# Effects of hot-water treatment, post-hot-water-treatment cooling and cold storage on the viability of dormant grafted grapevines under field conditions

**D. GRAMAJE*** **and J. ARMENGOL**

Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain

* *Present address:* Institute of Sustainable Agriculture (IAS), Spanish National Research Council (CSIC), Alameda del Obispo S/N, PO Box 4084, 14080 Córdoba, Spain.

Corresponding author: Dr David Gramaje, fax +34 95 7499252, email dgramaje@ias.csic.es

## Abstract

**Background and Aims:** Hot-water treatment (HWT) is an effective control method for black-foot and Petri disease, in grapevine propagating material. However, plant hydration and cold storage have emerged as critical factors in the production of quality planting material. The effects of HWT protocols on the performance of dormant plants ready to be sold to producers under field conditions were investigated.

**Methods and Results:** The effects of HWT at 53°C for 30 min, cooling (post-HWT cooling or no post-HWT cooling) and cold storage (0, 1, 2 and 4 weeks) on sprouting, and shoot length and weight in dormant grafted plants (Tempranillo cultivar grafted onto 110 Richter rootstock) were evaluated. Eight bundles of ten cuttings were treated for each factor combination, and eight additional bundles of ten untreated cuttings were prepared as controls (no HWT). Dormant grafted plants were immediately planted in two field sites in March 2010. The number of plants that emerged from dormancy was counted in July 2010. In January 2011, shoot length and fresh weight were evaluated. Although significant, the percentages of plants emerging from dormancy among treatments were relatively small. A significant reduction was observed in shoot length and weight for all treatments compared with the control, particularly in all variables for non-hydrated hot-water-treated cuttings kept in cold storage for 4 weeks immediately after HWT.

**Conclusions:** The findings obtained in this study indicate that long-term cold storage could be detrimental to planting material, especially when plants have not been previously hydrated following HWT.

**Significance of the Study:** This study represents the first approach for evaluation of different HWT protocols under field conditions. It improves the knowledge of the different steps used in the HWT process and provides valuable information about the most reliable protocol that can be used successfully in a commercial situation.

***Keywords:*** *black-foot*, *nursery*, *Petri disease*, Vitis vinifera

## Introduction

Hot-water treatment (HWT) is known to be an effective, practical and relatively inexpensive method 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), 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).

The use of HWT has also been reported as a promising method for the control of black-foot and Petri disease pathogens in grapevine propagating material. 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) has been strongly recommended as a means of reducing infection levels in nursery plants. However, there have been, and continue to be, irregular reports of unacceptably high losses when standard HWT protocol (50°C for 30 min) is applied to commercial batches of cuttings and rootlings (Waite and Morton 2007).

In Spain, Gramaje et al. (2008, 2009, 2010) determined that 53°C for 30 min is the most effective treatment to reduce conidial germination and mycelial growth of black-foot and Petri disease pathogens without detrimental effects to grapevine cuttings. However, HWT of dormant nursery plants has not yet been embraced as a standard treatment in Spanish nurseries. This is largely attributed to the confusion in industry about the efficacy and safety of HWT. Because of the large number of cuttings processed by commercial nurseries, it is a standard industry practice to store cuttings in cold rooms until required. However, in recent years, cold storage has emerged as a critical factor in the production of quality grapevine material (Waite and Morton 2007). It is thought that there may be an interaction between HWT and cold storage that has a

doi: 10.1111/j.1755-0238.2012.00185.x

detrimental effect on cuttings (Waite et al. 2004). Additionally, the mucilage that oozes from the ends of the cuttings following HWT provides the substrate for cold-adapted microorganism to grow (Waite and Morton 2007). Probst et al. (2012) recently observed that increasing periods of cold storage increased the disease incidence and severity of grapevine cuttings by Cylindrocarpon black-foot in nurseries. Fermentation of cuttings and rooted vines after HWT is another problem experienced in cold storage. HWT causes cuttings to become temporarily anaerobic and also result in long-term changes to respiration rates that persist through at least 14 weeks of cold storage (Waite et al. 2004).

Another factor that may influence the variability of hot-water-treated planting material is post-HWT cooling (hydration (Hyd)). Dormant cuttings or grafted plants are usually plunged into cold water immediately following HWT to quickly lower the temperature and minimize heat damage to the tissue. Some researchers, however, suggest eliminating this stage of the HWT protocol because water used in commercial cool-down tanks is not sterile, and it is a potential source of microbial contaminants including Petri disease pathogens (Waite et al. 2005).

Therefore, the objective of this study was to investigate the effects of HWT at 53°C for 30 min., Hyd and cold storage on the performance of dormant grapevine plants under field conditions.

## Methods and materials

### *Planting material and treatments*

A total of 720 dormant grafted plants ready to be sold to producers (*Vitis vinifera* (*V. vinifera*) cv. Tempranillo grafted onto 110 Richter rootstock) were obtained from a commercial nursery in Aielo de Malferit (Valencia, Spain). This planting material was allocated at random to 72 bundles of 10 cuttings. Eight bundles (80 grafted plants) were assigned to no HWT (control). The remaining 64 bundles (640 grafted plants) were assigned at random to either HWT/Hyd (32 bundles) or HWT/no Hyd (32 bundles). 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 subjected to Hyd 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 cuttings.

Each group of 32 bundles (320 grafted plants) was further divided into four groups of eight bundles each (80 grafted plants per group) and subjected to either 4 weeks cold storage (CS4), 2 weeks cold storage (CS2), 1 week cold storage (CS1) or no cold storage (NS). For cold storage, plants were sealed in perforated plastic bags for 24 h at 25°C and stored in a cool room at 1–2°C for the experimental storage times described earlier. Plants were then removed from storage and left in the bags overnight to stabilize to ambient temperature (approximately 15°C). The groups that did not receive cold storage were sealed in perforated plastic bags for 24 h at 25°C. All the treatments were performed successively in time to have all graftlings ready for plantation at the same time.

Grafted plants were immediately planted in March 2010 in two field sites where grapevines had not been grown, according to the local farmers, in Rotglà i Corberà (Valencia, Spain). Each bundle (10 grafted plants) was spaced 100 cm from other bundles, with grafted plants 30 cm apart from centre to centre and an inter-row spacing of 100 cm. Each field plot was 66 m long and included two rows, each with 18 plant bundles (360 plants per field). In both sites, the experimental design consisted of four randomized blocks, each containing 10 bundles of grafted vines each treatment. Standard cultural practices were used at both sites during the grapevine growing season. Plots were less than 1 km apart and had very similar climates and soil types.

### *Assessment*

The number of plants that emerged from dormancy was counted in July 2010. In January 2011, at the end of the growing season, dormant plants were uprooted, washed and assessed for shoot length and fresh weight.

### *Statistical methods*

The number of plants in each treatment that emerged from dormancy was expressed as a percentage, and shoot length and weight were expressed in centimetres and grams, respectively. Statistical analysis of the results was done using one-way analysis of variance with treatment as independent variable and the following dependant variables: plants emerging from dormancy (%), shoot length (cm) and shoot weight (g). The Student's Least Significant Difference test was used to compare the overall means of each treatment at $P < 0.05$. Linear single-degree-of-freedom contrasts were computed to test the effect of selected treatment combinations (Mead et al. 2003). In all cases, SAS (version 9.0, SAS Institute, Cary, NC, USA) was used.

## Results

There were no significant differences in the percentage of plants emerging from dormancy ($P = 0.4365$), shoot length ($P = 0.1194$) and shoot weight ($P = 0.5729$) between the two field sites, so the data were combined for analysis (Table 1). The

**Table 1.** Analysis of variance for the effects of the treatments on the percentage of plants emerging from dormancy, shoot length and weight.

| Treatments | df | Plants emerging from dormancy (%) | | Shoot length (cm) | | Shoot weight (g) | |
|---|---|---|---|---|---|---|---|
| | | MS | *P* > F† | MS | *P* > F | MS | *P* > F |
| Experiment | 1 | 88.89 | 0.4365 | 60.50 | 0.1194 | 2.72 | 0.5729 |
| Treatments | 8 | 756.59 | <0.001 | 607.89 | <0.001 | 37.49 | <0.001 |
| Residual | 63 | 144.05 | | 27.04 | | 3.39 | |
| Total | 71 | | | | | | |

†Probabilities associated with individual *F*-tests. df, degrees of freedom; MS, mean square.

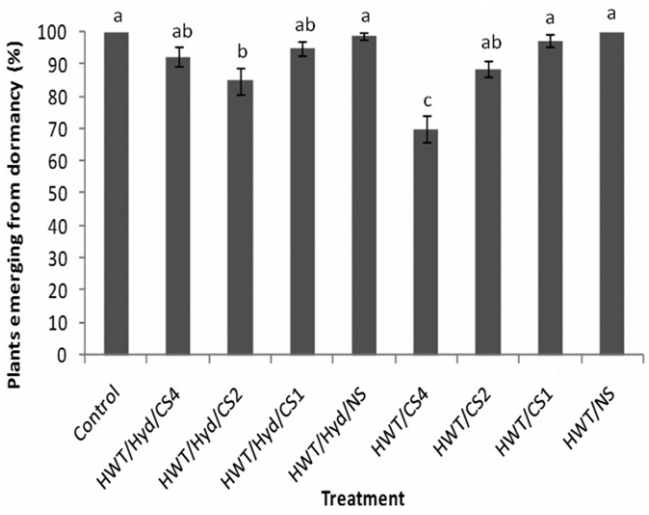


**Figure 1.** Percentage of grafted plants emerging from dormancy subjected to the following treatments: Control (no hot-water treatment (HWT)); HWT/post-HWT cooling (hydration (Hyd))/4 weeks cold storage (CS4); HWT/Hyd/2 weeks cold storage (CS2); HWT/Hyd/1 week cold storage (CS1); HWT/Hyd/no storage (NS). Vertical bars are the standard error of the means. Bars with a different letter are significantly different according to the Student's Least Significant Difference.

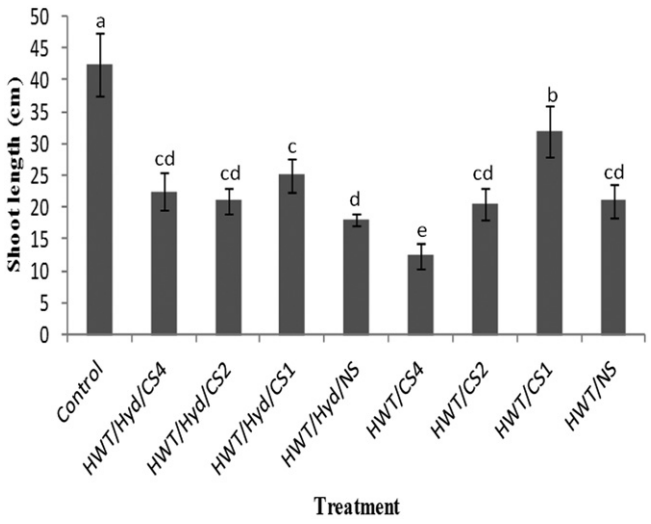


**Figure 2.** Shoot length of grafted plants subjected to the following treatments: Control (no hot-water treatment (HWT)); HWT/post-HWT cooling (hydration (Hyd))/4 weeks cold storage (CS4); HWT/Hyd/2 weeks cold storage (CS2); HWT/Hyd/1 week cold storage (CS1); HWT/Hyd/no storage (NS) Vertical bars are the standard error of the means. Bars with a different letter are significantly different according to the Student's Least Significant Difference.

effects of the treatments on the percentage of plants emerging from dormancy, shoot length and weight were all significant ($P < 0.001$).

In general, the percentage of plants that emerged from dormancy was similar in all treatments (Figure 1). The factors HWT/Hyd/CS2 (85.0 ± 4.0) and HWT/CS4 (70.0 ± 4.0) differed significantly from the control when measuring the percentage of plants emerging from dormancy. However, most of the treatments resulted in a significant reduction in shoot length and weight compared with the control (no HWT) (Figures 2,3). The factors HWT/Hyd/CS2 (85.0 ± 4.0) and HWT/CS4 (70.0 ± 4.0) differed significantly from the control when measuring the percentage of plants emerging from dormancy (Figure 1). In the case of the shoot length, all treatments differed significantly from the control (Figure 2). The factor HWT/CS4 differed significantly from all other treatments and presented the lowest values for shoot length (11.9 ± 2.0). In the case of the shoot weight, all treatments differed significantly from the control, with the exception of factors HWT/CS2 and HWT/CS1 (Figure 3). The factor HWT/CS4 differed significantly from the other treatments and presented the lowest values of shoot weight (5.0 ± 0.5).

Linear single-degree-of-freedom contrasts showed that plants assigned to HWT/Hyd/CS1 and HWT/CS1 combinations differed significantly from plants assigned to HWT/CS1 and HWT/NS combinations, respectively, when measuring shoot length ($P < 0.05$) (Table 2). Plants assigned to HWT/Hyd/CS4 and HWT/CS4 combinations differed significantly from plants assigned to HWT/CS4 and HWT/NS combinations, respectively, when measuring the percentage of plants emerging from dormancy ($P < 0.01$), shoot length ($P < 0.05$) and shoot weight ($P < 0.05$). Plants assigned to HWT/Hyd/CS2 combination differed significantly from plants assigned to HWT/Hyd/NS combination when measuring the percentage of plants emerging from dormancy ($P < 0.05$). Plants that were not subjected to HWT differed significantly from plants assigned to HWT/Hyd/NS and HWT/NS combinations when measuring shoot length ($P < 0.01$) and shoot weight ($P < 0.05$). The remaining contrasts performed among treatment combinations showed no significant differences.

## Discussion

In this study, the effects of HWT at 53°C for 30 min, Hyd and cold storage on the viability of grafted plants under field conditions have been investigated. In general, there was relatively little variability in the percentages of plants emerging from dormancy among treatments. In contrast, significant reductions were observed in shoot length for all treatments and in shoot weight in all except HWT/CS2 and HWT/CS1 with respect to the control (not hot-water-treated cuttings), indicating some effect of the HWT on plant development. This agrees with the findings of Gramaje et al. (2009), who evaluated the effect of different temperature and time combinations on the viability of five grapevine rootstocks and observed only some reduction of sprouting at treatments of up to 54°C for 30 min and a significant reduction in shoot weight in 161-49 Courderc and 110 R rootstocks after one growing season. Waite and May (2005) pointed out that hot-water-treated cuttings, particularly sensitive varieties, are generally slower to establish, than cuttings that have not been treated with hot water, and suffer retarded growth in the early part of the growing season. As reported by Waite et al. (2004) and Waite and Jaudzems (2005), HWT possibly interferes with the ability of cutting to utilize the starch reserves needed for budburst by disrupting the amyloplasts (starch grains), possibly causing the delayed early growth observed in hot-water-treated planting material.

Our results showed a significant reduction in all variables studied for non-soaked cuttings stored during 4 weeks in cold rooms immediately after HWT. This finding indicates that long-time cold storage could be detrimental to planting material, especially when plants have not been soaked following HWT. Waite and Jaudzems (2005) concluded that grapevine cuttings, including those that are not treated in any way, undergo significant changes over time during storage and that they vary with the imposition of HWT protocols. These authors also reported the appearance of halos with membrane around amy-

**Table 2.** Linear single-degree-of-freedom contrasts among selected treatment combinations.

| Contrasts | Plants emerging from dormancy (%) | Shoot length (cm) | Shoot weight (g) |
|---|---|---|---|
| HWT/Hyd/NS vs HWT/NS | 98.7† vs 100 ns | 18.0 vs 21.0 ns | 8.0 vs 8.0 ns |
| HWT/Hyd/CS1 vs HWT/CS1 | 95.0 vs 97.5 ns | 25.0 vs 32.0* | 7.0 vs 8.5 ns |
| HWT/Hyd/CS2 vs HWT/CS2 | 85.0 vs 88.7 ns | 21.0 vs 20.5 ns | 6.8 vs 9.0* |
| HWT/Hyd/CS4 vs HWT/CS4 | 92.5 vs 70.0** | 22.5 vs 11.9* | 8.3 vs 5.0* |
| HWT/Hyd/CS1 vs HWT/Hyd/NS | 95.0 vs 98.7 ns | 25.0 vs 18.0* | 7.0 vs 8.0 ns |
| HWT/Hyd/CS2 vs HWT/Hyd/NS | 85.0 vs 98.7* | 21.0 vs 18.0 ns | 6.8 vs 8.0 ns |
| HWT/Hyd/CS4 vs HWT/Hyd/NS | 92.5 vs 98.7 ns | 22.5 vs 18.0 ns | 8.3 vs 8.0 ns |
| HWT/CS1 vs HWT/NS | 97.5 vs 100 ns | 32.0 vs 21.0* | 8.5 vs 8.0 ns |
| HWT/CS2 vs HWT/NS | 88.7 vs 100 ns | 20.5 vs 21.0 ns | 9.0 vs 8.0 ns |
| HWT/CS4 vs HWT/NS | 70.0 vs 100** | 12.5 vs 21.0* | 5.0 vs 8.0* |
| No HWT vs HWT/Hyd/NS | 100 vs 98.7 ns | 42.5 vs 18.0** | 13.0 vs 8.0* |
| No HWT vs HWT/NS | 100 vs 100 ns | 42.5 vs 21.0** | 13.0 vs 8.0* |

*$P < 0.05$; **$P < 0.01$; ns, not significant ($P > 0.05$) according to t statistic. †Values represent the means of eight replications (80 plants) for each treatment combination; four per experiment. CS1, 1 week cold storage (CS1); CS2, 2 weeks cold storage; CS4, 4 weeks cold storage; HWT, hot-water treatment; Hyd, hydration/post-HWT cooling; NS, no storage.

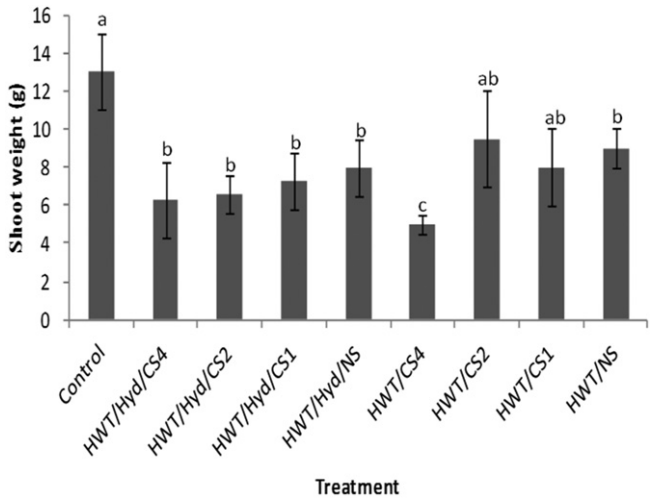


**Figure 3.** Shoot weight of grafted plants subjected to the following treatments: Control (no hot-water treatment (HWT)); HWT/post-HWT cooling (hydration (Hyd))/4 weeks cold storage (CS4); HWT/Hyd/2 weeks cold storage (CS2); HWT/Hyd/1 week cold storage (CS1); HWT/Hyd/no storage (NS). Vertical bars are the standard error of the means. Bars with a different letter are significantly different according to the Student's Least Significant Difference.

loplasts in tissue during storage, thus suggesting an increase of metabolic activity, and hence, oxygen consumption. Waite et al. (2004) investigated the effects of HWT and hydration on cutting respiration to determine if HWT or hydration cause cuttings to ferment, and they concluded that HWT cuttings had become fermentative, but not the hydrated or untreated cuttings. 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).

The consequences of hydrating planting material prior to or after HWT have been intensively investigated and discussed among researchers. It is generally accepted by the vine nursery industry in Australia that the standard practice of soaking propagating material overnight is beneficial and enhances the tolerance of cuttings to HWT (Nicholas et al. 2001). However, there is indirect evidence that presoaking plant material prior to HWT may reduce tolerance to HWT (Baker 1962). Additionally, the water used in cool-down tanks is not sterile and may be a source of waterborne microorganisms (Waite and Morton 2007). Viable propagules of Petri disease pathogens have been obtained from hydration tanks in Spain (Aroca et al. 2010, Gramaje et al. 2011). Crocker et al. (2002) reported a variable response of cuttings to hydration (+/HWT) in an experiment to determine the effects of hydration (0, 1 and 8 h) and HWT on root initiation on six *V. vinifera* cultivars and concluded that adequate watering of mother-vines between vintage and leaf fall and protecting cuttings from dehydration during processing was a better strategy for successful propagation than hydration. Waite (2002) observed that cuttings that were hydrated overnight prior to HWT changed their colour from a light, bright brown to a dull black and that the nodes tended to be soft and mushy. In a small preliminary trial, Waite and May (2005) demonstrated that Semillon cuttings showed suppression of rooting after 6- and 15-h soaking compared with bundles that were not soaked.

Based on the results reported here, planting material can be stored in cold rooms after HWT of up to 2 weeks without detrimental effects on sprouting and just some reduction on shoot length and weight. The order of storage with respect to the HWT has also been investigated and discussed among researchers, but no clear consensus has emerged. In Australia, some nurseries reported that grapevine cuttings and young vines subjected to HWT at 50°C for 30 min after cold storage are less likely to suffer a loss of quality than material that is treated with hot water before cold storage (Waite and May 2005). However, Cabernet Sauvignon cuttings treated at 52, 54, 56, 58 and 60°C for 10, 20 or 30 min and stored at 3–4°C thereafter generally showed faster budburst (Wample 1993) and better root development (Wample 1997) than cuttings treated after storage. This researcher suggested that the results of that trial held good only for Cabernet Sauvignon grown in Washington state, where winters are very cold, and that other cultivars growing in other climates might perform differently. The hypothesis that some *V. vinifera* varieties are more sensitive to HWT than others depending on the climate they grow has been suggested by different researchers. Crocker et al. (2002) indicated that grape-

vine cuttings taken from vines grown in warm climates are of better quality to cuttings taken from vines grown in cool climates and are better able to withstand HWT. Recent studies carried out by Graham (2007) showed that cuttings grown in cool climates in New Zealand were susceptible to damage at 50°C for 30 min. The effects of different hydration times and HWT protocols on cuttings of Chardonnay and Cabernet Sauvignon at callusing phase under glasshouse conditions were evaluated by Waite and May (2005), with different responses observed between varieties. In both varieties, callus development was consistently greater in cuttings that were treated with hot water post-storage than in cuttings that were treated with hot water pre-storage or stored without HWT. In Cabernet Sauvignon, cuttings hydrated for 4 h before cold storage also showed great callus development. Root development in Chardonnay was most advanced in HWT cuttings that received no hydration and in all cuttings that were hydrated for 15 h regardless of HWT. In contrast, the most advanced root development in Cabernet Sauvignon was in the group of plants that were not treated with hot water, indicating that HWT suppressed early root development in this variety.

All the protocols tested in our study could be used successfully in a commercial situation, with the exception of non-soaking of cuttings stored in cold rooms for periods of up to 2 weeks. These may suffer retarded plant growth compared with cuttings that were soaked prior to storage. Nurseries commonly plunge cuttings into cold water immediately following HWT treatment to reduce the temperature of the cuttings as quickly as possible to avoid any heat damage that may occur as a result of slow cooling (air-cooling) of the cutting tissue. However, it is difficult to justify the use of soaking prior or after HWT when the supposed benefits are doubtful and there is a real risk of spreading pathogens such as black-foot and Petri disease pathogens. To reduce the risk of contamination or reinfection by potential pathogens during the cool-down process, it may be possible to allow cuttings to air-cool, provided the slower cooling does not affect cutting viability. In this regard, Waite et al. (2005) examined the effects of post-HWT air-cooling versus water-cooling on dormant cuttings of four *V. vinifera* cultivars, Pinot Noir, Chardonnay, Semillon and Cabernet Sauvignon. All cuttings were viable and produced adequate numbers of roots at callusing to enable establishment. However, the volume of cuttings treated in commercial HWT plants is very much larger than that of the experimental batch. A commercial-scale trial needs to be carried out to evaluate the effects of rapid versus slow cooling of hot-water-treated planting material and to determine if this alternative could be applicable to the HWT process.

## Acknowledgements

This research was financially supported by the Projects RTA2007-00023-C04-03 and RTA2010-00009-C03-03 (Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, INIA, Spain) and the European Regional Development Fund (ERDF). The authors acknowledge V. Garrigues and R. Gandia for technical assistance.

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*Manuscript received:* 29 November 2011
*Revised manuscript received:* 15 January 2012
*Accepted:* 23 January 2012