Szegedi, E. (1995) Elimination of mites from the buds of dormant grapevine cuttings by hot water treatment. Vitis **34**, 65–66.

Vigues, V., Yobregat, O., Barthélémy, B., Dias, F., Coarer, M., Girardon, K., Berud, F., Muller, M. and Larignon, P. (2010) Wood decay diseases: tests of disinfection methods in French nursery. Phytopathologia Mediterranea **49**, 130–131.

Von Broembsen, S. and Marais, P.G. (1978) Eradication of *Phytophthora cinnamomi* from grapevine by hot water treatment. Phytophylactica **10**, 25–27.

Waite, H. (2002) The Effects of Hot Water Treatment on Grapevine Propagating and Planting Material. Master of Applied Science (Horticulture) Thesis, University of Melbourne, Victoria, Australia.

Waite, H. (2010) Trunk diseases and vine failure: The costs of poor quality propagating and planting material. The Australian and New Zealand Grapegrower and Winemaker Annual Technical Issue **555**, 21–22.

Waite, H. and May, P. (2005) The effects of hot water treatment, hydration and order of nursery operations on cuttings of *Vitis vinifera* cultivars. Phytopathologia Mediterranea **44**, 144–152.

Waite, H. and Morton, L. (2007) Hot water treatment, trunk diseases and other critical factors in the production of high-quality grapevine planting material. Phytopathologia Mediterranea **46**, 5–17.

Waite, H., Crocker, J., Wright, P., Fletcher, G. and Delaine, A. (2001) Hot water treatment in commercial nursery practice – an overview. The Australian Grapegrower and Winemaker **449**, 39–43.

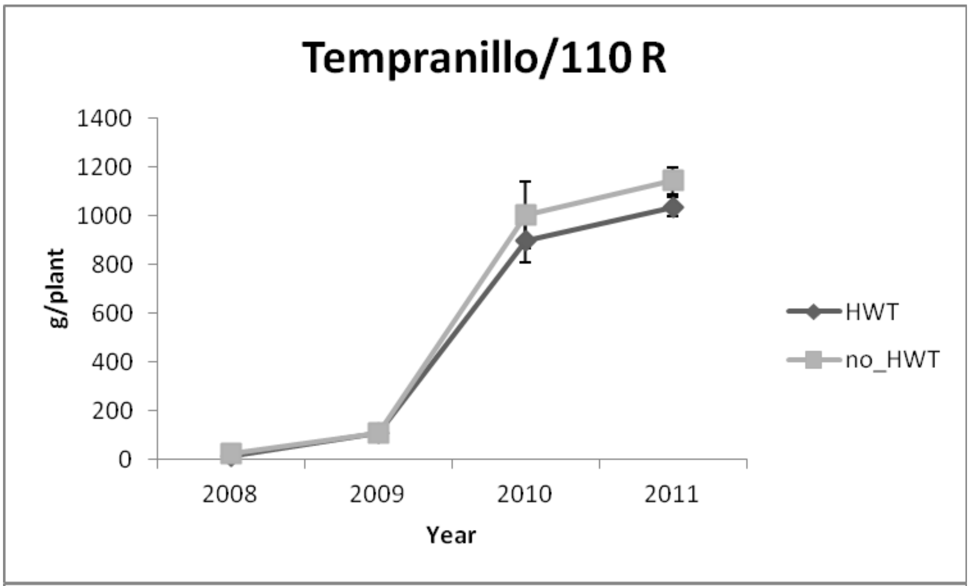
Wample, R. (1993) Influence of Pre- and Post-Treatment storage on budbreak of hot water treated cuttings of Cabernet Sauvignon. American Journal of Enology and Viticulture **44**, 153–158.

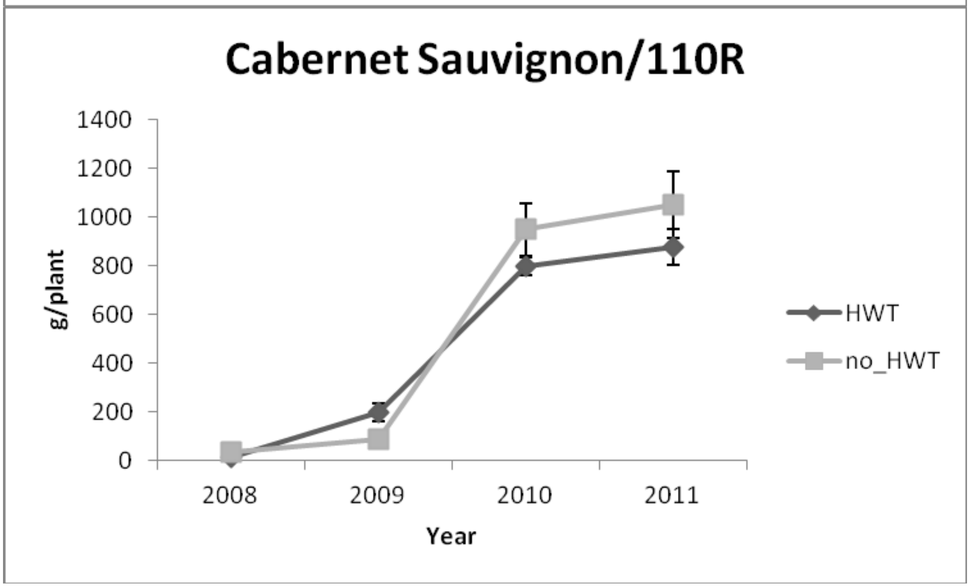
Wample, R. (1997) Influence of pre- and post-treatment storage on rooting of hot-water-treated cuttings of Cabernet Sauvignon, American Journal of Enology and Viticulture **48**, 131–136.

Table 1. Yield parameters and must quality indicators of Tempranillo/110 R combination in the 3rd and 4th growing seasons.

	3rd growing season		4th growing season	
Variables studied	**HWT**	**No HWT**	**HWT**	**No HWT**
Yield components				
Yield (kg/plant)	4.9† ± 1.5a	5.4 ± 0.8a	8.0 ± 1.8a	7.8 ± 1.8a
Ravaz index (g fruit/g pruning)	6.6 ± 3.7a	5.8 ± 0.8a	8.6 ± 3.0a	7.6 ± 2.6a
Berry sample weight (g)	1.4 ± 0.13a	1.4 ± 0.18a	1.6 ± 0.19a	1.6 ± 0.16a
Must quality indicators				
Total acidity (g H_2SO_4/L)	2.8 ± 0.6a	3.3 ± 0.4a	3.9 ± 0.3a	4.0 ± 0.2a
Tartaric acid (g/L)	5.4 ± 0.6a	5.9 ± 0.4a	5.9 ± 0.1a	6.2 ± 0.3a
Malic acid (g/L)	1.4 ± 0.1a	1.6 ± 0.3a	2.1 ± 0.1a	2.0 ± 0.3a
Anthocyanins (g/L)	0.24 ± 0.02a	0.229 ± 0.03a	0.163 ± 0.02a	0.173 ± 0.02a
Reducing sugars (g/L)	0.222 ± 0.02a	0.237 ± 0.02a	0.245 ± 0.06a	0.253 ± 0.03a
Colour intensity (AU)	9.9 ± 1.2a	9.5 ± 1.1a	7.9 ±1.2a	8.1 ± 3.5a
Total soluble solids (ºBaumé)	12.3 ± 1.4a	12.9 ± 0.9a	13.2 ±0.2a	13.7 ± 0.2b
Total phenol content	36.8 ± 1.8a	35.2 ± 0.9a	35.8 ± 1.5a	33.3 ± 2.7a
Volumetric mass (g/mL)	10.8 ± 0.3a	10.9 ± 0.1a	10.5 ± 0.5a	11.0 ± 0.01b
Assimilable nitrogen (mg/L)	192.6 ± 33.8a	217.6 ± 17.7a	200.7 ± 17.7a	214.4 ± 24.9a
ph	3.8 ± 0.1a	3.7 ± 0.07a	3.6 ± 0.08a	3.6 ± 0.07a
Potassium (g/L)	1.52 ± 0.14a	1.55 ± 0.15a	1.22 ± 0.01a	1.29 ± 0.02a

Results are expressed as means ± standard deviation. Analysis of variance to compare data: for each variable studied, values with different letters within each row and growing season are significantly different according to t statistic ($P < 0.05$). †Values represent the means of 8 replications of 20 grafted plants (160 grafted plants for each treatment).


Tempranillo/110 R
1400
1200
1000
800
600
400
200
0
g/plant
2008
2009
2010
2011
Year
HWT
no_HWT


Cabernet Sauvignon/110R
1400
1200
1000
800
600
400
200
0
g/plant
2008
2009
2010
2011
Year
HWT
no_HWT