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Die-hard seedlings. A global meta-analysis on the factors determining the effectiveness of drought hardening on growth and survival of forest plantations

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ABSTRACT

Drought hardening is a nursery technique aimed to enhance early forest plantation establishment under dry conditions, which is a main limiting factors for plantation success. However, the quantitative effectiveness of drought hardening remains unclear. We conducted a meta-analysis to evaluate the influence of different factors in the effectiveness of drought hardening on seedling post-planting survival and growth. Overall, drought hardening did not significantly affect survival or growth, as several factors induced great heterogeneity, but analyses of those factors explained its effectiveness, especially on survival. A longer time between hardening and transplanting strongly reduced survival. Indoor-grown seedlings did not benefit more from hardening than outdoor-grown seedlings. Evaluations of drought hardening effectiveness in pots showed positive effects on survival but negative effects on growth, while no effects were found in large bed experiments. In field experiments, hardening significantly increased survival and growth with site aridity. Survival benefits were independent of species drought tolerance, measured by osmotic potential at the turgor loss point (π_{tlp}), in moderate to high aridity sites. However, in low aridity sites, hardening increased survival in drought-tolerant species but decreased it in drought-intolerant species. Field results showed that hardening benefited shrubs more than trees in angiosperms. In conclusion, drought hardening at the end of nursery cultivation tend to increase post-planting seedling performance particularly in scenarios limiting post-planting root growth such as in arid climates and pot experiments. Our findings highlight the importance of future research on modelling the interaction between these technical features and species water use strategies..

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1. Introduction

Planting seedlings cultivated in nurseries is the main method for establishing forests worldwide. Water stress after planting due to transplant shock or extreme weather conditions is a main bottleneck for seedling survival and growth (Close et al., 2005; Grossnickle, 2005). Seedling outplanting performance also depends on field factors such as soil preparation, competition, and herbivory (Cuesta et al., 2010; Löf et al., 2012; Parsons et al., 2021). Additionally, outplanting performance is influenced by the morphological and physiological characteristics of seedlings at planting, such as reduced shoot:root ratio and stomatal conductance or enhanced osmotic adjustment, which greatly depend on nursery growing conditions and species' functional and ecological characteristics (van den Driessche, 1992; Grossnickle, 2012; Andivia et al., 2021). For example, fertilization or container size positively influence plant size, shoot to root mass ratio, and tissue nutrient concentration, which are traits that can increase early outplanting performance (Villar-Salvador et al., 2004a). Similarly, forest species usually show intrinsic differences in drought tolerance and growth rate (Cornelissen et al., 1998; Niinemets and Valladares, 2006), which partly determine their establishment capacity (Lopez-Iglesias et al., 2014).

Drought is a main constraint for seedling establishment in many planting sites worldwide and its importance is expected to increase due to climate change. Exposing seedlings to sublethal water stress in the nursery shortly before field planting has been proposed to enhance their short-term outplanting survival (Kaushal and Aussenac, 1989; van den Driessche, 1991a; Vilagrosa et al., 2003). This nursery technique, commonly termed drought hardening or drought conditioning, is applied with two main objectives. Firstly, it reduces plant growth and promotes bud set (Landis et al., 1989), two key processes for achieving the second aim, the promotion of the physiological mechanisms involved in drought tolerance (Chaves et al., 2003; Villar-Salvador et al., 2004b; Villar-Salvador et al., 2013) and frost tolerance (Medeiros and Pockman, 2011). Drought hardening can be implemented in the nursery in two ways: through repeated drought cycles followed by full seedling rehydration between drought cycles (Vilagrosa et al., 2003) or by permanently reducing irrigation to subject the seedlings to a constant water stress (Valliere et al., 2019).

Morphological and physiological responses to water stress in seedlings of woody species have been extensively studied (Abrams, 1990; Grossnickle et al., 1991a, 1991b; Ladjal et al., 2000; Vilagrosa et al., 2003). The intensity and duration of water stress can determine the extent of the acclimation to drought stress and the affected traits (Albouchi et al., 2001; Edwards and Dixon, 1995; Villar-Salvador et al., 2004b), with species-specific effects (Vilagrosa et al., 2003). For instance, species that have intrinsic low osmotic potential under optimal water status show greater osmotic adjustment in response to drought (Bartlett et al., 2014). Once water stress ceases and plants rehydrate, acclimation to water stress gradually relaxes (Blake et al., 1991; Ruehr et al., 2019), which potentially can reduce seedling establishment if outplanting is delayed.

Compared to physiological and morphological responses, the influence of drought hardening on the early field survival and growth of seedlings in forest species has received less attention with disparate outcomes across studies. While some studies show positive effects of drought hardening on outplanting performance (Rook, 1973; van den Driessche, 1992; Sánchez-Blanco et al., 2004; Valliere et al., 2019), others observed no effects or even reduced performance (Grossnickle et al., 1991a, 1991b; O'Reilly et al., 1994; Villar-Salvador et al., 2004b). These differences across studies may be due to multiple factors such as differences in drought hardening duration and intensity, the time elapsed since the end of hardening and planting date, or nursery microclimate cultivation conditions. For instance, in some studies, cultivation is primarily conducted outdoors under full sun, with low air relative humidity and cooler nighttime temperatures. These factors inherently promote seedling hardening more effectively compared to the stable and humid conditions of a greenhouse. (Poorter et al., 2016). In addition, studies differ on the method used to evaluate outplanting performance. While some studies tested outplanting performance under field conditions (Grossnickle et al., 1991a, 1991b; O'Reilly et al., 1994; Royo et al., 2001) others used large beds (i.e. large containers at least 50 cm deep and 1 m^2 surface filled with sandy soil) under controlled greenhouse conditions mimicking field conditions (van den Driessche 1991a; Villar-Salvador et al., 2004b; Villar-Salvador et al., 2013) or pots with limited rooting volume (Guarnaschelli et al., 2006; Sánchez-Blanco et al., 2004). These differences in experimental conditions may also affect the outcome of drought hardening by affecting the speed to which seedlings are exposed to drought stress after planting and rooting capacity, a key drought resistance trait (Andivia et al., 2019).

Many plant species are used in planted forests, encompassing a wide functional diversity (Hua et al., 2016). Functional traits, such as plant size, the shoot to root mass ratio and osmotic potential affect seedling resource economy, growth capacity, and stress tolerance (Wright et al., 2001; Reich, 2014), with potential consequences for species differences in outplanting establishment (Charles et al., 2018; del Campo et al., 2020). Species differences in drought tolerance and the aridity of plantation sites can also drive the disparity of reported outcomes of drought hardening. For example, the effectiveness of hardening may be small in wet climates where species are exposed to low drought stress or in species with inherent high drought tolerance. Similarly, functional differences between gymnosperms and angiosperms in their water economy (Choat et al., 2012; Niinemets and Valladares, 2006) can affect the efficacy of drought hardening.

Climate change will increase drought stress on planted seedlings (Harris et al., 2006; Vallejo et al., 2012) challenging the establishment of planted forests globally. Within this context, nursery cultivation techniques aimed at increasing seedling drought resistance, such as drought hardening, could play a key role in increasing the early establishment of forest plantations. Therefore, it is important to understand how drought hardening affects seedling establishment under harsh conditions. To date, no comprehensive review has evaluated the effectiveness of drought hardening on the performance of planted seedlings (see Grossnickle, 2012). To provide informed recommendations on its utility, it is necessary to understand the contexts and species most suitable for drought hardening based on their functional traits. Therefore, we conducted a quantitative review through a meta-analysis of published literature and unpublished data (i.e from research projects) to evaluate the following five factors influencing the effectiveness of drought hardening on seedling survival and growth in forest plantations: F1) Experimental evaluation conditions (i.e. field, large beds, or pots), F2) Aridity conditions of planting sites, F3) Species functional attributes (drought tolerance, life form, phylogenetic division), F4) Cultivation environment (indoor vs outdoors), and F5) Drought application characteristics (intensity, duration and time elapsed since hardening completion in the nursery until planting).

2. Material and methods

2.1. Literature search and selection of studies

Literature search was conducted on November 28th, 2021, across all the subscribed databases of the search engine in Web of Science. The search was organized according to the Population, Intervention, Control, Outcome scheme (known with the mnemonic PICO), which, even though was initially developed for clinical research (Richardson et al., 1995), has been proposed to formulate universally questions in every research area (Nishikawa-Pacher, 2022) including forest ecology (Lázaro-González et al., 2023; Skinner et al., 2023). Under this scheme, we defined *Population* as seedlings of forest species cultivated in the nursery and transplanted to the field, semi-controlled conditions, or pots. *Intervention* was considered as the application of water stress during nursery cultivation (drought hardening), *Control* as the optimal irrigation to which water stress treatments are compared, and *Outcome* as seedling survival and/or growth after transplanting. Thus, the research question configured according to PICO was: Does drought hardening treatments applied during nursery cultivation affect seedling outplanting performance (survival and or growth) of forest species?

To answer that question, we focused on *Population* and *Intervention* during the initial database search. Therefore, the terms used for the search were:

TS=(seedling* AND forest*) AND TS=(drought OR "water stress") AND TS=(hardening OR *conditioning).

In addition, we conducted a search using the same terms but replacing forest* with restor* to scan those cases in which non-tree species in the context of ecological restoration are studied, and in which the term forest might be absent. The results of both searches were merged and duplicated records eliminated. An additional search of literature in Finnish and Spanish was also conducted among reports of the Finnish Natural Resources Institute (Luke, formerly Metla) and the Proceedings of the Working Group on Afforestation of the Spanish Society for Forest Science.

The initial article pool found in the search were 2665 items, which were screened based on the title, discarding those off-topic, to a focused pool of 850 articles. This focused pool was further screened based on the material and methods section to retain only those studies that fulfilled the following conditions:

1. Based on *Intervention* and *Control* terms, studies should involve at least one nursery irrigation reduction treatment and a control treatment (full irrigation or standard optimal irrigation procedure).
2. Based on *Population*, studies should involve outplanting or transplanting to larger soil volumes to those in which they were cultured in the nursery.

   The refined article pool after this screening consisted of 33 articles. Finally, during data extraction articles not meeting all the following data quality criteria were further excluded:
3. Based on *Outcome*, survival and/or growth/size (biomass, height or root collar diameter) after outplanting/transplanting should be reported.
4. Finally, as a technical requirement to conduct the meta-analysis, it was necessary to report the number of planted seedlings used to assess outplanting survival. For post-planting growth/size measurements, the standard error or standard deviation along with the sample size from each pre-planting irrigation treatment, should be reported.

After refining the article pool according to the above-mentioned criteria, 22 articles were retained. In addition to the studies found in the literature search, we integrated unpublished data on the field performance of eight species from six independent field experiments conducted under Mediterranean-climate conditions. In six of these experiments, the characteristics of seedlings after drought hardening have been published: Chirino et al., (2004), Vilagrosa et al., (2003), and Villar-Salvador et al. (2013), (2004b), (1999). Two of these published papers (Villar-Salvador et al., 2013, 2004b) reported results of transplanting experiments conducted in large beds under greenhouse conditions. However, outplanting performance in field trials were not included in these five publications. The sixth unpublished field data set was provided by Tsakaldimi and Ganatsas, who conducted experiments in Greece. The data from this experiment, together with the unpublished field data related to the study by Vilagrosa et al. (2003) were summarized in the Final Report of the European Union project "Restoration of degraded ecosystems in Mediterranean Regions" (ENV4-CT97–0682/AMB99–0155-CE, 2001). Similarly, the unpublished field data linked to the study by Chirino et al. (2004) were summarized in the Final Report of the EU project "Conservation and Restoration of European Cork Oak woodlands: a unique ecosystem in the balance" (CREOAK; LK5-CT-2002–01594, 2006). A detailed description of the material and methods of the field experiments for each of the six unpublished datasets can be found in the supplementary material along with the results incorporated into the meta-analysis database (Appendix A).

2.2. Case study selection and data extraction

In many studies multiple cases were identified. When a study compared different species, planting sites, or seedlings grown under different nursery conditions (excluding irrigation), each combination of these factors was treated as a separate case study if the data was presented separately for each combination. Additionally, if a study examined more than one level of water stress (besides the control treatment), each level of water stress was considered a distinct case study. For example, if a study compared the field performance of two species planted at two sites and grown under three irrigation conditions before planting (full irrigation, stress level 1, and stress level 2), this would result in 8 case studies (2 species × 2 sites × 2 irrigation treatments) if the data for each combination were reported separately. In each of these case studies, the stress treatment was compared against the control.

Dependent variables data (survival, growth, seedling size), sample size and dispersion statistics, were extracted from text, tables, or figures. From figures, data were extracted using ImageJ (Schneider et al., 2012). In addition, information on the potential moderators of the response (independent variables) was recorded. Species were separated according to taxonomy (gymnosperms and angiosperms) and growth form (trees, shrubs and perennial herbs). Cultivation characteristics included outdoors or indoor (greenhouse) cultivation and the elapsed time between the end of water stress treatments and planting. Site management considered planting conditions (planting either in the field, large beds, or pots).

Site coordinates were approximated using Google Earth when not reported in the article. An aridity index for each location was obtained from the Global Aridity Index Geospatial Database (Trabucco and Zomer, 2019), which is calculated from the WorldClim 2.0 data set (Fick and Hijmans, 2017) for the interval 1970–2000 at a resolution of 30 arc-sec, using precipitation and potential evapotranspiration data as follows:

$$Aridity\ index = \frac{Precipitation}{Potential\ evapotranspiration} \quad [1]$$

To facilitate the interpretation of the results we considered the moderator *Aridity* as the inverse of [1].

In total, 28 studies (22 published papers and 6 unpublished studies) were included in the analysis comprising 211 case studies. Of these, 84 included exclusively survival data, 47 only growth, and 80 both growth and survival (164 case studies with survival data and 127 with growth data).

2.3. Calculation of a cross-study comparable metric of drought hardening intensity

Across studies, various metrics were used to quantify the level of water stress experienced by plants subjected to different drought hardening treatments. These metrics, in order of frequency, were: 1) container or pot weight, 2) seedling predawn water potential (ψ_{pd}), 3) growing medium water content (v:v,%), and 4) watering frequency. While many studies reported data for both container weight and crop ψ_{pd}, a subset of seven studies out of the total 21 selected studies for survival data only provided ψ_{pd} data. To analyze whether the intensity of drought hardening influenced transplanting performance, we established a common metric across all case studies by calculating the water stress intensity of the hardened treatment compared to the control as:

$$Intensity = \frac{C - H}{C} \times 100 \quad (\%), \quad [2]$$

where C represents the container weight or the water content of the growing medium or the irrigation frequency of the control group (well-watered plants), and H is the corresponding hydration metric for the water-stressed plants. *Intensity* values calculated using the hydration metrics 1, 3, and 4 yielded comparable values, especially metrics 1 and 3. However, *Intensity* derived from ψ_{pd} data are not directly comparable with other metrics, as container weight, growing medium water content, or watering frequency are not linearly related to ψ_{pd}. To address this issue, we used ψ_{pd} values to estimate container weight-based water stress intensity values using a model developed with the data obtained from studies reporting both container weight and ψ_{pd} (Appendix B). Given that peat was the main component of the growing media in all studies, we assumed that the relation was not significantly biased by small variations across studies in mixtures of peat with other materials such as vermiculite. In addition, to make the model more robust, we included in the final model unpublished data from three drought-hardening studies where the authors had built crop predawn water potential *vs.* container weight for irrigation scheduling of their drought hardening treatments (Villar-Salvador et al., 2004b; Villar-Salvador et al., 2013, 1999; Appendix B).

2.4. Species drought tolerance

We assessed whether species drought tolerance influences the effects of drought hardening on outplanting survival and growth. The osmotic potential at the turgor loss point (π_{tlp}) was used as a proxy for species drought tolerance. Since this parameter can be measured indirectly through fast measurements with an osmometer (Bartlett et al., 2012b), it is readily available for a large number of species. Although osmotic adjustment capacity might better reflect drought tolerance, it has been demonstrated that the magnitude of this parameter correlates strongly with initial (i.e. non-stressed) π_{tlp} (Bartlett et al., 2014). Species with lower (more negative) π_{tlp} values typically thrive and have higher survival under water stress than species with higher π_{tlp} values (Álvarez-Cansino et al., 2022; Bartlett et al., 2012a). We sourced species π_{tlp} values from published studies except for *Rosmarinus officinalis*, where we used unpublished data from the study by Vilagrosa et al. (2014). Appendix C presents the species used in this study along with their π_{tlp} values and the corresponding references to obtain these values. We could not assign π_{tlp} values for two species in the survival database, which accounts for 3 % of the 164 study cases, and eight species in the growth database, affecting 15 % of the 127 study cases.

2.5. Effect-size calculations and statistical analyses

Effect size statistics were computed for each case study and differently for survival and growth data. For survival, we calculated the rate ratio (*RR*), assuming that each case study is a 2 × 2 contingency table (Koricheva et al., 2013). The *RR* is calculated as:

$$RR = \ln \frac{S_{hardened}}{S_{control}},$$

where $S_{hardened}$ represents the survival of the hardened plants and $S_{control}$ represents the survival of control plants. Survival was calculated on a per capita basis. The variance estimate for each *RR* values was determined as follows:

$$Variance \ln RR = \frac{(1 - S_{hardened})}{(n_{hardened}) \times S_{hardened}} + \frac{(1 - S_{control})}{(n_{control}) \times S_{control}} \quad [3]$$

where $n_{hardened}$ and $n_{control}$ represent the number of replicates (planted seedlings) of the hardened and control treatments, respectively. We used *RR* because variance measures were absent in a significant number of studies.

For growth, the effect size was the response ratio (*R*) (Koricheva et al., 2013), which was calculated as:

$$R = \ln \frac{G_{hardened}}{G_{control}} \quad [4]$$

where $G_{hardened}$ and $G_{control}$ is the arithmetic mean of the growth metric of the hardened and control treatments, respectively. The variance estimate of *R* is:

$$Variance \ln R = \frac{S^2_{hardened}}{n_{hardened} \times G^2_{hardened}} + \frac{S^2_{control}}{n_{control} \times G^2_{control}} \quad [5]$$

where $S^2_{hardened}$ and $S^2_{control}$ are the standard deviations of the growth metric for the hardened and control treatments, respectively.

The extent of heterogeneity was evaluated with the I^2 statistic (Higgins and Thompson, 2002), which estimates the percentage of variability due to heterogeneity rather than sampling error. Effect sizes were analyzed using mixed models adjusted for a meta-analysis (Borenstein et al., 2009), which allows incorporating fixed (moderators), and true random effects and nesting factors. We evaluated the overall effect size by fitting a null mixed effect meta-analysis model assuming that each case study was a random sample of a larger overall population, and considering the article identity as a nesting factor to avoid violating the assumption that effect sizes are independent from each other.

We also assessed whether several moderators (intensity and duration of drought hardening, elapsed time since the end of nursery hardening and outplanting, cultivation environment -indoors vs. outdoors-, transplanting environment -pots, large beds and field sites-, species π_{tlp}, aridity of field planting site, taxonomic group -angiosperm vs. gymnosperm, growth form -perennial herb, shrub, and tree-) explained part of the heterogeneity in the true effect. For this, we incorporated these factors individually into the mixed effect meta-analysis model that always included hardening intensity as a covariate. The exception was for species π_{tlp} and aridity of planting conditions, which were included as a pairwise interaction. For those analyses including aridity of the planting field site as moderator, we excluded one extreme case with an aridity value of 0.30 (a *Tsuga heterophylla* study case) to allow model convergence. Four study cases had to be removed to achieve model convergence in survival analyses, so the final number of study cases was n=160.

The test of Egger and Rosenberg's Fail-safe number were used to assess publication bias. Significant meta-analytic result is robust if the fail-safe number is > 5k + 10, where k is the number of studies in the meta-analysis. In addition, we analyzed whether publication year biased the results by adding it as a moderator.

Seedling growth in the selected studies was evaluated using different metrics. If multiple growth metrics were reported, we prioritized those that better reflects plant size in the following order plant mass > shoot mass > stem volume > stem diameter > shoot height (Andivia et al., 2021). Consequently, 16 % of the study cases were based on plant mass, 8 % on shoot mass, 29 % on stem volume, 19 % on stem diameter, and 28 % on shoot height. The range of variation and mean and median values of the independent continuous variables are shown in Appendix D.

3. Results

3.1. Effect of drought hardening on outplanting survival

In the survival database, 42 % of the cases had positive effect size values, i.e. increased survival due to drought hardening, while 11 % of the cases were equal to zero, i.e. same survival rates for hardened and control seedlings. This resulted in a positive overall effect size, which was, however, not significantly different from zero (*RR* = 0.09, 95 % confidence interval -CI- = -0.09–0.27, p-value = 0.32). This indicates no significant differences on survival between control and hardened seedlings across studies. However, we observed high heterogeneity in the effect of drought hardening on survival (I^2 = 99 %, p<0.001) indicating that factors other than the sampling error contributed to this heterogeneity.

When we assessed the influence of drought hardening characteristics and the cultivation method on the effect size, we observed that hardening intensity had a positive effect on seedling survival, although the effect was weak according to the low value of the standardized estimate (predicted slope=0.009, CI=0.0084–0.0095, p<0.001). Conversely, the time elapsed between the end of nursery hardening and outplanting had a strong, negative influence on the effect size (see high value of standardized estimate of the slope in Fig. 1), indicating that delaying planting after hardening in the nursery reduces its effectiveness. Conversely, the duration of drought hardening did not influence the effect size of drought hardening on survival (predicted slope=-0.0001; CI=-0.0024–0.0024; p=0.96). Finally, cultivation environment, indoors or outdoors, did not differ in effect size of drought hardening on survival (Ln*RR* for indoors = 0.24, CI = -0.08–0.56; Ln*RR* for outdoors = 0.02, CI = -0.22–0.25; p=0.28).

The type of outplanting experiment influenced the effect size for outplanting survival. Specifically, we found significant positive effects of drought hardening in pots, with effect sizes that were significantly higher than those observed in field or large bed studies, which showed neutral effect sizes., i.e.- nonsignificant differences between control and hardened seedlings (Fig. 2).

Effect size of drought hardening on survival was significantly (p < 0.001) higher in angiosperms than in gymnosperms (Fig. 2) and higher in shrubs (Ln*RR* = 0.14, CI = -0.04–0.32) than in trees (Ln*RR* = 0.08, CI = -0.10–0.26), although in both comparisons, the effect size did not differ significantly from zero. When gymnosperms were excluded from the analysis of growth forms, the effect size for shrubs was positive, significantly different from zero, and significantly higher than the effect size of trees. In other words, drought hardening primarily benefits survival in shrub species.

When data analysis was restricted to field study cases, we observed that the effect size of drought hardening on survival significantly increased with aridity of planting sites. However, the effect of the aridity on the outcome of drought hardening depended on species π_{tlp} (significant aridity × π_{tlp} interaction; estimate=0.137, CI = 0.123–0.152, p<0.001). Species with π_{tlp} < –3.2 MPa benefited from drought hardening independently of climate aridity (Fig. 3). At low aridity sites (Aridity < 2), drought hardening had neutral or negative effects on species with moderate to low drought tolerance (π_{tlp} > -3.0 MPa). Drought hardening gradually benefited species with higher π_{tlp} (less drought tolerant species) as aridity increased (see contour line for neutral effects of drought hardening displacing to more positive π_{tlp} values with aridity increase in Fig. 3). At sites with high aridity conditions (aridity> 3.5), drought hardening had an overall positive effect on survival regardless of species π_{tlp}.

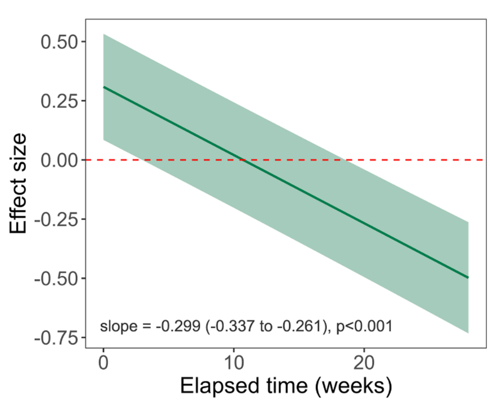

Fig. 1. Variation of the predicted effect size (Ln rate ratio, *-RR-*) of drought hardening on seedling outplanting survival relative to the elapsed time since the end of nursery hardening and planting date. n= 160. Positive values in the effect size denote an increase in survival due to drought hardening while negative values indicate a reduction in survival. Bands represent 95 confidence intervals.

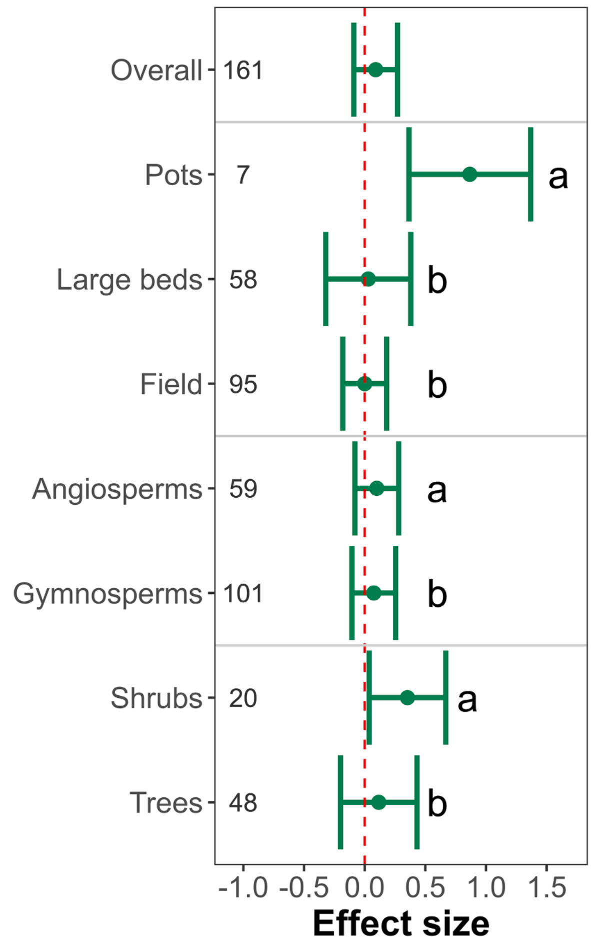

Fig. 2. Differences in the predicted effect size (Ln rate ratio) of drought hardening on seedling survival among the type of experiment in which outplanting growth was evaluated (pots, large beds, and field experiments), taxonomic group (angiosperms vs. gymnosperms), and growth forms (shrubs and trees). Values are predicted means ± 95 % confidence interval. Means with different letters indicate significant differences.

The test of Egger showed that our survival database has publication bias (estimate of the intercept = -0.003, CI = -0.004 to -0.0032, p<0.001). However, Rosenberg's Fail-safe number was 29970 suggesting that publication bias was small. Publication year did not bias the effect size (Estimate = 0.0013, CI = -0.0244–0.027, p=0.92).

3.2. Effect of drought hardening on outplanting growth

The overall mean effect size of seedling growth after outplanting was slightly negative but not significantly different from zero (Fig. 4, p = 0.53), indicating that there were no differences between control and hardened seedlings across studies. Heterogeneity of growth effect size values was high (I^2 = 91.2 %, p < 0.001). Neither hardening intensity (model slope = −0.021, CI = -0.080–0.037, p = 0.48), its duration (model slope = 0.004, CI = -0.100–0.107, p = 0.94), nor the time elapsed between the end of nursery hardening and outplanting (model slope = 0.013, CI = −0.088–0.103, p = 0.80) significantly affected the effect size. Similarly, no differences were observed between indoor and outdoor cultivation environments in the effect size (*R* = -0.046, CI = -0.289–0.196 for indoor studies; *R* = -0.014, CI = -0.211–0.182 for

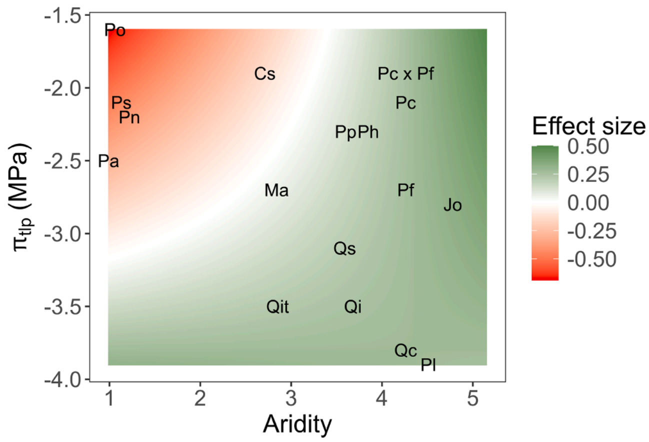

Fig. 3. Variation of the predicted effect size (Ln response ratio) of drought hardening on outplanting survival in species in relation to species osmotic potential at the turgor loss point (π_{tlp}) and outplanting aridity conditions. Labels inside the figure represent initial of species used in the analyses: Cs=*Ceratonia siliqua*; Jo=*Juniperus oxycedrus*; Ma=*Medicago arborea*; Pa=*Picea abies*; Ph=*Pinus halepensis*; Pn=*Pinus nigra;* Pp=*Pinus pinea*; Ps=*Pinus sylvestris*; Pl=*Pistacia lentiscus*; Po=*Platanus orientalis*; Pc=*Prosopis chilensis*; Pf=*Prosopis flexuosa*; Pc × Pf= *Prosopis chilensis* × *P. flexuosa*; Qc=*Quercus coccifera*; Qi=*Quercus ilex*; Qit=*Quercus ithaburensis*; Qs=*Quercus suber*.

outdoor studies, p = 0.84).

Regarding outplanting conditions, the effect size showed significantly more negative values in pot experiments compared to field or large bed outplanting experiments, where the effect size did not differ significantly from zero (Fig. 4). Aridity conditions in the field experiments increased the effect size of seedling growth after outplanting (Fig. 5).

Finally, considering functional differences among species, we detected that shrubs and trees had significantly higher effect size values than perennial herbs. In all growth forms, however, the mean effect size was not significantly different from zero (Fig. 4) and growth form differences did not change when excluding the gymnosperms (data not shown). Taxonomic group (LnR = 0.079, CI = -0.136–0.293 for angiosperms; R = -0.120 CI = -0.30–0.064 for gymnosperms, P = 0.16), species π_{tlp} (model slope = -0.010, CI = -0.128–0.107, p=0.86), or the interaction between species π_{tlp} and aridity (p = 0.34) did not influence growth effect size.

The test of Egger indicated that our growth database does not have publication bias (estimate of the intercept = 0.001, CI = -0.001 to -0.003, p= 0.44). In contrast we detected that publication year may have affected the effect size with an increased proportion of case studies with negative effect size in recent years (Estimate = -0.009, CI = -0.160 to −0.020, p = 0.01).

4. Discussion

Plant drought responses are complex, involving many physiological and morphological changes in response to varying drought conditions, which result from the interaction of meteorological and soil features (Tardieu, 2012). The efficacy of drought acclimation (i.e. hardening effect) on plant performance is even more challenging, as it depends on these complex responses and its persistence over changing drought scenarios (Yordanov et al., 2000). This complexity leads to variable results across studies (Grossnickle, 2012) and aligns with the lack of overall significant effects of drought hardening on seedling outplanting performance observed in our metanalysis. This is likely due to high heterogeneity of species, hardening application conditions and outplanting environments. Our results provide useful insights into this complexity, and quantitatively explain the factors that could determine the effectiveness of drought hardening treatments in improving

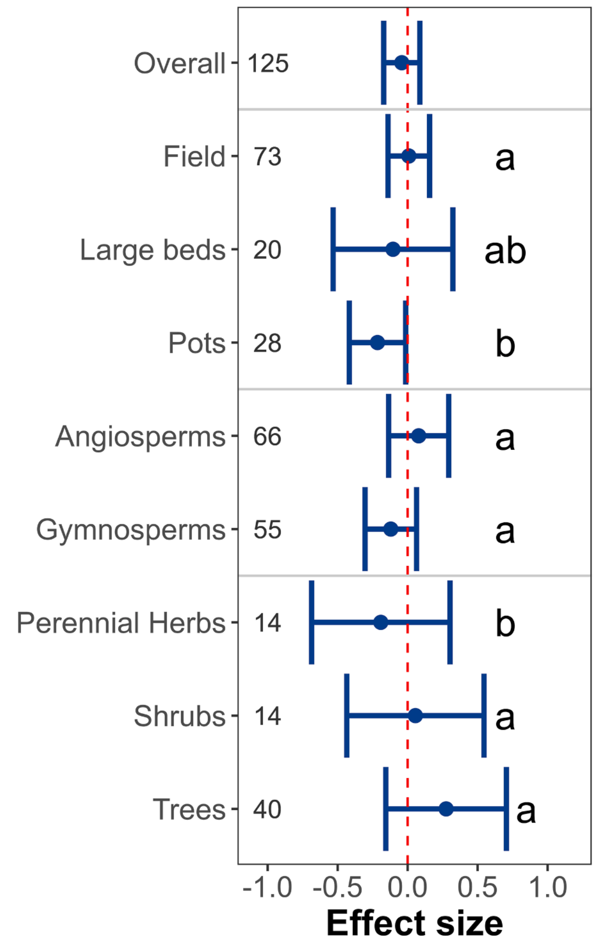

Fig. 4. Differences in the predicted effect size (Ln response ratio) of drought hardening on seedling outplanting growth among the type of experiment in which outplanting growth was evaluated (pots, large beds, and field experiments), taxonomic group (angiosperms vs. gymnosperms), and growth forms (perennial herbs, shrubs, and trees). Values are predicted means ± 95 % confidence interval. Means with different letters indicate significant differences.

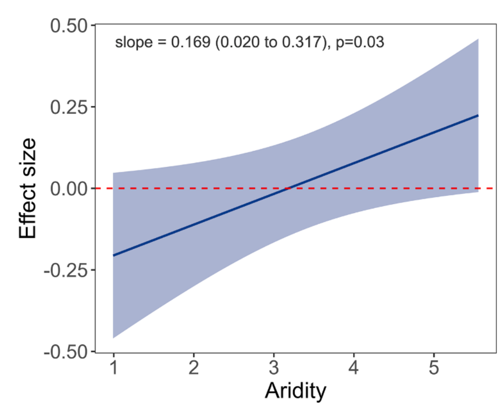

Fig. 5. Variation of the effect size (Ln response ratio) of the effect of drought hardening on seedling outplanting growth under field conditions. Bands represent 95 confidence intervals.

outplanting performance.

Transplanting conditions (F1) emerged as one of the most relevant factors in our study, with the effectiveness of drought hardening being higher in pot than in large beds or field experiments. Hardening induces morphological and physiological changes that can increase both the drought tolerance and avoidance capacity of seedlings (Lamhamedi et al., 2001; Stewart and Lieffers, 1993; Vilagrosa et al.,2003). Regarding drought avoidance capacity, the main positive effect of drought hardening is generally associated to plant water conservation mechanisms such as reduced shoot-to-root ratio, specific leaf area, or leaf water conductance (van den Driessche, 1991b; Villar-Salvador et al., 1999, 2004b). Extensive, deep rooting after transplanting drives drought avoidance of plants in dry climates by accessing humid soil layers. At the seedling stage, this strategy can be more crucial for survival than other water conservation mechanisms or increased drought tolerance (Villar-Salvador et al., 2012a; Andivia et al., 2019a; Padilla and Pugnaire, 2007). However, water stress can damage roots, reducing their capacity to grow new roots when water stress is relieved (Tinus, 1996). Thus, several studies report that drought hardening effects on root growth capacity are negligible or even negative (Villar-Salvador et al., 2004b; Villar-Salvador et al., 2013). When transplanted to relatively small pots, growth of roots becomes irrelevant as they cannot explore larger volumes of soil, especially in depth. In addition, plants deplete soil water faster (Turner, 2019) compared to the much larger rooting volumes existing under field conditions. Therefore, in pot transplanting experiments, the effect of drought hardening could be more pronounced due to restriction of root exploration of moist soil layers, giving more relevance to physiological mechanisms or changes in carbon allocation induced by conditioning (von Moler and Nelson, 2021). Additionally, the shorter time elapsed between the end of drought hardening and transplanting might explain the significant positive effect of drought hardening on survival in pots (Fig. 2), as it is usually shorter in pot experiments. The idea of restricted soil volume influencing drought hardening effects in pot-transplanting experiments was also supported by the observed negative effect of drought hardening on growth observed only within these experiments (Fig. 4). Under field conditions seedlings can grow unrestricted, which might decrease the differences in plant growth between hardened and non-hardened plants. In pots, seedlings cannot avoid water stress by root exploration and, as water conservation acclimation traits (reduced leaf area, stomatal conductance) generally impair growth (Valliere et al., 2019) and drought hardening can even hinder root growth capacity, this would explain less growth in hardened plants transplanted to pots. These results highlight the inadequacy of assessing the effect of drought hardening in pots and suggest that positive drought hardening effects could be more prominent in situations where new roots cannot explore large soil volumes due to site or species characteristics, planting timing or lack of intense soil preparation.

The positive effect of drought hardening on the survival of seedlings planted under field conditions (F2) increased with aridity Figs. 3 and 5). The window of opportunity for root growth after planting, when soil moisture is adequate and the plant is active, usually decreases with aridity. Thus, seedlings planted in arid sites could benefit most from water conservation traits conferred by drought hardening such as reduced stomatal and residual conductance (Guarnaschelli et al., 2006; Villar-Salvador et al., 2004b).This positive relationship between aridity and the effect of drought hardening occurred regardless of the drought tolerance of the species (F3). Species with low drought tolerance ($\pi_{tlp} >$ −3.0 MPa) such as the Mediterranean pines (*Pinus pinea, P. halepensis*, see Fig. 3) are associated with strong water conservative strategies, such as tightly regulating stomatal conductance in response to soil drying to avoid turgor loss or hydraulic failure. In contrast, drought-tolerant species like Mediterranean oaks rely on keeping cell turgor and stomata opening at low water potentials (Klein et al., 2013), contributing to root growth and, hence, drought avoidance. Therefore, the similar effect of drought hardening on survival in arid sites, regardless of species drought tolerance, suggests that drought hardening enhances survival in arid sites primarily by improving water conservation in scenarios where root exploration after transplanting is limited.

Drought hardening was ineffective or even detrimental in low aridity sites (Figs. 3 and 5). When root growth is not restricted by soil volume or drought conditions immediately after planting, plant size becomes a main driver of seedling performance (Andivia et al., 2021; Villar-Salvador et al., 2012). Given that drought hardening typically reduces plant size, its effects become uncertain when drought stress conditions are not severely limiting, as it diminishes root growth during the humid season, a critical process for survival during the dry season. For instance, seedling root growth after planting for pine species inhabiting high Mediterranean mountains like *Pinus sylvestris* and *P. nigra* (see Fig. 3), which already have slower root growth rates than typically Mediterranean pine species (Andivia et al., 2019), can be impaired in small, drought hardened seedlings, which can compromise their survival in summer.

Shrubs benefited more from drought hardening than broadleaf trees (F3). Differences in physiological drought responses between growth forms has been reported in different plant communities (Apgaua et al., 2017; Castro et al., 2023). Shrubs have, in general, higher drought resistance than trees (Peñuelas et al., 2001), lower hydraulic efficiency, and slower shoot growth (Zhang et al., 2023). These water-conserving characteristics may be more enhanced by drought hardening in shrubs than in trees (Fig. 2). Conversely, drought hardening did not differentially affect shrub and tree growth, but it had a negative effect on the growth of perennial herbs (not represented in the survival database). Since herbs are generally more sensitive to water stress than woody plants (Klimeš et al., 2022), impaired pre-planting growth likely translates into a lower post-planting growth. However, due to insufficient data we could not conclude if there is a trade-off between survival and growth for this functional group as observed in pot experiments or field studies in arid sites.

Cultivation environment (outdoors vs indoors) did not significantly affect the outcome of drought hardening (F4). Restricted irrigation before planting is recognized as necessary when cultivating forest seedlings inside greenhouses or growth chambers (Dumroese et al., 2021). Seedlings grown indoors generally receive less light and grow under higher temperature and air humidity than those grown outdoors. This results in phenotypes which may be actively growing at planting time and thus are less acclimated to frosts and the water stress caused by the transplanting shock. Stress hardiness is critical for plantations in cold climates (Jacobs and Landis, 2009) and in xerogardening seedling production (Franco et al., 2006), where most production occurs in greenhouses. However, our results do not show that this is particularly relevant in greenhouse cultivation, and drought-hardening effects are overall indistinguishable from those observed in outdoors cultivation.

We found that the intensity and duration of drought application had a weak effect or non-significant effect on post-planting survival (F5). This was unexpected because variability in drought treatment application has been identified as a major source of outcome differences among drought hardening studies (Grossnickle, 2012; Von Moler and Nelson, 2021). However, drought effects are highly species-specific, so the same intensity and duration can induce varied effects depending on the acclimation capacity in response to water stress. Other seedling features, such as the ratio between leaf area and pot volume or plant age, can dramatically change the impact of the same duration and intensity of drought application (Varone et al., 2012). Additionally, meteorological conditions, in particular evaporative demand during drought treatment application can affect the intensity of plant water stress (Bañon et al., 2006; Sánchez-Blanco et al., 2004). However, we observed that more intense drought hardening tended to improve post-planting survival. This suggests that many studies might fail to apply a significant degree of water stress, resulting in small morphological and physiological changes in the seedling. Nursery handbooks recommend application of 'mild' drought stress (Landis and Wilkinson, 2009), which might lead researchers and practitioners to be overly cautious, resulting in insignificant effects. As a practical consequence, drought intensity and duration need to be tailored to the specific conditions of the nursery and target species.

In contrast to other drought hardening application conditions, the elapsed time between treatment application and plantation (F5) had a strong negative effect on survival (Fig. 1). Drought acclimation involves persistent developmental responses, generally leading to improved plant water conservation, such as reduced shoot-to-root ratio or increased specific leaf area, as well as temporal physiological changes. The effectiveness and persistence of physiological acclimations depends strongly on the species and the physiological trait (Galmés et al., 2007). For instance, some species fully recover pre-stress levels of stomatal conductance within hours after re-watering (Liu et al., 2001), while in others, abscisic acid accumulation depresses stomatal conductance for days (Tombesi et al., 2015). While the effects of drought hardening on stomatal conductance are relatively short-lived, osmotic adjustment (i.e. the active accumulation of solutes to maintain cell turgor under decreasing water potentials) can be maintained longer. However, the magnitude of osmotic adjustment and its effectiveness in maintaining plant turgor under water stress vary among species (Abrams, 1988; Bartlett et al., 2014). This variability could explain the negative effect of delaying plantation after drought hardening. However, this effect disappeared when species were included as a random factor in the statistical analysis (data not shown), indicating potential bias from specific species or group of species within our dataset. Specifically, boreal trees, which are usually cold-stored before planting, had the longest elapsed times between hardening and planting. For those species, hardening effects might not be relevant when planted after cold storage in sites generally free from drought stress.

5. Conclusions

Since correct application of drought hardening is challenging, its use must be restricted to specific planting scenarios where post-planting root growth can be limited by water stress or constrained rooting volume in shallow soils. In those cases, drought hardening might improve survival. Nevertheless, our results suggests that in those situations where drought hardening could be suitable, intensity and duration of drought stress should be carefully adjusted. Future research should address the interaction between these drought application features and species water use strategies. Modeling these interactions could help to tailor specific drought hardening protocols depending on functional traits of the target species. However, it must be highlighted that drought hardening treatments evaluated through pot-transplanting experiments need to be interpreted with caution. Therefore, transplanting to soil volume that do not restrict root growth can be prescribed in this type of studies.

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CRediT authorship contribution statement

Jaime Puertolas: Writing – review & editing, Writing – original draft, Supervision, Investigation, Data curation, Conceptualization. **Pedro Villar-Salvador:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Enrique Andivia:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ishita Ahuja:** Writing – review & editing, Investigation. **Claudia Cocozza:** Writing – review & editing, Investigation. **Branislav Cvjetković:** Writing – review & editing, Investigation. **Jovana Devetaković:** Writing – review & editing, Investigation. **Julio J. Diez:** Writing – review & editing, Investigation. **Inger S. Fløistad:** Writing – review & editing, Investigation. **Petros Ganatsas:** Writing – review & editing, Resources. **Barbara Mariotti:** Writing – review & editing, Investigation. Marianthi Tsakaldimi: Writing – review & editing, Resources. Alberto Vilagrosa: Writing – review & editing, Resources. **Johanna Witzell:** Writing – review & editing, Investigation. **Vladan Ivetić:** Project administration, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data is available on Figshare (https://dx.doi.org/10.6084/m9.figshare.27073717).

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.foreco.2024.122300.

References

Abrams, M.D., 1988. Sources of variation in osmotic potential with special reference to North American tree species. For. Sci. 34, 1030–1046.
Abrams, M.D., 1990. Adaptations and responses to drought in *Quercus* species of North America. Tree Physiol. 7, 227–238.
Albouchi, A., Sebei, H., Mezni, M.Y., El Aouni, M.H., 2001. Influence de la durée d’acclimatation sur l’endurcissement à la sécheresse d’Acacia cyanophylla Lindl. Ann. Sci. 58, 519–528.
Álvarez-Cansino, L., Comita, L.S., Jones, F.A., Manzané-Pinzón, E., Browne, L., Engelbrecht, B.M.J., 2022. Turgor loss point predicts survival responses to experimental and natural drought in tropical tree seedlings. Ecology 103. https://doi.org/10.1002/ecy.3700.
Andivia, E., Villar-Salvador, P., Oliet, J.A., Puértolas, J., Dumroese, R.K., Ivetić, V., Molina-Venegas, R., Arellano, E.C., Li, G., Ovalle, J.F., 2021. Climate and species stress resistance modulate the higher survival of large seedlings in forest restoration worldwide. Ecol. Appl., e02394 https://doi.org/10.1002/eap.2394.
Andivia, E., Zuccarini, P., Grau, B., de Herralde, F., Villar-Salvador, P., Savé, R., 2019. Rooting big and deep rapidly: the ecological roots of pine species distribution in southern Europe. Trees 33, 293–303. https://doi.org/10.1007/s00468-018-1777-x.
Apgaua, D.M.G., Tng, D.Y.P., Cernusak, L.A., Cheesman, A.W., Santos, R.M., Edwards, W. J., Laurance, S.G.W., 2017. Plant functional groups within a tropical forest exhibit different wood functional anatomy. Funct. Ecol. 31, 582–591. https://doi.org/10.1111/1365-2435.12787.
Bañon, S., Ochoa, J., Franco, J.A., Alarcón, J.J., Sánchez-Blanco, M.J., 2006. Hardening of oleander seedlings by deficit irrigation and low air humidity. Environ. Exp. Bot. 56, 36–43. https://doi.org/10.1016/J.ENVEXPBOT.2004.12.004.
Bartlett, M.K., Scoffoni, C., Sack, L., 2012a. The determinants of leaf turgor loss point and prediction of drought tolerance of species and biomes: a global meta-analysis. Ecol. Lett. 15, 393–405. https://doi.org/10.1111/j.1461-0248.2012.01751.x.
Bartlett, M.K., Scoffoni, C., Ardy, R., Zhang, Y., Sun, S., Cao, K., Sack, L., 2012b. Rapid determination of comparative drought tolerance traits using an osmometer to predict turgor loss point. Methods Ecol. Evol. 3, 880–888. https://doi.org/10.1111/j.2041-210X.2012.00230.x.
Bartlett, M.K., Zhang, Y., Kreidler, N., Sun, S., Ardy, R., Cao, K., Sack, L., 2014. Global analysis of plasticity in turgor loss point, a key drought tolerance trait. Ecol. Lett. 17, 1580–1590. https://doi.org/10.1111/ele.12374.
Blake, T.J., Bevilacqua, E., Zwiazek, J.J., 1991. Effects of repeated stress on turgor pressure and cell elasticity changes in black spruce seedlings. Can. J. Res 21. https://doi.org/10.1139/x91-187.
Borenstein, M., Hedges, L.V., Higgins, J.P.T., Rothstein, H.R., 2009. Introduction to meta-analysis. Introd. Meta-Anal. https://doi.org/10.1002/9780470743386.
Castro, H., Dias, M.C., Sousa, J.P., Freitas, H., 2023. Functional groups response to water deficit in Mediterranean ecosystems. Plants 12, 1471. https://doi.org/10.3390/plants12071471.

Charles, L.S., Dwyer, J.M., Smith, T.J., Connors, S., Marschner, P., Mayfield, M.M., 2018. Species wood density and the location of planted seedlings drive early-stage seedling survival during tropical forest restoration. J. Appl. Ecol. 55, 1009–1018. https://doi.org/10.1111/1365-2664.13031.
Chaves, M.M., Maroco, J.P., Pereira, J.S., 2003. Understanding plant responses to drought-from genes to the whole plant. Funct. Plant Biol. 30, 239–264. Func Plant Biol 30, 239–264.
Chirino, E., Carmona, A.V., Rubio, E., 2004. Efectos de la reducción del riego y la fertilización en las características morfológicas de *Quercus suber*. Cuad. Soc. Esp. Cienc. For. 17, 51–56.
Choat, B., Jansen, S., Brodribb, T.J., Cochard, H., Delzon, S., Bhaskar, R., Bucci, S.J., Feild, T.S., Gleason, S.M., Hacke, U.G., Jacobsen, A.L., Lens, F., Maherali, H., Martínez-Vilalta, J., Mayr, S., Mencuccini, M., Mitchell, P.J., Nardini, A., Pittermann, J., Pratt, R.B., Sperry, J.S., Westoby, M., Wright, I.J., Zanne, A.E., 2012. Global convergence in the vulnerability of forests to drought. Nature 491, 752–755. https://doi.org/10.1038/nature11688.
Close, D.C., Beadle, C.L., Brown, P.H., 2005. The physiological basis of containerised tree seedling 'transplant shock': a review. Aust 68, 112–120. https://doi.org/10.1080/00049158.2005.10674954.
Cornelissen, J.H.C., Castro-Díez, P., Carnelli, A.L., 1998. Variation in relative growth rate among woody species. In: Lambers, H., Poorter, H., Van Vuuren, M.M.I. (Eds.), Inherent Variation in Plant Growth. Physiological Mechanisms and Ecological Consequences. Backhuys. Publishers, Leiden, pp. 363–392.
Cuesta, B., Villar-Salvador, P., Puértolas, J., Jacobs, D.F., Rey Benayas, J.M., 2010. Why do large, nitrogen rich seedlings better resist stressful transplanting conditions? A physiological analysis in two functionally contrasting Mediterranean forest species. Ecol. Manag. 260, 71–78. https://doi.org/10.1016/j.foreco.2010.04.002.
del Campo, A.D., Segura-Orenga, G., Ceacero, C.J., González-Sanchis, M., Molina, A.J., Reyna, S., Hermoso, J., 2020. Reforesting drylands under novel climates with extreme drought filters: The importance of trait-based species selection. Ecol. Manag. 467, 118156. https://doi.org/10.1016/j.foreco.2020.118156.
Dumroese, R.K., Landis, T.D., Luna, T., 2021. Raising native plants in nurseries: Basic concepts. USDA, Forest Service Rocky Mountain Research Station, Fort Collins. https://doi.org/10.2737/RMRS-GTR-274.
Edwards, D.R., Dixon, M.A., 1995. Mechanisms of drought response in Thuja occidentalis L. I. Water stress conditioning and osmotic adjustment. Tree Physiol. 15, 121–127. https://doi.org/10.1093/treephys/15.2.121.
Fick, S.E., Hijmans, R.J., 2017. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. Int. J. Climatol. 37, 4302–4315. https://doi.org/10.1002/JOC.5086.
Franco, J.A., Martínez-Sánchez, J.J., Fernández, J.A.A., Bañon, Bañón, S., 2006. Selection and nursery production of ornamental plants for landscaping and xerogardening in semi-arid environments. J. Hort. Sci. Biotech. 81, 3–17.
Galmés, J., Flexas, J., Savé, R., Medrano, H., 2007. Water relations and stomatal characteristics of Mediterranean plants with different growth forms and leaf habits: responses to water stress and recovery. Plant Soil 290, 139–155.
Grossnickle, S.C., 2005. Importance of root growth in overcoming planting stress. New 30, 273–294. https://doi.org/10.1007/s11056-004-8303-2.
Grossnickle, S.C., 2012. Why seedlings survive: Influence of plant attributes. New 43, 711–738. https://doi.org/10.1007/s11056-012-9336-6.
Grossnickle, S.C., Arnott, J.T., Major, J.E., Tschaplinski, T.J., 1991a. Influence of dormancy induction treatments on western hemlock seedlings. I. Seedling development and stock quality assessment. Can. J. Res 21, 164–174.
Grossnickle, S.C., Arnott, J.T., Major, J.E., 1991b. Influence of dormancy induction treatments on western hemlock seedlings. II. Physiological and morphological response during the first growing season on a reforestation site. Can. J. Res 21, 175–185. https://doi.org/10.1139/X91-021.
Guarnaschelli, A.B., Prystupa, P., Lemcoff, J.H., 2006. Drought conditioning improves water status, stomatal conductance and survival of *Eucalyptus globulus* subsp. *bicostata* seedlings. Ann. Sci. 63, 941–950. https://doi.org/10.1051/FOREST:2006077.
Harris, J.A., Hobbs, R.J., Higgs, E., Aronson, J., 2006. Ecological restoration and global climate change. Restor. Ecol. https://doi.org/10.1111/j.1526-100X.2006.00136.x.
Higgins, J.P.T., Thompson, S.G., 2002. Quantifying heterogeneity in a meta-analysis. Stat. Med 21, 1539–1558. https://doi.org/10.1002/SIM.1186.
Hua, F., Wang, X., Zheng, X., Fisher, B., Wang, L., Zhu, J., Tang, Y., Yu, D.W., Wilcove, D. S., 2016. Opportunities for biodiversity gains under the world's largest reforestation programme. Nat. Comm. 7, 12717. https://doi.org/10.1038/ncomms12717.
Jacobs, D.F., Landis, T.D., 2009. Hardening. In: Nursery Manual for Native Plants. USDA. Forest Service, Washington, pp. 216–227.
Kaushal, P., Aussenac, G., 1989. Transplanting shock in Corsican pine and cedar of Atlas seedlings: Internal water deficits, growth and root regeneration. Ecol. Manag. 27, 29–40.
Klein, T., Shpringer, I., Fikler, B., Elbaz, G., Cohen, S., Yakir, D., 2013. Relationships between stomatal regulation, water-use, and water-use efficiency of two coexisting key Mediterranean tree species. Ecol. Manag. 302, 34–42. https://doi.org/10.1016/J.FORECO.2013.03.044.
Klimeš, A., Šímová, I., Zizka, A., Antonelli, A., Herben, T., 2022. The ecological drivers of growth form evolution in flowering plants. J. Ecol. 110, 1525–1536. https://doi.org/10.1111/1365-2745.13888.
Koricheva, J., Gurevitch, J., Mengersen, K., 2013. Handbook of meta-analysis in Ecology and Evolution. Princeton University Press. https://doi.org/10.1515/9781400846184.
Ladjal, M., Epron, D., Ducrey, M., 2000. Effects of drought conditioning on thermotolerance of photosystem II and susceptibility of photosynthesis to heat stress in cedar seedlings. Tree Physiol. 20, 1235–1241.
Lamhamedi, M., Lambany, G., Margolis, H., Renaud, M., Veilleux, L., Bernier, P.Y., 2001. Growth, physiology, and leachate losses in *Picea glauca* seedlings (1+0) grown in air-slit containers under different irrigation regimes. Can. J. For. Res. 31, 1968–1980.
Landis, T., Tinus, R., McDonald, A.J.S., Barnett, J.P., 1989. Seedling nutrition and irrigation, The container tree nursery manual. USDA. Forest Service.
Landis, T.D., Wilkinson, K.M., 2009. Water quality and irrigation. In: Dumroese, R.K., Luna, T., Landis, T.D. (Eds.), Nursery Manual for Native Plants: A Guide for Tribal Nurseries. USDA. Forest Service, Washington, USA, pp. 177–199.
Lázaro-González, A., Andivia, E., Hampe, A., Hasegawa, S., Marzano, R., Santos, A.M.C., Castro, J., Leverkus, A.B., 2023. Revegetation through seeding or planting: A worldwide systematic map. J. Environ. Manag. 337, 117713. https://doi.org/10.1016/j.jenvman.2023.117713.
Liu, L., McDonald, A.J.S., Stadenberg, I., Davies, W.J., 2001. Abscisic acid in leaves and roots of willow: significance for stomatal conductance. Tree Physiol. 21, 759–764. https://doi.org/10.1093/treephys/21.11.759.
Löf, M., Dey, D.C., Navarro, R.M., Jacobs, D.F., 2012. Mechanical site preparation for forest restoration. New 43, 825–848. https://doi.org/10.1007/s11056-012-9332-x.
Lopez-Iglesias, B., Villar, R., Poorter, L., 2014. Functional traits predict drought performance and distribution of mediterranean woody species. Acta Oecol 56, 10–18. https://doi.org/10.1016/j.actao.2014.01.003.
Medeiros, J.S., Pockman, W.T., 2011. Drought increases freezing tolerance of both leaves and xylem of *Larrea tridentata*. Plant Cell Environ. 34, 43–51. https://doi.org/10.1111/j.1365-3040.2010.02224.x.
Niinemets, U., Valladares, F., 2006. Tolerance to shade, drought, and waterlogging of temperate northern hemisphere trees and shrubs. Ecol. Monogr. 76, 521–547.
Nishikawa-Pacher, A., 2022. Research questions with PICO: a universal mnemonic. Publications 10, 21. https://doi.org/10.3390/publications10030021.
O'Reilly, C., Owens, J.N., Arnott, J.T., Dunsworth, B.G., 1994. Effect of nursery culture on morphological development of western hemlock seedlings during field establishment. II. Survival, shoot length components, and needle length. Can. J. Res 24, 61–70.
Padilla, F.M., Pugnaire, F.I., 2007. Rooting depth and soil moisture control Mediterranean woody seedling survival during drought. Funct. Ecol. 21, 489–495.
Parsons, J., Motta, C., Sehgal, G., Miller-ter-Kuile, A., Young, H., Orr, D., 2021. Interactive effects of large herbivores and climate on California oak seedling outcomes. Ecol. Manag. 502. https://doi.org/10.1016/j.foreco.2021.119650.
Peñuelas, J., Lloret, F., Montoya, R., 2001. Severe drought effects on mediterranean woody Flora in Spain. Sci 47, 214–218. https://doi.org/10.1093/forestscience/47.2.214.
Poorter, H., Fiorani, F., Pieruschka, R., Wojciechowski, T., Van Der Putten, W.H., Kleyer, M., Schurr, U., Postma, J., 2016. Tansley review Pampered inside, pestered outside? Differences and similarities between plants growing in controlled conditions and in the field. N. Phytol. 212, 838–855. https://doi.org/10.1111/nph.14243.
Vallejo, R.V., Smanis, A., Chirino, E., Fuentes, D., Valdecantos, A., Vilagrosa, A., 2012. Perspectives in dryland restoration: approaches for climate change adaptation. New 43, 561–579. https://doi.org/10.1007/s11056-012-9325-9.
Reich, P.B., 2014. The world-wide 'fast-slow' plant economics spectrum: a traits manifesto. J. Ecol. 102, 275–301. https://doi.org/10.1111/1365-2745.12211.
Richardson, W., Wilson, M., Nishikawa, J., Hayward, R., 1995. The well-built clinical question: a key to evidence-based decisions. ACP J. Club 123, A12–A13. https://doi.org/10.7326/ACPJC-1995-123-3-A12.
Rook, D.A., 1973. Conditioning radiata pine seedlings to transplanting, by restricted watering. N. Z. J. Sci. 3, 54–69.
Royo, A., Gil, L., Pardos, J.A., 2001. Effect of water stress conditioning on morphology, physiology and fiel performance of *Pinus halepensis* Mill. seedlings. New 21, 127–140.
Ruehr, N.K., Grote, R., Mayr, S., Arneth, A., 2019. Beyond the extreme: recovery of carbon and water relations in woody plants following heat and drought stress. Tree Physiol. 39, 1285–1299. https://doi.org/10.1093/TREEPHYS/TPZ032.
Sánchez-Blanco, M.J., Ferrández, T., Navarro, A., Bañon, S., Alarcón, J.J., 2004. Effects of irrigation and air humidity preconditioning on water relations, growth and survival of *Rosmarinus officinalis* plants during and after transplanting. J. Plant Physiol. 161, 1133–1142. https://doi.org/10.1016/j.jplph.2004.01.011.
Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods 9 (7), 671–675. https://doi.org/10.1038/nmeth.2089.
Skinner, G., Cooke, R., Keum, J., Purvis, A., Raw, C., Woodcock, B.A., Millard, J., 2023. Dynameta: A dynamic platform for ecological meta-analyses in R Shiny. SoftwareX 23, 101439. https://doi.org/10.1016/j.softx.2023.101439.
Stewart, J.D., Lieffers, V.J., 1993. Preconditioning effects of nitrogen relative addition rate and drought stress on container-grown lodgepole pine seedlings. Can. J. Res 23, 1663–1671. https://doi.org/10.1139/x93-207.
Tardieu, F., 2012. Any trait or trait-related allele can confer drought tolerance: just design the right drought scenario. J. Exp. Bot. 63, 25–31. https://doi.org/10.1093/jxb/err269.
Tinus, R., 1996. Root growth potential as an indicator of drought stress history. Tree Physiol. 16, 795–799.
Tombesi, S., Nardini, A., Frioni, T., Soccolini, M., Zadra, C., Farinelli, D., Poni, S., Palliotti, A., 2015. Stomatal closure is induced by hydraulic signals and maintained by ABA in drought-stressed grapevine. Sci. Rep. 5, 12449. https://doi.org/10.1038/srep12449.
Trabucco, A., Zomer, R., 2019. Global Aridity Index and Potential Evapotranspiration (ET0) Climate Database v2 – CGIAR-CSI [WWW Document]. https://doi.org/https://doi.org/10.6084/m9.figshare.7504448.v3.
Turner, N.C., 2019. Imposing and maintaining soil water deficits in drought studies in pots. Plant Soil 439, 45–55. https://doi.org/10.1007/s11104-018-3893-1.

Valliere, J.M., Zhang, J., Sharifi, M.R., Rundel, P.W., 2019. Can we condition native plants to increase drought tolerance and improve restoration success? Ecol. Appl. 29, e01863. https://doi.org/10.1002/eap.1863.

van den Driessche, R., 1991a. Influence of container nursery regimes on drought resistance I Survival and growth. Can. J. Res 21, 555–565.

van den Driessche, R., 1991b. Influence of container nursery regimes on drought resistance of seedlings following planting. II Stomatal conductance, specific leaf area, and root growth capacity. Can. J. Res 21, 566–572.

van den Driessche, R., 1992. Changes in drought resistance and root growth capacity of container seedlings in response to nursery drought, nitrogen, and potassium treatments. Can. J. For. Res. 22, 740–749. https://doi.org/10.1139/x92-100.

Varone, L., Ribas-Carbo, M., Cardona, C., Gallé, A., Medrano, H., Gratani, L., Flexas, J., 2012. Stomatal and non-stomatal limitations to photosynthesis in seedlings and saplings of Mediterranean species pre-conditioned and aged in nurseries: Different response to water stress. Environ. Exp. Bot. 75, 235–247. https://doi.org/10.1016/J.ENVEXPBOT.2011.07.007.

Vilagrosa, A., Cortina, J., Gil-Pelegrín, E., Bellot, J., 2003. Suitability of drought-preconditioning `techniques in Mediterranean climate. Restor. Ecol. 11, 208–216.

Vilagrosa, A., Hernández, E.I., Luis, V.C., Cochard, H., Pausas, J.G., 2014. Physiological differences explain the co-existence of different regeneration strategies in Mediterranean ecosystems. N. Phytol. 201. https://doi.org/10.1111/nph.12584.

Villar-Salvador, P., Ocaña, L., Peñuelas, J.L., Carrasco, I., 1999. Effect of water stress conditioning on the water relations, root growth capacity, and the nitrogen and non-structural carbohydrate concentration of *Pinus halepensis* Mill. (Aleppo pine) seedlings. Ann. Sci. 56, 459–465. https://doi.org/10.1051/forest:19990602.

Villar-Salvador, P., Peñuelas, J.L., Jacobs, D.F., 2013. Nitrogen nutrition and drought hardening exert opposite effects on the stress tolerance of *Pinus pinea* L. seedlings. Tree Physiol. 33, 221–232. https://doi.org/10.1093/treephys/tps133.

Villar-Salvador, P., Planelles, R., Enríquez, E., Rubira, J.P., 2004a. Nursery cultivation regimes, plant functional attributes, and field performance relationships in the Mediterranean oak *Quercus ilex* L. Ecol. Manag. 196, 257–266. https://doi.org/10.1016/j.foreco.2004.02.061.

Villar-Salvador, P., Planelles, R., Oliet, J., Penuelas-Rubira, J.L., Jacobs, D.F., Gonzalez, M., 2004b. Drought tolerance and transplanting performance of holm oak (*Quercus ilex*) seedlings after drought hardening in the nursery. Tree Physiol. 24, 1147–1155. https://doi.org/10.1093/treephys/24.10.1147.

Villar-Salvador, P., Puértolas, J., Cuesta, B., Peñuelas, J.L., Uscola, M., Heredia-Guerrero, N., Rey Benayas, J.M., 2012a. Increase in size and nitrogen concentration enhances seedling survival in Mediterranean plantations. Insights from an ecophysiological conceptual model of plant survival. New 43, 755–770. https://doi.org/10.1007/s11056-012-9328-6.

Von Moler, E.R., Nelson, A.S., 2021. Perspectives on drought preconditioning treatments with a case study using Western larch. Front Plant Sci. 12, 741027. https://doi.org/10.3389/fpls.2021.741027.

Wright, I.J., Reich, P.B., Westoby, M., 2001. Strategy shifts in leaf physiology, structure and nutrient content between species of high-and low-rainfall and high-and low-nutrient habitats. Funct. Ecol. 15, 423–434.

Yordanov, I., Velikova, V., Tsonev, T., 2000. Plant responses to drought, acclimation, and stress tolerance. Photosynthetica 38, 171–186. https://doi.org/10.1023/A:1007201411474.

Zhang, H., McDowell, N.G., Li, X., Huo, J., Li, Y., Wang, Z., 2023. Hydraulic safety and growth rather than climate of origin influence survival in desert shrubs and trees. Ecol. Manag. 543, 121130. https://doi.org/10.1016/j.foreco.2023.121130.