Effect of hot-water treatment on grapevine viability, yield components and composition of must

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Abstract

Background and Aims: Hot-water treatment (HWT) has been shown to be effective for the control of several endogenous and exogenous grapevine pests and diseases in dormant grapevine cuttings and young rooted vines. Little is still known, however, about the long-term effect of HWT on plant viability under field conditions. The effect of HWT on the performance of dormant plants in a four-growing seasons study was investigated.

Methods and Results: The effect of HWT at 53°C for 30 min on shoot mass, yield parameters and composition of must in dormant grafted plants (Tempranillo cultivar grafted onto 110 Richter rootstock) was evaluated. Eight bundles of 20 grafted plants were assigned to HWT, and eight additional bundles of 20 untreated grafted plants were prepared as a control (non-HWT). Dormant grafted plants were immediately planted in two field sites in April 2007. Shoot fresh mass was evaluated during winter in four consecutive growing seasons. Yield parameters and must composition were evaluated in the third and fourth growing seasons. In general, there was no significant difference in shoot mass at pruning, yield parameters and must components between treatments, with the exception of the must total soluble solids and volumetric mass in the fourth growing season.

Conclusions: The findings obtained in this study indicate that HWT at 53°C for 30 min did not affect plant viability, yield parameters and the main components of must composition, and could be successfully used commercially.

Significance of the Study: This study represents the first approach to investigate the long-term effect of HWT on plant development, yield and composition of must under field conditions. It suggests that the success of HWT depends not only on the most adequate protocol applied by nurseries, but also on management practices before, during and after the propagation process that 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 because of hot-water treatment (HWT) are an ongoing problem for both grapegrowers and nurseries worldwide (Waite and May 2005, Waite and Morton 2007). Hot-water treatment has been shown to be effective for the control of several 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).

Questions about the efficacy of HWT, however, 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). Hot-water treatment 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 (140 Ruggeri and 1103 Paulsen grafted with the Negroamaro cultivar) treated at 50°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 (50°C for 30 min) sometimes damage young plants, possibly because of poor heat acclimatisation in the cool climate of New Zealand. Conversely, Waite and May (2005) investigated the effect of different hydration times and HWT protocols on cuttings of Chardonnay and Cabernet Sauvignon at the callusing phase

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under glasshouse conditions and, despite the variable responses of the two cultivars to HWT when measuring callus, shoot and root development, no cuttings of either cultivar 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 the level of fungal infection 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 effect of HWT on cuttings and rootlings; these investigations, however, 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 effect 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 a temperature of up to 53°C. Little is still known, however, about the long-term effect 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 53°C for 30 min on grapevine development, yield parameters and must composition in a four-growing seasons study (2008–2011).

Materials and methods

Planting material and treatment

A total of 320 grafted plants ready to be sold to producers of the scion/rootstock combination Tempranillo/110 Richter was 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 eight bundles were assigned to HWT. For HWT, planting material was placed in a hydrating bath for 1 h in order to pre-soak 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 control plants were immediately planted in April 2007 in two field sites (four bundles of 20 plants per treatment, 160 grafted plants per field site) at Las Tiesas experimental farm where grapevines had not been grown. The farm is located at the city limits between Barrax and Albacete (Spain), and has the average geographical coordinates of latitude 39°14'north, longitude 2°5'west and 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 randomised blocks, each containing two rows of grafted vines (one bundle each of HWT or of control in each row) (40 plants per block). Standard cultural practices were applied at both sites during the grapevine growing season.

Assessment of plant growth, fruit sampling and must analysis

Plants were pruned in four consecutive growing seasons to two buds per spur and eight spurs along the cordon, during normal winter pruning time. Shoots of all treatments were immediately wrapped and taken back to the laboratory for mass assessment. In the third and fourth growing seasons, the fruit of each plant were weighed (yield) at harvest, and the berry sample mass and Ravaz index (yield/pruning mass) were calculated per plant. In addition, a sample of 500 berries per bundle was taken at random for must analysis. 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 (TSS), total polyphenol index, volumetric mass (density), yeast assimilable nitrogen (YAN), pH and potassium. The must was analysed by LIEC Agroalimentaria S. L. (Ciudad Real, Spain) by the FT-IR spectroscopy technique using a WineScan FT120 (Foss, Hillerød, Denmark) using ready-made calibrations and the WinISI software package (Infrasoft International LLC, State College, PA, USA).

Statistical methods

The results within each growing season were statistically analysed using one-way analysis of variance with treatment as the independent variable and the following dependent variables: shoot mass (g/plant), yield (g/plant), Ravaz index (g fruit/g pruning mass), berry sample mass (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), TSS (°Baume), total polyphenol index (abs_{280}), 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 mass and yield parameters

There were no significant differences in shoot mass 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 mass at pruning between treatments (HWT and control) within each growing season ($P > 0.05$) (Figure 1). Shoot mass was similar among treatments throughout the four growing seasons of the study, with a slight, but not significant ($P > 0.05$) reduction of shoot mass in HWT-treated plants in 2010 and 2011 (834.8 and 967.3 g/plant, respectively) compared with that of control plants (946.2 and 1080.5 g/plant, respectively).

There were no significant differences in yield and Ravaz index between the HWT and control in the third and fourth growing seasons ($P > 0.05$) (Table 1). In the third growing season, the yield was 4.9 and 5.4 kg/plant in HWT and control

Table 1. Yield components and must composition in the third and fourth growing seasons of control grafted Tempranillo/110 R plants and those subjected to hot-water treatment at 53°C for 30 min.

Variables	Third growing season		Fourth growing season	
	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 mass)	6.6 ± 3.7a	5.8 ± 0.8a	8.6 ± 3.0a	7.6 ± 2.6a
Berry sample mass (g)	1.4 ± 0.13a	1.4 ± 0.18a	1.6 ± 0.19a	1.6 ± 0.16a
Must composition				
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 (°Baume)	12.3 ± 1.4a	12.9 ± 0.9a	13.2 ± 0.2a	13.7 ± 0.2b
Total polyphenol index (Abs_{280})	36.8 ± 1.8a	35.2 ± 0.9a	35.8 ± 1.5a	33.3 ± 2.7a
Volumetric mass (g/mL)	1.08 ± 0.03a	1.09 ± 0.01a	1.05 ± 0.05a	1.10 ± 0.001b
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

†Values represent the means of eight replications of 20 grafted plants (160 grafted plants for each treatment). 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 ($P < 0.05$).

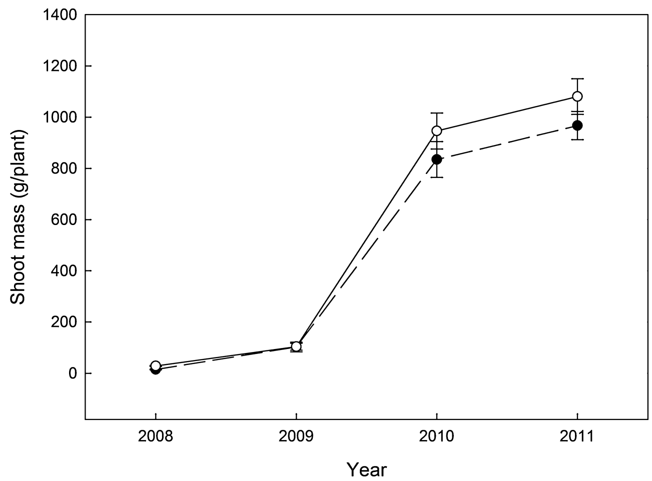

Figure 1. Shoot mass of control (○) grafted plants (Tempranillo/110 Richter) and those subjected to hot-water treatment at 53°C for 30 min (●) during four growing seasons. Vertical bars are the standard error of the means.

plants, respectively. The Ravaz index was 6.6 and 5.8 g fruit/g pruning mass in HWT and control plants, respectively, in this season. In the fourth growing season, yield was 8.0 kg/plant in the HWT plants and the Ravaz index was 8.6 g fruit/g pruning mass, while the yield was 7.8 kg/plant and the Ravaz index was 7.6 g fruit/g pruning mass in control plants.

Must analyses

Must composition of the Tempranillo/110 R combination in the third and fourth growing seasons is shown in Table 1. There was no significant difference in must composition between the field sites in each growing season, so the data were combined for analyses ($P > 0.1$). A significant difference between HWT and control plants in must composition was observed only for TSS ($P = 0.0440$) and volumetric mass ($P = 0.0454$) in the fourth growing season.

Discussion

This study analyses the effect of HWT on grapevine viability, yield parameters and must composition under field conditions over the first four growing seasons after planting in the vineyard. Although shoot mass at pruning was similar among treatments throughout the study, there was a slight reduction of shoot mass in HWT plants in the third and fourth 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 HWT cuttings, particularly sensitive cultivars, are generally slower to establish than cuttings that have not been treated with hot water, and suffer delayed early growth. Waite (2002) argued that HWT 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 53°C for 30 min, sprouting was delayed and, as a consequence, shoot mass was significantly reduced in HWT plants compared with that of the untreated controls. On the basis of our results, we did not find a statistically significant difference among treatments when measuring shoot mass, but HWT appeared to still produce an effect on other aspects of plant development after four growing seasons. The retarded growth of HWT plants, however, did not result in a decrease in yield parameters, and the levels obtained for yield, Ravaz index and berry sample mass were not significantly different among treatments through the study.

We evaluated the effect of HWT on viability, yield components and must composition of a Tempranillo/110 R combination; Tempranillo is the most planted red wine cultivar 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 rootstocks most often demanded by Spanish grape growers (Hidalgo 2002). Little variability in the tolerance among grapevine cultivars (Bobal, Merlot and Tempranillo) to temperature in Spain has been reported previously (Gramaje et al. 2009). Waite et al. (2001), however, studied the sensitivity of several grapevine cultivars to HWT in Australia and concluded that Pinot Noir was the most sensitive cultivar; Chardonnay, Riesling and Merlot were moderately sensitive; and Cabernet Sauvignon was the least sensitive. Further studies with additional grapevine cultivars are needed to evaluate the long-term effect of HWT post the initial years of vineyard establishment. This is the first study to investigate the effect of HWT on must composition. 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 53°C for 30 min did not affect the main must attributes, and could be successfully applied commercially. A slight reduction was observed in total acidity and tartaric acid in both growing seasons, and a statistically significant reduction in the TSS and volumetric mass in the fourth growing season for HWT plants compared with that of the control plants.

Must acidity normally varies with the cultivar, the climate and grape maturity (Ribéreau-Gayon et al. 2000a). These authors reported that in musts from northerly vineyards, the concentration of tartaric acid is often over 6 g/L, whereas in the south, it may be as low as 2–3 g/L because respiration (malic acid loss) is more effective when the grape bunches are maintained at a high temperature. In our study, the difference observed in total acidity and tartaric acid among treatments (≤ 0.5 g/L) was not sufficient to cause a significant variation in wine composition, because a maximum addition 1.5 g/L of tartaric acid is permitted to further correct the acidity and effect a positive impact on wine stability and flavour (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 analysis, respectively (Iland et al. 2004). Crippen and Morrison (1986) reported that °Brix is a good indicator of berry sugar concentration at a level above 18°Brix (>10°Baume), when sugars become the predominant soluble solids in grapes. The small, but significant difference observed in TSS (≤ 0.6°Baume) and volumetric mass (≤ 0.7 g/mL) in the fourth growing season could be because of aspects of sampling (variable °Baume among berries within the same fruit sample, sampling time), yield storage (dehydration), measurement error or even a slight difference in type 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 applied with confidence by nurseries. In Australia, many nurseries have experienced costly failures of HWT 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 failure. Recent research indicated that many management practices before, during and after the propagation process could affect the viability of grapevine propagating material subjected to HWT. These include management of mother vines (level of fertilisation, crop load, pests and diseases); pre-HWT processes, such as harvesting and transporting cuttings and rootlings, hydration of propagation material, water quality, and anaerobic conditions; and post-HWT nursery processes, such as 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 HWT 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-term 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, it is not statistically significant. This suggests that the success of HWT depends not only 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; these nursery practices that are used in the propagation process, however, 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 are 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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