

CHAPTER 5

Quality matters. A quantitative review on the effect of seedling morphology and nursery practices on the outplanting performance of forest plantings

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Introduction: Background, challenges, and objectives

Most forest plantings worldwide are performed through the establishment of seedlings grown in nurseries. During nursery cultivation, seedlings are exposed to several environmental conditions, such as temperature and growing media pH, and are supplied with resources such as water, light, and mineral nutrients for optimal growth (Duryea,

^a Retired.

1984; Landis et al., 1989). Plants acclimate to these varied nursery conditions by adjusting morphological and physiological characteristics, leading to distinct phenotypes with specific morphological and physiological traits. Once outplanted, these morphological and physiological traits, referred to as functional attributes, determine seedling water, carbon, and mineral nutrient economy, as well as their resistance and resilience to stress, and consequent ability to establish and grow (Cuesta et al., 2010b; Grossnickle, 2012; Grossnickle and MacDonald, 2018a, 2018b; Villar-Salvador et al., 2012). For instance, leaf nitrogen concentration is often directly related to photosynthesis rate, which in turn drives new organ growth and carbon storage (Uscola et al., 2015; Villar-Salvador et al., 2015). Similarly, large 1 + 0 (i.e., 1-year-old), high nutrient content *Pinus canariensis* seedlings showed 35% higher survival and twofold growth on a semiarid field site compared to small 1 + 0 seedlings with little nutrient content (Luis et al., 2009). The main cultivation practices that made the difference between the *P. canariensis* stocktypes were the fertilization rate and the type of growing medium. In summary, seedling growers have tools to shape key functional attributes that drive seedling survival and growth in forest plantings.

The ecological characteristics of each species can constrain the extent to which nursery professionals can tailor these attributes through cultivation and subsequently influence seedling field establishment (Andivia et al., 2021; Toca et al., 2018, 2019). For instance, 1 + 0 seedlings of *Quercus ilex* cultivated in nurseries located on mild-winter sites are more prone to frost damage when planted on cold-winter sites than plant batches cultivated in cold-winter nurseries, despite originating from acorns sourced from the same population and cultivated with the same containers and fertilization regime (Mollá et al., 2006). Outplanting practices, such as soil preparation, planting date, and removal of competing vegetation, determine the availability of resources at the planting microsite level, thus potentially influencing the performance of plant batches with differing functional attributes. An illustrative example is that large 1 + 0 *Q. ilex* seedlings survived better than small seedlings when both are outplanted at the end of the planting window, whereas both stocktypes survived the same when outplanted at the beginning of the window (Palacios et al., 2009), which is the typically recommended planting period for many sites of the Mediterranean basin.

Seedlings that perform “satisfactorily” on a forest site are considered high quality because their functional attributes maximize establishment in that particular environment. The “target plant concept” (TPC) emphasizes matching seedling functional attributes and nursery cultivation to the planting environment. In this conceptual framework of “good” forestation practices, the selection of species and provenances, reproductive material, and outplanting practices align with the objectives and environmental conditions of forest restoration (Dumroese et al., 2016; Landis, 2011). Consequently, defining suitable seedling functional attributes or phenotypes for different planting scenarios is an important challenge of the TPC (Montagnoli et al., 2018; Stanturf et al., 2014).

On a global scale, a high diversity of woody plant species is used in forest plantings (Cagnoni *et al.*, 2023). In some regions, the number of species grown in nurseries for forestry purposes is small, partly due to the specific objectives of forestation projects and because of the low regional diversity of woody plants. For instance, tree seedling production and planted areas in the boreal regions of Europe and North America are huge, but the variety of cultivated species is, in general, small (Clark *et al.*, 2023). Conversely, in other regions, such as many tropical and subtropical areas or in the Mediterranean basin, the variety of woody plant species used in forest plantings is comparatively higher, particularly when the main objective is biodiversity recovery (da Cruz *et al.*, 2020; Elliott *et al.*, 2023; Turrión *et al.*, 2021). This high plant diversity, also often associated with a diverse array of ecological field conditions, poses notable challenges to implementing the TPC.

In the last 50 years, a vast body of scientific and technical literature has been published on how nursery practices affect the field performance of outplanted seedlings. This knowledge should be part of the foundation upon which the TPC should rest. However, the application of the TPC is often hampered by two knowledge limitations. The first one is the lack of consensus regarding the functional attributes that planted seedlings should have to optimize their establishment under different outplanting scenarios (South *et al.*, 2016). The second limitation is the lack of information regarding how species ecology drives the optimal attributes that maximize outplanting performance (Del Campo *et al.*, 2022; Toca *et al.*, 2018, 2019; van den Driessche, 1991a, 1992), as well as cultivation practices (Climent *et al.*, 2011; Mariotti *et al.*, 2020, 2015a; Puértolas *et al.*, 2009; Tsakalidimi *et al.*, 2009). For instance, are the desired functional attributes for an evergreen broadleaf tree the same as those for an evergreen shrub on a high weed competition site? Will an increase in container volume affect the survival of a gymnosperm in the same way as an angiosperm species on dry planting sites?

Several reviews have examined the impacts of cultivation practices on the establishment of forests, variations among stocktypes, and the relevance of seedling attributes (Davis and Jacobs, 2005; Grossnickle, 2000; Grossnickle and El-Kassaby, 2016; Oliet *et al.*, 2013; South *et al.*, 2016; South and Nadel, 2020; Wilson and Jacobs, 2006). Many of these reviews have focused on specific geographical regions, with fewer having a global perspective (Andivia *et al.*, 2021; Grossnickle and El-Kassaby, 2016; Haase *et al.*, 2021a, 2021b; Mariotti *et al.*, 2023). Furthermore, most are systematic reviews, precluding the establishment of quantitative generalizations, which is essential for developing a sound basis on plant quality theory (Andivia *et al.*, 2019). An urgent challenge for the forestry sector is to identify general patterns in how nursery practices and seedling functional attributes affect outplanting performance, especially in the context of climate change. Notably, this includes understanding the interaction of functional attributes with species ecology, and planting site conditions and practices such as soil preparation and irrigation.

A starting point to achieve these goals is performing global, quantitative, meta-analytical reviews of the vast published literature on forestation (for instance, [Andivia et al., 2021](#); [Mariotti et al., 2023](#)). An important difficulty of conducting such reviews is to handle the high diversity of plant species used in forestation worldwide. One approach is to simplify such taxonomic diversity by grouping species into functional groups based on their ecophysiology. For instance, angiosperms and gymnosperms show notable physiological differences in water use and growth, mediated by strong differences in their xylem and leaf properties ([Choat et al., 2012](#); [Niinemets and Valladares, 2006](#); [O'Brien et al., 2017](#)). These differences may influence their response to nursery practices and outplanting performance.

Functional groups can also be based on easily measurable functional attributes (soft traits), which summarize main ecophysiological capacities of plant species. The specific leaf area (SLA), i.e., the ratio between the leaf area and its mass, is a key attribute of the plant economic spectrum, a framework where plant species can be aligned along a gradient of growth, resource acquisition and processing, and stress resistance ([Reich, 2014](#)). Thus, the reduction of SLA across species is typically related to slower growth and gas-exchange capacity but higher stress resistance ([Bartlett et al., 2012](#); [Greenwood et al., 2017](#)). SLA has been used, for instance, to select tropical species for mine restoration plantings ([Gastauer et al., 2020](#); [Toledo-Aceves et al., 2022](#)). In addition, differences in SLA are linked to the leaf habit of woody species, with low to mid-SLA values related to the evergreen habit, while high SLA values are linked to the deciduous habit. SLA and other soft traits, such as wood density ([Andivia et al., 2021](#); [Charles et al., 2018](#); [Werden et al., 2018](#)), can also be a valid framework for classifying similar functional species for quantitative reviews on forest practices and giving informed recommendations.

The main objective of this chapter is to conduct a quantitative review of the effects of morphological and functional attributes, as well as several cultivation practices, on the early establishment of forests worldwide. We used global analysis to provide useful, informed recommendations on a local scale. To achieve our goals, we have compiled several databases by extracting quantitative information from papers published in scientific journals, gray literature, and, in some cases, unpublished data. Several of these databases were developed within the framework of the COST Action PEN-CAFoRR (CA19128), while others were developed independently by some of the authors involved in this chapter. In addition to this introductory section, the chapter is organized into six further sections. The second one focuses on the relation of seedling morphological attributes (seedling size and shoot-to-root ratio) with outplanting survival. The third section analyzes factors that account for differences in survival between container and bareroot (BR) stocktypes. The remaining sections quantitatively explore the effects of different nursery practices on seedling outplanting performance. Thus, the fourth section analyzes the effects of water stress hardening and photoperiod reduction (blackout) on seedling survival and growth. The fifth section addresses the influence of container

properties on the survival of oak and pine seedlings. The sixth section examines the impact of innovative alternative growing media on the survival of forest species compared to peat-based cultivation. Finally, the last section aims to provide informed recommendations based on the results of the main outcomes of the previous sections. Each section provides a brief overview of the current state of the art and the main challenges of the topic. We briefly inform on the methodology used to obtain and analyze the data, present the main findings, and discuss their implications for nursery cultivation and forest establishment.

Relation between seedling morphological attributes and outplanting survival

Introduction and challenges

Quantitative and qualitative morphological traits are commonly used for assessing seedling quality and removing plants with low potential for outplanting performance (Grossnickle, 2012; Mataruga et al., 2023; Mexal and Landis, 1990; Ritchie and Landis, 2010). Among these traits, shoot height and root collar diameter (proxies of “seedling size”) are the most common quantitative attributes due to their ease of measurement and suitability for screening a large number of seedlings. Many countries have established standards for these traits that seedlings of important tree species must meet to be considered acceptable for use in forest plantings (Mataruga et al., 2023).

Seedling height and root collar diameter are positively correlated with leaf area and root mass, which are involved in photosynthesis and in water and nutrient uptake, respectively. These physiological processes drive new organ growth and nutrient and carbon storage building, crucial for seedling establishment and stress recovery (Cuesta et al., 2010b; Grossnickle, 2012; Grossnickle and MacDonald, 2018a, 2018b; Villar-Salvador et al., 2015). Thus using larger seedlings can potentially enhance survival mediated by increased seedling establishment capacity (Grossnickle, 2005; Villar-Salvador et al., 2012). However, larger seedling size can also increase transpiration, making seedlings more vulnerable to drought stress (Grossnickle, 2012; Oliet et al., 2019) and potentially reducing seedling survival, especially on dry sites (Trubat et al., 2011).

Practitioners and foresters often disagree on the optimal seedling size (South et al., 2016). This lack of consensus extends to the scientific community, as studies comparing plants of the same age within a species have yielded different results. While many studies have reported that large seedlings show higher outplanting survival than small seedlings (Cuesta et al., 2010b; Kabrick et al., 2015; Mason et al., 1996; Pinto et al., 2012; South et al., 2005; Tsakaldimi et al. 2013; Villar-Salvador et al., 2008, 2013b, 2012), others reported the opposite trend, particularly on harsh sites (Rose et al., 1993; Trubat et al., 2011; Tuttle et al., 1988).

The balance between the size of the shoot and the root of planted seedlings is another morphological attribute that has received much attention in the forestry sector. In scientific and technical literature, the balance between both seedling organs is calculated as the shoot-to-root mass ratio (S/R) (sometimes it is calculated as the root-to-shoot mass ratio). The widespread consensus is that seedlings with a disproportionately large shoot relative to the root system should be avoided in plantings. Some countries, such as those in the European Union, have ruled against the use of strongly unbalanced plants, although these regulations do not provide clear criteria for defining such imbalances, particularly considering species differences (Mataruga et al., 2023). Strong shoot-to-root imbalance increases transpiration relative to water uptake capacity, potentially making seedlings more vulnerable to stress during outplanting and establishment, particularly on dry sites or locations where soil retains low moisture. The relationship between seedling S/R and outplanting survival within species is frequently either neutral or, if significant, varies in direction among studies (Bayley and Kietzka, 1997; Bernier et al., 1995; Landhäusser et al., 2012b; Mexal and Landis, 1990; Navarro et al., 2006; South, 2000; South et al., 2005; Thompson, 1985; Tuttle et al., 1988; van den Driessche, 1992; Villar-Salvador et al., 2004a).

The contradictory results regarding the relationship of seedling size and S/R at outplanting with subsequent survival may stem from inherent differences in species' functional characteristics (Gardiner et al., 2019). A recent meta-analysis by Andivia et al. (2021) involving 86 species concluded that planting larger seedlings within the same age range increases outplanting survival in forest plantings worldwide. This relationship was independent of phylogeny, and the pattern is consistent for both angiosperm and gymnosperm species across diverse aridity conditions. The effect of seedling morphology on outplanting performance, however, might also vary depending on the environmental conditions at the planting sites (Charles et al., 2018), planting practices, or the stocktype (Cuesta et al., 2010a, 2010b; Palacios et al., 2009; Pinto et al., 2012). For instance, BR seedlings often show greater vulnerability to stressful planting conditions than container seedlings (Grossnickle and El-Kassaby, 2016). Similarly, seedlings of different ages can have different outplanting performance, especially when prolonging container seedling cultivation beyond the standard duration for a given species (Harayama et al., 2023; Siles et al., 2010). Therefore stocktype selection may influence how variations in plant morphology affect outplanting survival. In addition, differences in planting site quality, such as those between croplands and woodlands, and soil preparation intensity (del Campo et al., 2007; Löf et al., 2012; South et al., 2023; Villar-Salvador et al., 2013b), both of which can alter resource availability and competition of planted seedlings, may also modulate the effect of seedling morphology on outplanting performance. However, to our knowledge, these features have not been addressed, and as far as we know, no quantitative review of the relationship between seedling S/R and field survival has been conducted.

This section aims to assess, on a global scale, how different field environmental factors and practices influence the direction (i.e., positive, negative, or neutral) and magnitude of the effect of seedling size and the S/R ratio at planting on field survival. Specifically, we examined how aridity interacts with the type of field site (cropland vs. woodland) and soil preparation intensity to affect the relationships between seedling morphology and field survival. We also assessed whether species' functional ecology, as indicated by SLA, modulated the relationship between seedling morphology and outplanting survival. Understanding the interactions between seedling morphology, species functional strategies, environmental conditions, and planting practices can provide valuable guidelines for designing plantings and cultivation methods to match forestation objectives across a range of conditions and species.

To address these questions, we used a partially published database (Andivia et al., 2021) to conduct a quantitative review of the literature using a meta-analytic approach (see Box 5.1 for statistical explanation) to evaluate the relationship of seedling size and S/R with outplanting survival. The published database for seedling size comprised 324 case studies encompassing 86 species across 142 locations worldwide. The unpublished database for S/R included 130 cases, encompassing 51 species in 76 locations. The meta-analytic metric of effect size used in both databases was Fisher's z -transformation, calculated using the correlation coefficient between survival and seedling size as well as seedling S/R, for each case study. Positive values of the effect size (positive values of z) indicated that survival was positively correlated with seedling size or S/R. Negative values indicated the opposite, while values near zero indicated no significant correlation between survival and plant morphology traits. The value of the effect size quantifies the strength of the correlation, either positive or negative. For each calculated effect size, we also report the 95% confidence interval (CI). Detailed methodological information regarding data extraction from published literature, computation of the effect size metric, and other general methodological aspects can be consulted in Andivia et al. (2021) and Box 5.1.

We used the aridity index, the ratio between annual precipitation and the potential evapotranspiration (Middleton and Thomas, 1997), to distinguish between dry (aridity index ≤ 0.5) and humid climates (aridity index > 0.5) and analyzed if the aridity of planting sites affected the relationship between survival and seedling morphology. While almost all gymnosperms in our database were conifers, some, such as cycads, *Ginkgo biloba*, and species of the genus *Ephedra*—which are used in forest restoration in some regions—are not. Similarly, while most of our angiosperms were broadleaves, not all have flat, wide leaves, and *sensu stricto* cannot be classified as broadleaf species. Therefore, we preferred to use the terms “gymnosperms” and “angiosperms” in this and other sections of the chapter to provide a broader and more precise functional distinction between these groups of plants.

BOX 5.1 A guide to the statistics used in this chapter: A primer for unfamiliar readers

Part of the quantitative review in this chapter uses meta-analytic approaches. Meta-analysis is an informative and unbiased statistical tool for quantitatively summarizing evidence on any research question (Koricheva et al. 2013; Andivia et al., 2019). It combines data from independent scientific and technical studies to identify general patterns and evaluate factors modulating result heterogeneity. Meta-analysis relies on extracting data and on the magnitude and direction of the studied phenomenon (e.g., the correlation between two variables) for each case study. Because different variables can measure the same phenomenon across studies (e.g., seedling growth via shoot weight, length, or diameter), outcomes are standardized on a common scale, known as effect sizes (Rosenberg et al., 2013).

This chapter uses two different effect sizes. Section 2 applies Fisher's z-transformation, based on the correlation coefficient (r) between survival and seedling size or S/R. Section 4 uses response ratios, calculated as the survival or growth ratio of hardened seedlings relative to controls (Fig. B5.1). The contribution of each case study to the overall effect size is weighted by its precision estimate (e.g., variance, standard error, or CI) and calculated alongside a CI. In other words, the contribution of each case study to the overall effect size depends on its number of replicates and variance. We can also calculate data heterogeneity (I^2 , %), which quantifies the variability in the effect size across case studies. Its magnitude reflects the potential contribution of other factors, such as aridity, planting practices, or species functional ecology, on such variability.

We can determine whether the overall effect differs significantly from zero by assessing if the CI does not overlap 0 or if it does it only marginally. In addition, we can test whether any covariate explains heterogeneity among studies (Andivia et al., 2019). The CI represents the range within which the true population parameter (e.g., the z-value) is expected to fall with 95% certainty. A wide CI indicates high uncertainty, often due to substantial variation across studies or a small sample size. For instance, if the CI of the z-value in gymnosperms in Section 2 overlaps zero but not in angiosperms, this means that seedling size affects survival only in angiosperms.

Another statistic used in Sections 3 and 6 is the chi-square (χ^2) test. In Section 3, it was applied to assess whether observed survival outcome frequencies differ between container and BR stock from the expected frequencies under the assumption of no differences between stocktypes. To illustrate with a more friendly example, consider a chocolate maker investigating whether bittersweet chocolate preference is linked to age (children-teenagers vs. adults). A blind test is conducted with 100 randomly selected individuals from each group. If age has no influence on chocolate preference, bittersweet chocolate preference should be around 50% within each group (the expected frequency). However, if young people prefer sweeter options while adults choose bittersweet chocolate, the observed frequencies will strongly deviate from expected frequency (difference between white and blue bars in the figure below) (Fig. B5.2). If the deviation exceeds a critical threshold—determined by sample size and deviation magnitude—we conclude that age significantly affects chocolate preference.

Box 5.1 A guide to the statistics used in this chapter: A primer for unfamiliar readers—cont'd

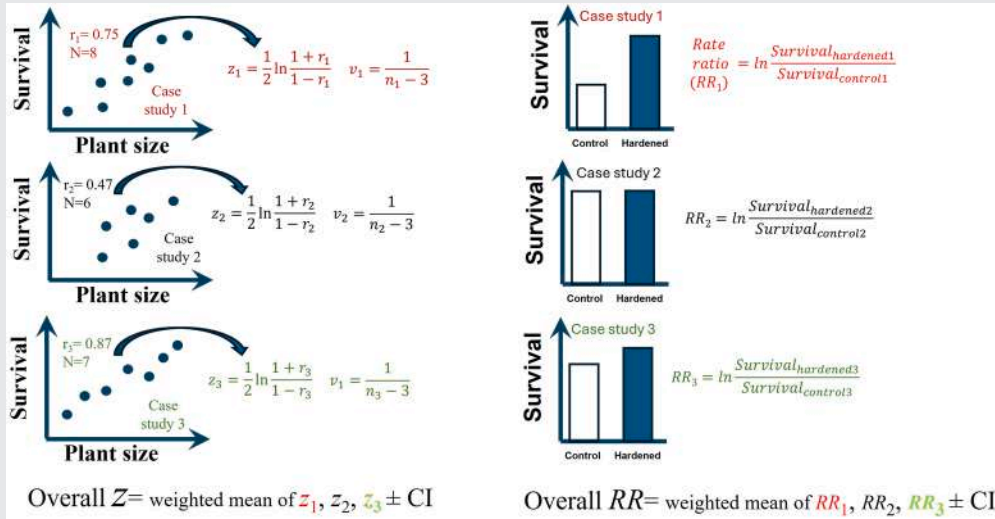


Figure B5.1 Summary of the calculations of the two effect sizes used in this chapter. The example on the left is the calculation of Fisher’s z-transformation from the correlation coefficient along the effect size variance (v), while the example on the right is a response ratio—the rate ratio—(RR) used in the hardening section. The examples assume there are only three study cases.

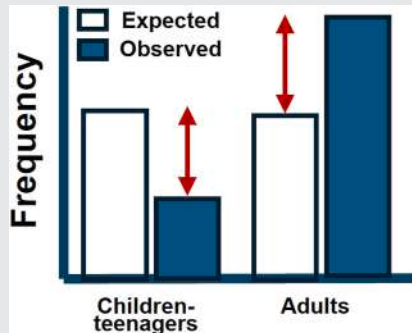


Figure B5.2 Illustrative example of a chi-square test of the relationship between age and preference for bittersweet chocolate. Expected frequencies (assuming there is no difference between populations) are compared with observed frequencies across age groups (children-teenagers vs. adults). The magnitude of the deviation between both frequencies (red arrows) determines significance.

Factors modulating the relationships between seedling morphology and field survival

Seedling size at outplanting was positively and significantly related to field survival (estimated effect size $z = 0.69$, $P < 0.001$, which corresponded to an average estimated $r = 0.60$) (Andivia et al., 2021). In contrast, seedling S/R at planting was not related to field survival (estimated effect size $z = 0.061$, CI = -0.110 to 0.233, $P = 0.48$, which corresponded to an average estimated $r = 0.061$; $n = 130$). Seedling size and seedling S/R data had high heterogeneity ($I^2 = 91\%$, CI = 88–94% for seedling size relationships and $I^2 = 58\%$, CI = 41–73% for seedling S/R relationships) (see Box 5.1 for the meaning of I^2).

The relationship between seedling size and survival was unaffected by climate aridity, with no significant difference between dry (aridity index ≤ 0.5) and humid climates (aridity index > 0.5) as the CI of the z -value for the dry and humid climates overlapped (Fig. 5.1, upper row). In contrast, relationships between seedling S/R and survival were slightly but significantly more positive on dry planting sites than on humid sites, although in both cases the CI of the effect size overlapped zero, especially in humid climates. This means that in both dry and humid climates, S/R showed no significant relationship with survival. These results consequently do not support the idea that increased seedling size and S/R enhance the vulnerability of planted seedlings to drought stress (Grossnickle, 2012; Landhäusser et al., 2012b; Thompson, 1985; Trubat et al., 2008) and suggest that in most study cases, seedlings were not morphologically imbalanced to reduce their survival. Some factors can, however, affect this general pattern, as we will address later.

The increased survival with seedling size was likely attributable to the higher establishment and competitive capacity of large seedlings (Cuesta et al., 2010b, 2010c; Grossnickle, 2012; Lamhamedi et al., 1998). Establishment is often linked to rapid and high root and shoot growth, for which plants require substantial resources derived from current photosynthesis and storage (Burdett, 1990; Landhäusser et al., 2012a; Villar-Salvador et al., 2015, 2012). Compared to small seedlings, large seedlings display greater photosynthetic area and store more mineral nutrients and carbohydrates. The main argument against using large seedlings or plants with high S/R is that when exposed to stress conditions, such as dry soil at planting or no rain for many weeks after planting, they are more vulnerable to water stress. In other words, the negative effects of having high transpiration exceed the benefits of high resource mobilization, photosynthesis, and rapid and abundant early root and shoot growth, processes that are the basis for seedling establishment. However, our findings that seedling morphology–survival relationships were not influenced by aridity suggest that this issue is not a common phenomenon.

Water shortage in many dry climates, such as in the Mediterranean or dry tropical biomes, is usually restricted to specific periods of the year, whose length varies among

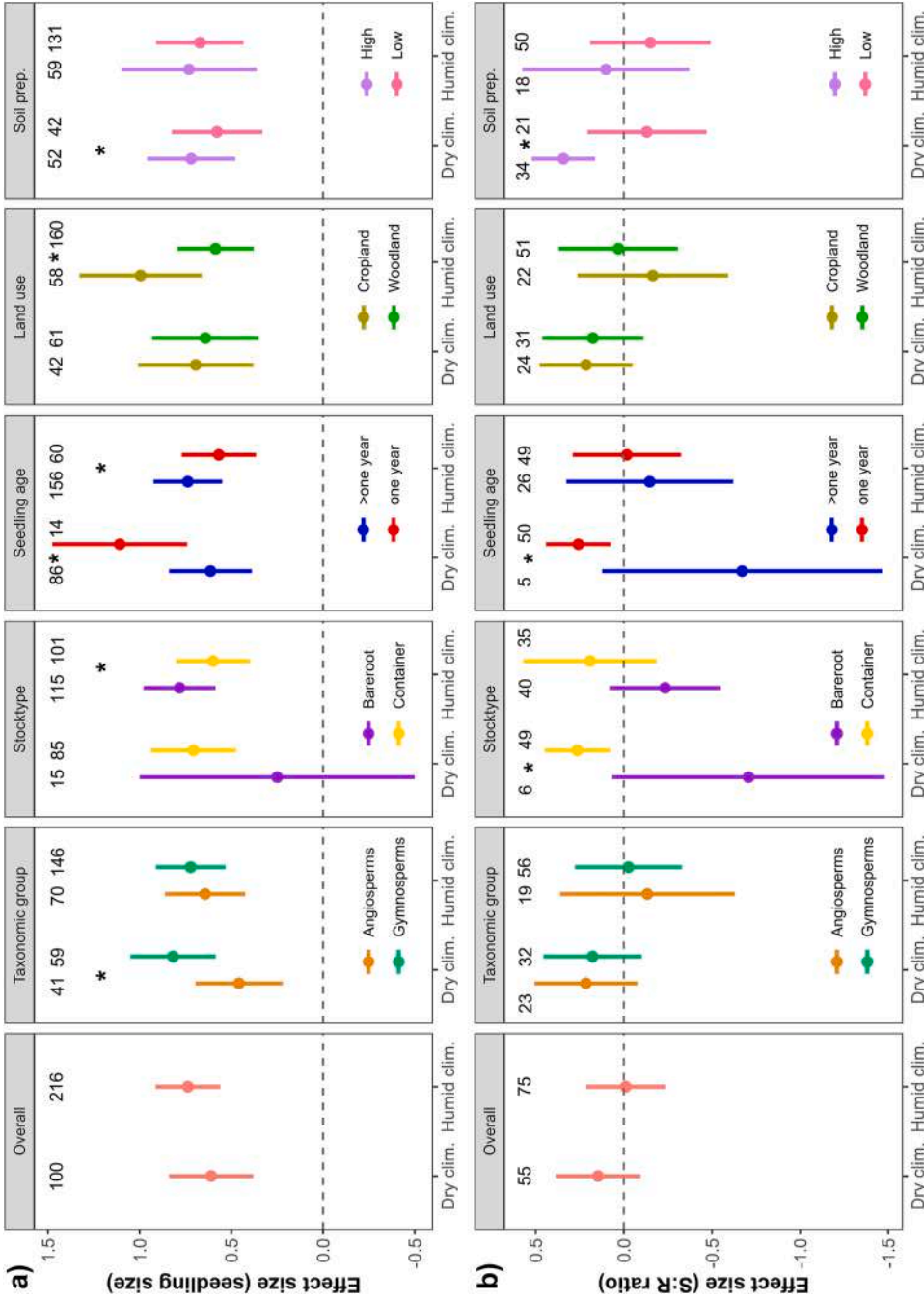


Figure 5.1 Predicted effect size ($z \pm 95\%$ confidence interval) for the relationship between seedling size (upper row) and the shoot-to-root mass ratio (S/R) (lower row) and their outplanting survival in relation to the aridity of planting site, and the interaction of aridity with taxonomic group (gymnosperms vs. angiosperms), stocktype (bareroot vs. container), plant age (1-year and older than 1-year-old), planting environment (woodland vs. cropland), and site preparation intensity (low vs. high). Asterisks indicate statistically significant ($P < 0.05$) differences between treatments. The number of case studies for each combination and the potential evapotranspiration (Middleton and Thomas, 1997), was ≤ 0.5 and the aridity index, the ratio between annual precipitation and the potential evapotranspiration (Middleton and Thomas, 1997), was ≤ 0.5 and humid if the aridity index was > 0.5 . Intense soil preparation included grouped mounding, subsoiling, and mechanical holes, while low-intensity soil preparation included no preparation and handmade holes. Positive effect size values indicate that the overall relationship of seedling size or S/R with survival is positive, while negative values indicate the reverse trend.

regions, but the rest of the year is usually wet (Bhaskar and Ackerly, 2006). In dry climates, seedling outplanting is conducted during the wet season to avoid drought stress (Mediavilla and Escudero, 2004; Tognetti et al., 2000). During the wet season, planted seedlings undertake a race against time to root and establish before the onset of the dry season (Cuesta et al., 2010a). In this context, having a large and deep root system at the end of the wet season is key for avoiding drought stress during the subsequent dry season (Cuesta et al., 2010b; Villar-Salvador et al., 2012). Seedlings can achieve this if outplanted early in the wet season, but also by using seedlings with high photosynthesis and resource mobilization capacity, such as large seedlings, to support high, fast growth during the wet season (Cuesta et al., 2010b). In addition, most woody species native to dry locations show drought-resistant traits (Anderegg et al., 2016; Bartlett et al., 2012), which can mitigate outplanting stress and might also explain the positive effect of seedling size on survival and the lack of significant seedling S/R–field survival relationships in dry-climate sites.

The relationship between seedling size and survival occurred across phylogenetic groups (Andivia et al. 2021), pointing to the same general trend across woody plants. In dry-climate locations, the relationship between seedling size and outplanting survival was, however, stronger in gymnosperms than in angiosperms (Fig. 5.1). Compared to angiosperms, gymnosperms have a lower transpiration rate for individuals of equivalent size (Choat et al. 2012; Niinemets and Valladares 2006; O'Brien et al. 2017), which can reduce the stress experienced by large gymnosperm seedlings during establishment compared to large angiosperm seedlings. Conversely, angiosperms and gymnosperms showed similar magnitude for the relationship between seedling S/R and survival, independent of aridity (Fig. 5.1).

If seedling survival depends on the balance of opposite ecophysiological processes mediated by plant size (namely high growth and resource mobilization that boost establishment *versus* high transpiration and low water-use efficiency that increase vulnerability to drought) then nursery or planting practices, plant functional traits, or environmental conditions that reduce the negative effects of high transpiration should enhance the establishment of large seedlings compared to small ones. In this framework, we expected that positive relationships of seedling size and S/R with outplanting survival would be stronger in container plants than in BR plants, with high soil preparation intensity, and on sites with deeper and more fertile soils. Consistent with this idea, stocktype (BR vs. container) had a marked effect on the relationship of seedling size and especially of S/R with seedling survival. Interestingly, these differences between stocktypes depended on planting site aridity (Fig. 5.1). In humid climates, seedling size was positively related to survival in both stocktypes, with the relationship being slightly but significantly more positive for BR plants. In contrast, in dry climates, while the seedling size–survival relationship was clearly positive for container plants, the relationship was neutral for BR stock.

Notably, effect sizes for BR stock in dry climates showed a high variation across studies as indicated by the wide variation in the 95% CI. This means that while the general pattern of increased survival with seedling size in dry climates was strongly and unambiguously supported for container plants, it was not supported for BR plants, which showed a strong variation in the directions of this relationship. The lack of a plug that buffers against short-term plant desiccation and protects roots from mechanical damage (Grossnickle and El-Kassaby, 2016; Landis et al., 1990; McNabb and Pike, 2019; Preisig et al., 1979) was likely the main explanation for the result of BR stock in a dry climate. The higher vulnerability of BR plants in a dry climate is also corroborated by the results on the relationship between seedling S/R and survival. In general, for container plants, this relationship was significantly more positive than for BR plants, which showed negative effect size values, especially in dry-climate sites (although CI intervals slightly overlap zero). These results have practical implications for nursery production protocols, as they suggest that target seedling size and S/R specifications must be smaller for BR stock, especially if planted in dry sites, while for container stock, target sizes, and S/R values may be increased to maximize survival independent of climate conditions.

In most woody species, seedlings are ready to be outplanted at the end of the first cultivation season as they have reached a suitable size and, in container stock, the integrity of the plug is not compromised. However, sometimes not all nursery stock can be used in a campaign, and part of it remains in the nursery for a second growing season. In these cases, older seedlings of the same species tend to be larger, and container stock usually has higher S/R than younger seedlings (Iverson, 1984) whenever cultivation practices do not change significantly. Therefore, it can be expected that the morphological characteristics of 2-year-old seedlings and likely other practices linked to longer cultivation may make them more vulnerable to outplanting conditions, resulting in less positive or even negative relationships of survival with plant size and S/R. Our results partially support this idea, but the modulating effect of seedling age on these relationships seems to depend on aridity. On humid sites, the seedling size-survival relationships were always positive with no significant difference among ages. However, in dry climates, 1-year-old seedlings showed significantly more positive size-survival relationships than seedlings older than one year. These latter results must be taken with caution due to the low number of cases of seedlings older than one year in our database. Seedling age did not significantly affect the survival-S/R relationships in humid locations. On the contrary, in dry climates, 1-year-old seedlings showed positive relationships, while seedlings older than one year showed negative relationships. These results suggest that a strong imbalance due to high S/R values can be detrimental for older seedlings in dry climates but not in humid ones.

Many new forest plantings worldwide have been performed on abandoned cropland (Gutiérrez Rodríguez et al., 2016; Zheng et al., 2023). These afforestation areas often

show higher fertility and more favorable soil physical properties that can promote seedling growth compared to woodland soils (Brudvig et al., 2013; Flinn and Vellend, 2005). Despite these differences, the relationship between seedlings' size and survival differed little between the two land uses. In humid climates, a more positive relationship was found in croplands compared to woodlands (Fig. 5.1), while in arid climates, previous land use did not affect the strength or the direction of the relationships between survival and seedling size or S/R.

Site and soil preparation affects seedling establishment by reducing competition and soil compaction and increasing water infiltration and nutrient availability (Löff et al., 2012; Örländer et al., 1996; Querejeta et al., 2000, 2001), which can enhance seedling rooting and outplanting performance (Barberá et al., 2005; Bocio et al., 2004; Fleming et al., 1996; Harayama et al., 2023). Consistent with these benefits on the soil environment, the positive effects of seedling size and S/R on survival were significantly greater in plantings with high-intensity soil preparation (mounding, subsoiling, and mechanical holes) than on sites with low-intensity soil preparation (no preparation or manual holes). These differences, however, were only detected in dry climates (Fig. 5.1), especially for the S/R-survival relationships, highlighting the importance of intense soil preparation on sites where drought limits the success of forest plantings. In dry climates, intense soil preparation strongly increases soil water availability, especially in deep layers (Querejeta et al., 2000, 2001) and rooting depth (Dea et al. 2001; Dassot and Collet 2021). This likely facilitates the establishment of large, high S/R seedlings (Palacios et al., 2009; South et al., 2001) by mitigating the negative effects of their higher water consumption.

We found evidence that species' functional characteristics drive the effect of morphology on seedling outplanting performance. SLA modulated seedling size-survival relationships. For angiosperms planted in dry climates, the relationship between seedling size and survival increased with SLA reduction (Fig. 5.2a). Particularly, the seedling size-survival relationship was significantly positive for SLA values $< 80 \text{ cm}^2 \text{ g}^{-1}$, while it was not significantly different from zero for species with SLA values $> 80 \text{ cm}^2 \text{ g}^{-1}$. In our database, low SLA values mainly correspond with evergreen sclerophyllous trees and shrubs, while SLA values $> 80 \text{ cm}^2 \text{ g}^{-1}$ comprised mostly deciduous woody species. Across species, reduced resistance to drought is usually associated with high SLA (Anderegg et al., 2016; Greenwood et al., 2017) and high transpiration rate, which may increase vulnerability to transplant shock. In addition, deciduous species leaf out earlier than evergreen species (species with mid to low SLA values; Abramoff and Finzi, 2015), and shoot growth often inhibits early new root growth due to competition for stored resources (Riedacker, 1976; Reich et al., 1980). All these factors can increase transplant shock vulnerability of high SLA species in dry climates, a process that can be exacerbated in large seedlings.

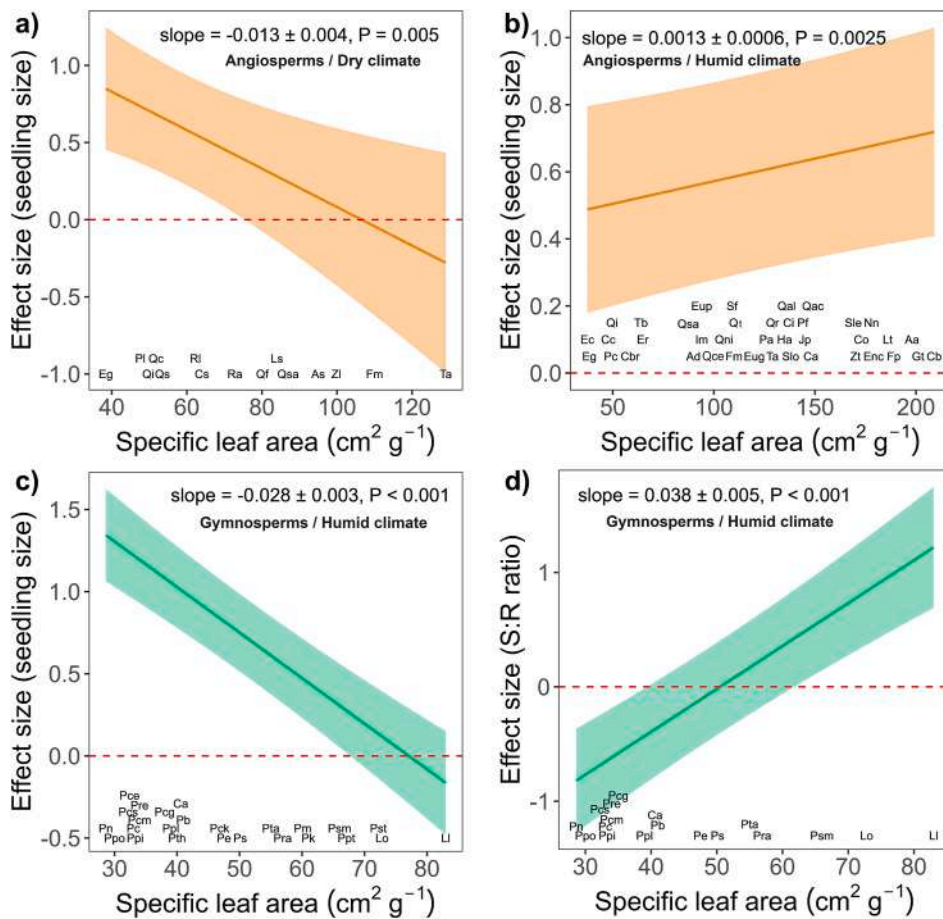


Figure 5.2 Regressions between the predicted effect size ($z \pm 95\%$ confidence interval) of the relationships of seedling field survival with seedling size (a–c) and S/R (d) at planting and the specific leaf area in angiosperms (upper rows) and gymnosperms (lower rows). Panel (a) shows results for case studies in dry climates (aridity index < 0.5), and panels (b–d) show results for case studies in humid climates (aridity index ≥ 0.5). The dashed line at zero indicates no significant effect of seedling size or S/R and outplanting survival. Labels inside the figure represent the initials of species used in the analyses, with their position corresponding to their SLA value. Angiosperms: Aa = *Ailanthus altissima*; Ad = *Acacia dealbata*; As = *Acacia salicina*; Ca = *Cordia alliodora*; Cb = *Catalpa bignonioides*; Cc = *Corymbia citriodora*; Ci = *Carya illinoensis*; Co = *Cedrela odorata*; Cs = *Ceratonia siliqua*; Cbr = *Calophyllum brasiliense*; Ec = *Eucalyptus camaldulensis*; Eg = *Eucalyptus globulus*; Er = *Eucalyptus regnans*; Enc = *Enterolobium contortisiliquum*; Eug = *Eucalyptus urograndis*; Eup = *Eucalyptus urophylla*; Fm = *Fraxinus mandshurica*; Fp = *Fraxinus pennsylvanica*; Gt = *Gleditsia triacanthos*; Ha = *Hieronyma alchorneoides*; Im = *Inga marginata*; Jp = *Jacaranda puberula*; Lt = *Liriodendron tulipifera*; Ls = *Lavandula stoechas*; Nn = *Nothofagus alpina*; Pa = *Platanus acerifolia*; Pc = *Prosopis chilensis*; Pf = *Prosopis flexuosa*; Pl = *Pistacia lentiscus*; Qac = *Quercus acutissima*; Qal = *Quercus alba*; Qc = *Quercus coccifera*; Qce = *Quercus cerris*; Qf = *Quercus faginea*; Qi = *Quercus ilex*; Qni = *Quercus nigra*; Qr = *Quercus rubra*; Qt = *Quercus texana*; Qs = *Quercus suber*; Qsa = *Quillaja saponaria*; Ra = *Rhamnus alaternus*; Rl = *Rhamnus lycioides*; Sf = *Shorea faguettiana*; Slo = *Shorea johorensis*; Sle = *Shorea leprosula*; Tb = *Terminalia bellirica*; Ta = *Tilia amurensis*; Zt = *Zeyheria tuberculosa*; Zl = *Ziziphus lotus*. Gymnosperms: Ca = *Cedrus atlantica*; Ll = *Larix laricina*; Lo = *Larix gmelinii*; Pb = *Pinus banksiana*; Pc = *Pinus canariensis*; Pe = *Pinus elliotii*; Pk = *Pinus koraiensis*; Pm = *Pinus massoniana*; Pn = *Pinus nigra*; Pce = *Picea engelmannii*; Pcg = *Picea glauca*; Pck = *Picea koraiensis*; Pcm = *Picea mariana*; Pcs = *Picea sitchensis*; Ppi = *Pinus pinea*; Ppl = *Pinus palustris*; Ppo = *Pinus ponderosa*; Ppt = *Pinus patula*; Pra = *Pinus radiata*; Pre = *Pinus resinosa*; Ps = *Pinus sylvestris*; Psm = *Pseudotsuga menziesii*; Pst = *Pinus strobus*; Pta = *Pinus taeda*; Pth = *Pinus thunbergii*.

Notably, on wet sites, the positive effect of seedling size on the survival of angiosperms was maximized by high SLA (i.e., contrary to dry sites; Fig. 5.2). Plant species with high SLA show rapid growth and high resource uptake and processing capacity, but low abiotic stress resistance (Reich, 2014; Reich et al., 1997). On wet sites, competition with natural vegetation is a key limitation for seedling establishment (Grossnickle, 2012, 2000). In competitive scenarios, the advantage of large seedlings is more pronounced in fast-growing, low stress-resistant species that can outcompete site vegetation by taking up resources more effectively than small seedlings (Cuesta et al., 2010b; Jobidon et al., 2003).

Andivia et al. (2021) showed that wood density variation across species is a trait that can also modulate the relationships between seedling size and field survival. Specifically, these authors reported that for angiosperms in wet climates, the positive effect of seedling size on their survival increased in species with lower wood density in concomitance with higher SLA. This combination of traits, high SLA and low wood density, is part of the syndrome associated with fast growth and high resource acquisition capacity among plants (Reich, 2014), traits that confer high competition capacity and seem to be maximized using large seedlings in forestations.

In gymnosperms, SLA affected the seedling size–survival relationship only in humid climates, where it was more positive as SLA decreased (Fig. 5.2c). It is possible that gymnosperms planted in wetter climates are often used in frost-prone locations or on sites with shallow, poor soils (e.g., mountain pines such as *Pinus nigra* in southern Europe and *Pinus ponderosa* on southerly aspects in the northern Rocky Mountains) or high herbivory. In such environments, seedlings may face moderately harsh conditions, where larger seedlings of low-SLA species have an advantage in establishment due to their greater stress tolerance. However, to maximize this advantage, these seedlings should be cultivated to achieve a low S/R ratio as suggested by the significant negative relationship between seedling S/R and survival for gymnosperm species with low SLA (Fig. 5.2d).

In summary, seedling size has a significant global and positive effect on survival, while increasing S/R in seedlings of the same age usually does not compromise survival. Some planting practices, stocktype, and species functional ecology can modulate the effect of seedling morphology on survival, particularly in interaction with the aridity of the planting site. In dry climates, mitigating water stress, either by planting on more humid microsites or conducting more intensive soil preparation, as well as using container plants while avoiding very old ones, facilitates the use of larger plants. These findings provide valuable insights into how to cultivate and use target seedlings of different species. The final section of this chapter provides recommendations on how to use this knowledge for the cultivation of target seedlings.

Drivers of survival differences between bareroot and container stocktypes

Introduction and challenges

Forests can be established using container or BR stock. The main advantage of container seedlings is that during storage, transport, and at planting, the root system is protected within the plug from environmental and mechanical damage. The plug also stores water and nutrients, mitigating seedling post-planting shock stress. In addition, container plants have a dense, fibrous root system that has a high-water uptake capacity, reducing post-planting water stress (Davis and Jacobs, 2004; Grossnickle and El-Kassaby, 2016). In contrast, the roots of BR plants lack any protection at planting. Consequently, the roots can be more easily damaged by rough handling during lifting, storage, transport, and planting (Grossnickle and El-Kassaby, 2016), which can further exacerbate post-planting shock (Struve and Joly, 2011; Yamashita et al., 2016). Although container seedlings are usually more expensive than BR seedlings, they can be planted faster, reducing planting costs. The consensus is that container stock usually has better outplanting performance than BR plants on poor-quality sites, particularly under water-limited conditions (Barnett and McGilvray, 1993; Grossnickle and El-Kassaby, 2016; Haywood and Barnett, 1984; Morrissey et al., 2010). Nevertheless, several studies have demonstrated that BR stock showed satisfactory performance on high-quality sites (Davis and Jacobs, 2004), where it may have economic advantages (Burkett et al., 2005).

Currently, container plants in Europe are predominantly used in the boreal and Mediterranean climate zones, which are characterized by harsh climatic conditions. In contrast, in Central Europe, where climatic conditions are less severe than in the boreal and Mediterranean regions, BR plants have significant demand and coexist with container plants (Pemán et al., 2017). This high use of BR stock is also influenced by lower financial investments and the generally smaller seedling sizes used in some countries, where it is favored on fertile, vegetation-rich sites (Ivetić, pers. comm.). In other wet temperate regions of the world, BR stock is still widely used. For instance, around 73% of forest seedlings in the USA are BR, being a major stocktype in the Southeast, South Central, and Pacific Northwest (Haase et al., 2021a, 2021b). Current patterns will likely change with climate change.

The study by Grossnickle and El-Kassaby (2016) represents the most comprehensive systematic review of the scientific and technical literature comparing BR and container stock to date. However, the review lacked a quantitative approach and did not examine potential differences between conifers and broadleaf species, nor did it consider how planting site characteristics and planting practices might affect these differences.

In this section, we conducted a quantitative analysis to investigate factors influencing survival differences between container and BR stocktypes. Specifically, we examined whether container plants showed superior survival under more challenging planting conditions, such as arid environments, or woodlands as opposed to croplands, the absence of soil preparation, or competition from surrounding vegetation. In addition, we also analyzed if differences between stocktypes depended on whether seedlings were angiosperms or gymnosperms. BR angiosperms may be more susceptible to post-planting stress due to their higher transpiration capacity compared to gymnosperms (Choat et al., 2012; O'Brien et al., 2017). To achieve these aims, we conducted a quantitative review of the scientific and technical literature using a vote count approach. The literature search was conducted in December 2019 in *Web of Science*, *TreeSearch* (www.fs.usda.gov/), *Google Scholar*, and the Canadian Forest Service (www.cfs.nrcan.gc.ca/publications). In addition, we used references from Tables 4 and 6 in Grossnickle and El-Kassaby (2016). After removing duplicates, we identified 125 studies. Within each study, different case studies were established if different species or planting sites were evaluated, resulting in a total of 354 study cases encompassing a total of 42 species, mainly in temperate and boreal biomes. The voting direction was categorized as positive when container (C) plants demonstrated significantly superior survival relative to BR counterparts ($C > BR$). Conversely, a negative voting direction was assigned when BR plants outperformed container plants ($C < BR$). When no statistically significant differences were reported, the voting direction was designated as neutral ($C = BR$) (Floress et al., 2019). Data were analyzed using chi-square statistics applied to contingency tables (see Box 5.1).

Drivers of survival differences between bareroot and container seedlings

Nearly half of the study cases in our database reported no significant differences between container and BR plants (Fig. 5.3). The frequency of cases showing higher survival for container stock compared to BR stock ($C > BR$) was significantly higher (four times higher) than the frequency of cases showing the opposite result ($C < BR$) (Fig. 5.3). This latter result aligns with previous reviews on the topic (Barnett and McGilvray, 1993; Grossnickle and El-Kassaby, 2016; Haywood and Barnett, 1984; Morrissey et al., 2010) and highlights the establishment limitations of BR stock compared to container stock. The high proportion of cases with no differences between both stocktypes indicated, however, that there were situations where BR seedlings can be used without significant survival limitations.

An increase in aridity of planting sites (indicated by lower aridity index values, see Section 2.1 of this chapter for its definition), increased the probability of higher survival for container seedlings in comparison to BR seedlings (Fig. 5.3). Only on markedly wet

sites (aridity index >3.0), was the probability of container seedlings outperforming BR plants lower than 50%. This result corroborated previous observations that container stock has better performance than BR stock in challenging environments. Water stored in the plugs in container plants meets the plant water demand during transportation and the first days after planting, which can reduce the intensity of post-planting shock. In addition, fine roots in container stock are less damaged and can resume growth more rapidly after outplanting (Grossnickle and El-Kassaby, 2016; Mena-Petite et al., 2001; Pendl and D'Anjou, 2011), facilitating the hydraulic connection of the seedling to the surrounding soil.

Previous land use did not affect the greater advantage of container stock over BR stock, with differences between both stocktypes being similar in woodlands and croplands. It is possible that soil quality showed little difference between both types of planting sites in our stocktype database, or that environmental differences between woodlands and croplands were less important than other factors such as climate.

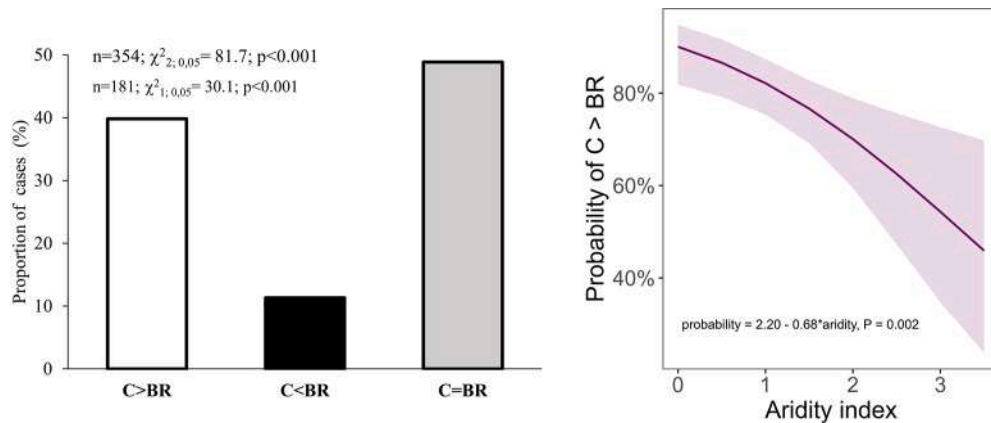


Figure 5.3 *Left plot:* Proportion of cases where container seedlings showed higher survival than bare-root seedlings (C>BR), lower survival than bareroot plants (C<BR), and no survival difference with bareroot plants (C=BR). The first line of statistical results is for the observed frequency of cases of the three possible outcomes (C>BR, C<BR, and C=BR) relative to the expected frequency, assuming no differences in frequency among the possible outcomes. The second line of statistical results refers to the differences in the observed frequency of positive (C>BR) and negative (C<BR) outcomes relative to their expected frequency, assuming no differences in frequency among these two outcomes. *Right plot:* Logistic regression depicting the probability of C>BR relative to the aridity of the planting site. Each point represents a case study for which the outcome was C>BR or C<BR. Decrease in aridity index values indicates drier climates. The purple band around the fitted model represents its confidence interval.

Soil preparation ameliorates several physical and chemical soil limitations that potentially hinder seedling establishment (Löf et al., 2012). The proportion of cases showing $C > BR$ was significantly higher in plantings without soil preparation and in sites where weeds were not removed, and consequently, seedlings likely faced higher competition. These results partially supported the hypothesis that differences between container and BR stocktypes were more pronounced under challenging planting conditions.

The proportion of cases where $C > BR$ did not differ between gymnosperms and angiosperms is shown in Fig. 5.4. However, these results must be taken with caution due to the limited representation of angiosperms in our dataset (28 cases out of 181 cases). As indicated in Section 1 of this chapter, gymnosperms have lower transpiration rates than angiosperms, potentially making them less susceptible to desiccation and post-planting shock. Nevertheless, except for two species, all angiosperms were deciduous, and seedlings of deciduous trees typically lack leaves at planting. This absence of foliage can reduce seedling planting shock stress in this group of plants.

In summary, container stock benefits more from soil preparation than BR stock and is less negatively impacted by weed competition and arid climatic conditions, while previous land use does not affect the advantage of container stock over BR stock.

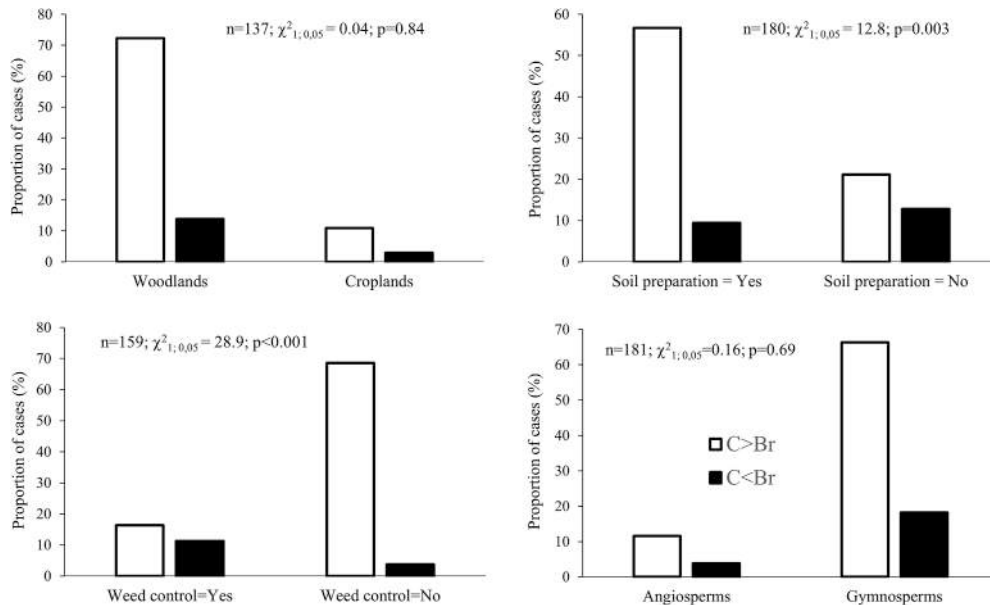


Figure 5.4 Proportion of cases where container stock had higher survival than bareroot stock ($C > BR$) and container stock had lower survival than bareroot ($C < BR$) in relation to previous land use of planting sites (woodland vs. cropland), soil preparation, and weed control (yes vs. no), and species taxonomy (angiosperms vs. gymnosperms). In each figure, we show statistical results and the number of study cases.

Nursery hardening and seedling outplanting performance

Introduction and challenges

Seedlings for forest planting projects must resist the main stress factors in the planting sites or during plant transportation and handling (Landis et al., 2010a, 2010b). Nursery cultivation practices can enhance seedling stress tolerance, a process referred to as hardening, conditioning, or acclimation. These practices involve exposing plants to environmental conditions that trigger physiological and morphological stress tolerance mechanisms (Lambers et al., 2008; Levitt, 1980). Nursery hardening serves three important objectives (Landis et al., 1998): (1) modifying seedling morphology and inducing dormancy, (2) acclimating seedlings to field environmental conditions, and because of the previous objectives, (3) improving outplanting survival and growth.

Hardening is typically conducted during the final weeks of cultivation, coinciding with the proximity of the planting or seedling storage season. Nursery hardening practices mimic what occurs in nature, where plants acclimatize to variations in environmental conditions to maintain their performance. For instance, woody plants in temperate and boreal regions increase their frost resistance as the cold season approaches, stimulated by longer nights and decreasing night temperatures in late summer and throughout the autumn (Beck et al., 2004; Puttonen and Arnott, 1994). This occurs simultaneously with stem growth slowdown and bud set (in species bearing buds), an important condition for hardening to occur (Greer et al., 2000).

Several hardening practices are used in nurseries. For plants grown in greenhouses, the mere exposure to outdoor environmental conditions (increased light and ultraviolet radiation, reduced temperatures, wind, and drier air) in the late cultivation stages increases seedling stress resistance. Exposing seedlings to short photoperiod by covering the crop with opaque curtains curtails shoot height growth, induces bud set, and increases cold and drought tolerance (Puttonen and Arnott, 1994; Tan, 2007). This type of hardening is referred to as blackout, and it is mainly used to condition seedlings for cold storage (Landis et al., 1998), but also for summer or early fall planting in the boreal biome and in temperate climates with cold winters (Luo et al., 2021; Tan, 2007). Finally, restricting irrigation to expose seedlings to sublethal levels of drought can increase short-term water stress tolerance (Kaushal and Aussenac, 1989; van den Driessche, 1991a; Vilagrosa et al., 2003).

The effect of drought hardening and blackout on the early outplanting performance of seedlings in forest species has received little attention. While some studies have shown positive effects of drought hardening on outplanting performance (Sánchez-Blanco et al., 2004; Valliere et al., 2019; van den Driessche, 1992), others observed no effects or even reduced performance (Grossnickle et al., 1991; O'Reilly et al., 1994; Villar-Salvador et al., 2004b; Villar-Salvador et al., 2013a). Differences among studies may be due to variations in the duration and intensity of drought hardening, whether the

seedlings were cultivated indoors or outdoors, or the time elapsed since the end of hardening and planting date. In addition, differences in species ecology, such as drought tolerance and environmental conditions of plantation sites, can also explain the disparity of reported outcomes of drought hardening.

Climate change will challenge the success of new forests in many regions (Busch et al., 2024). In this context, it is important for practitioners to understand how nursery practices, such as drought hardening or blackout, affect seedling establishment under harsh conditions. The implementation of drought hardening is a complex nursery practice. It is unclear if drought hardening and blackout increase outplanting performance, if they increase seedling field performance, and under which planting conditions or for which species they can be recommended.

In this section, we address the following questions: (1) Does the intensity and duration of nursery drought or blackout hardening, along with the time elapsed since hardening completion until planting, influence post-planting survival and growth? (2) Does drought hardening benefit seedlings cultivated indoors more than seedlings cultivated outdoors? (3) Do species drought tolerance and the aridity conditions of planting sites drive the effectiveness of hardening? To respond to these questions, we conducted a meta-analysis of published literature and unpublished data showing field results on both drought hardening and blackout. The literature search procedure and paper acceptance criteria for drought-hardening papers are explained in Puértolas et al. (2024). The drought-hardening database includes information for 36 species, while the blackout database only included five species, four conifers (*Chamaecyparis nootkatensis*, *Picea abies*, *Pinus tabuliformis*, *Tsuga heterophylla*) and one broadleaf species (*Populus tremuloides*).

We calculated the Ln rate ratio (RR) as the effect size for survival and the Ln response ratio (R) for growth data. Both effect sizes represent the ratio between the survival or growth of hardened seedlings relative to that of control seedlings (Box 5.1). Thus, significant positive values indicate higher performance of hardened seedlings compared to the control, while negative values indicate the opposite. We also compiled information on the osmotic potential at the turgor loss point (ψ_{TLP}) of each species. This physiological trait is a proxy for the potential drought tolerance of a plant. Species with low (more negative) ψ_{TLP} values typically thrive and have higher ψ survival under water stress than species with high ψ_{TLP} values (Álvarez-Cansino et al., 2022; Bartlett et al., 2012; Maréchaux et al., 2015). Complete methodological details on the drought hardening meta-analysis can be found in Puértolas et al. (2024).

Effects of drought hardening on seedling performance

The mean effect size for survival across all studies was not significantly different from zero, indicating no significant differences in survival between drought-hardened and control seedlings across studies (Fig. 5.5). This does not mean that drought hardening has no effect on outplanting performance. It is possible that species responses, hardening

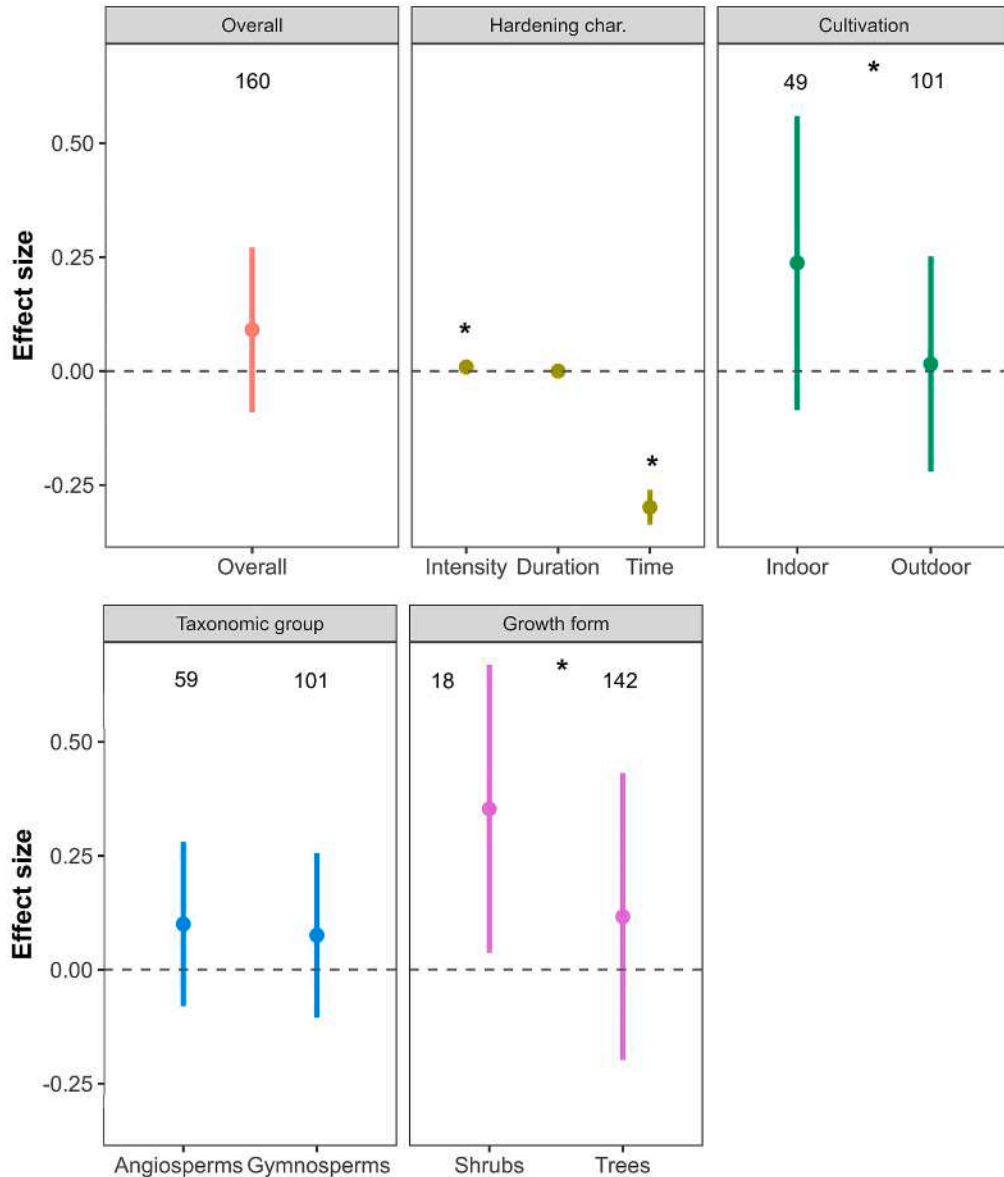


Figure 5.5 Predicted effect size (Ln rate ratio) \pm 95% confidence interval of drought hardening on the overall seedling outplanting survival and in relation to drought-hardening intensity, duration, elapsed time since the end of nursery hardening, and planting date, as well as differences between indoor and outdoor cultivation, angiosperms and gymnosperms, and shrubs and trees among angiosperm species. The number of case studies for each factor is indicated in the upper part of the panels. Positive effect size values denote an increase in survival due to drought hardening, while negative values indicate a reduction in survival. Asterisks indicate significant differences between treatments. Drought-hardening intensity is represented as the relative reduction of crop hydration state relative to the control (see Puértolas et al., 2024).

characteristics, and field conditions are interacting with hardening treatments in opposite ways, resulting in neutral hardening effects. Therefore, it is important to assess modulators of hardening effects on seedling performance.

We found that the time elapsed between the end of nursery hardening and the planting date had a negative effect on the effectiveness of drought hardiness. This result was due to case studies where the seedlings were cold-stored after nursery hardening in boreal species. It was possible that drought physiological mechanisms activated during hardening can relax after rewatering the plants (Liu et al., 2001; Villar-Salvador et al., 2004b) and left in cold storage, a practice that lasts weeks. This might counteract the drought-hardening effects. Conversely, drought-hardening intensity had a weak, positive but significant effect on seedling survival. Drought effects are highly species-specific, so the same intensity and duration can induce varied effects depending on the species' acclimation capacity to water stress. Additionally, meteorological conditions, in particular evaporative demand during the application of drought, can affect the intensity of plant water stress (Bañon et al., 2006; Sánchez-Blanco et al., 2004). Despite this result, we do not recommend nursery growers to use strong water stress levels to harden seedlings because they hinder the rehydration of organic growing media such as peat or coir. Neither the duration of drought hardening nor the cultivation environment (indoors vs. outdoors) significantly influenced the effect of drought hardening on survival. This means that growing the seedlings most or all the time inside greenhouses does not influence the effectiveness of drought hardening.

Part of the study cases in our database included outplanting experiments conducted under real field conditions, but others were conducted in large pots. The consequences of both experimental approaches on the effect of drought hardening on outplanting performance were discussed in Puértolas et al. (2024). When focusing on studies performed under field conditions, we observed that drought hardening benefited the survival of shrubs more than trees among angiosperms. Shrubs have, in general, higher drought resistance than trees and lower hydraulic efficiency (Zheng et al., 2023). These water-conserving characteristics may be enhanced more by drought hardening in shrubs than in trees. The effect of hardening on survival was significantly higher for angiosperms than for gymnosperms (Fig. 5.5), although in both cases the CI of the effect size was not different from zero, i.e., no evidence of positive effects of hardening on seedling survival in any case.

The benefits of nursery drought hardening on outplanting survival increased with planting site aridity. However, this effect depended on species drought tolerance (Fig. 5.6). Specifically, drought hardening benefited species with inherent high drought tolerance ($\psi_{TLP} < -2.3$ MPa) in a wide variety of aridity conditions. Conversely, drought hardening only enhanced the survival of species with low drought tolerance ($\psi_{TLP} > -2.3$ MPa) when planted on severely dry planting sites, with this effect

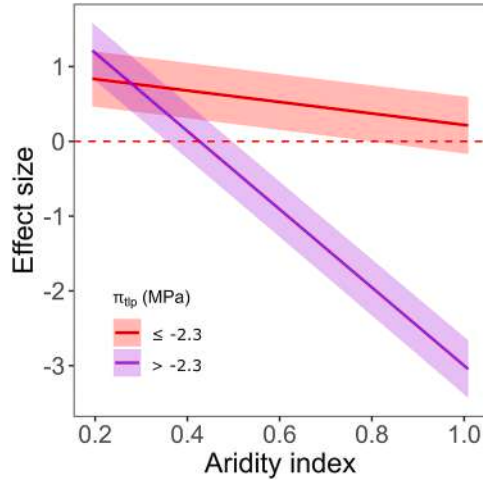


Figure 5.6 Variation of the predicted effect size (Ln rate ratio, $RR \pm 95\%$ confidence interval) of the effect of drought hardening on outplanting survival in relation to the aridity index of the planting sites. The effect sizes are distinguished between low drought-tolerant species (osmotic potential at the turgor loss point (π_{tlp}) > -2.3 MPa) and high drought-tolerant species ($\pi_{\text{tlp}} \leq -2.3$ MPa). The aridity index is defined as the ratio of precipitation to potential evapotranspiration, with low values corresponding to arid sites. The selected threshold of $\pi_{\text{tlp}} = -2.3$ MPa approximates the mid-point of π_{tlp} variation across plant species (Bartlett et al., 2012).

vanishing for moderate aridity sites and becoming negative on low aridity sites (i.e., high aridity index sites).

Many low drought-tolerant species, such as the Mediterranean pines *Pinus pinea* and *P. halepensis*, have strong water conservation strategies to avoid turgor loss or hydraulic failure (Martínez-Vilalta et al., 2004; Salazar-Tortosa et al., 2018). In these species, hardening enhances drought avoidance mechanisms such as reduced stomatal conductance (Stewart et al., 1995; Villar-Salvador et al., 2013a). A decrease in stomatal conductance lowers the transpiration rate, which can be beneficial under severely dry conditions. However, it also results in a reduction in the photosynthesis rate, which may have negative effects on sites with moderate or low aridity, where competition may be more limiting than water stress. In contrast, many drought-tolerant species rely on keeping cell turgor and stomata open at low water potentials in response to drought hardening through osmotic adjustment (Abrams, 1990; Bartlett et al., 2014; Klein et al., 2013), to keep growing and, hence, avoid drought stress. These results suggest that specific drought hardening protocols should be tailored depending on the drought tolerance of the target species or even genotypes (Knutzen et al., 2015), especially for species to be planted on moderate dry sites.

Similarly to survival, drought hardening did not have a significant impact on growth, as reflected by an overall effect size that was not significantly different from zero (Fig. 5.7). Neither hardening intensity, nor its duration, nor the time elapsed between the end of nursery hardening and outplanting significantly affected drought hardening. No significant differences in the effect size were observed between indoor and outdoor cultivation environments. The effect size of drought hardening on growth decreased with the aridity index of the planting site, indicating that drought hardening helped to increase growth in dry locations. This variation of the effect size with aridity was independent of species π_{lp} .

Finally, we detected that the shrubs had significantly higher effect size values than perennial herbs or trees, which did not differ between them. In all growth forms, however, the mean effect size was not significantly different from zero (Fig. 5.7). The taxonomic group of species (angiosperms *vs.* gymnosperms) had no influence on the effect size of hardening on growth.

Effects of blackout on seedling outplanting growth

We could not evaluate the effect of blackout on survival because in most cases survival was high (>85%) with no differences between blackout treatments and/or most of the papers did not report it (73% of the study cases). Consequently, we show only results for growth. In general, the number of studies reporting field performance results after blackout was low, because these studies usually focus on physiological variables such as frost resistance, carbohydrate content, or bud setting and do not test outplanting performance. Only nine studies with outplanting growth results were included, which represented 45 study cases. The overall mean effect size of blackout on growth was slightly negative but not significantly different from zero, which indicates that blackout did not affect growth after outplanting. The duration of blackout treatments did not influence the effect (Fig. 5.8). Similarly, the elapsed time between the end of blackout and planting time did not influence the effect of blackout on growth, despite differences of up to 40 weeks across studies.

The effect size of blackout on the growth of the seedlings cultivated indoors did not differ from that of seedlings cultivated outdoors, despite previous studies supporting this difference for *Populus tremuloides* (Landhäusser et al., 2012a). The transplanting conditions used to evaluate the effects of blackout influenced field growth outcomes. Experiments conducted under field conditions showed significantly lower effect size values compared to those conducted under pot conditions. Notably, the effect of blackout on growth was negative, and the effect size value was significantly different from zero for experiments evaluated under field conditions. This result has important implications as it indicates that blackout can hinder short-term outplanting growth. A similar result on the effect of transplanting conditions was also observed in drought-hardening studies

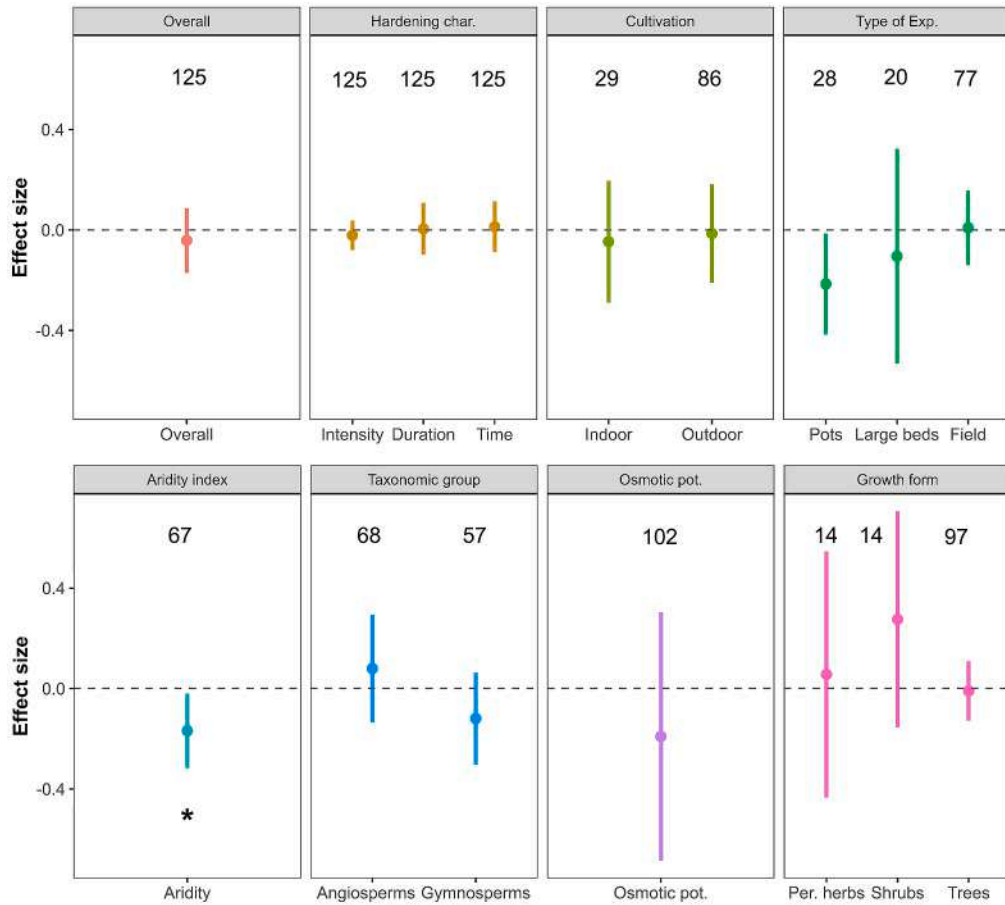


Figure 5.7 Predicted effect size (Ln response ratio) \pm 95% confidence interval of drought hardening on seedling outplanting growth relative to drought-hardening intensity, duration, elapsed time since the end of nursery hardening, and planting date, as well as differences between indoor and outdoor cultivation, angiosperms and gymnosperms, and angiosperm shrubs and trees. The number of case studies for each factor is indicated in the upper part of the panels. Positive effect size values denote an increase in growth due to drought hardening, while negative values indicate a reduction in growth. Drought-hardening intensity is represented as the relative reduction of crop hydration (see Puértolas et al., 2024).

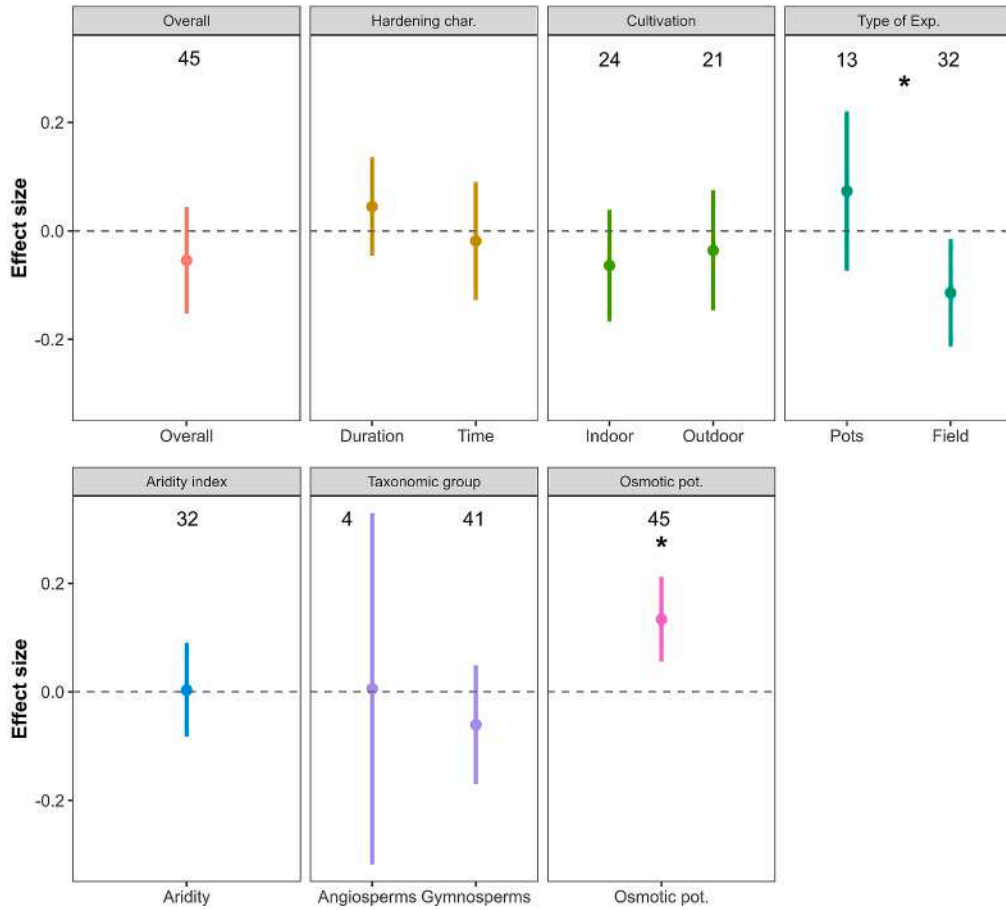


Figure 5.8 Predicted effect size (Ln response ratio) \pm 95% confidence interval of blackout on seedling outplanting growth relative to drought-hardening intensity, duration, elapsed time since the end of nursery hardening, and planting date, as well as differences between indoor and outdoor cultivation, angiosperms and gymnosperms, angiosperm shrubs and angiosperm trees. The number of case studies for each factor is indicated in the upper part of the panels. If numbers are not shown, it means that the number of case studies is equal to the one shown in the overall panel. Positive effect size values denote an increase in growth due to drought hardening, while negative values indicate a reduction in growth.

that evaluated transplanting growth (Puértolas et al., 2024). Therefore how the hardening effect is evaluated matters, and we suggest that future studies should only evaluate hardening treatments under field conditions.

The reduction of growth due to blackout might be mediated by a reduction of nonstructural carbohydrates of seedlings (Landhäusser et al., 2012a; Luo et al., 2021), especially if daytime temperature during application of blackout treatments keeps respiration and root growth high (Luo et al., 2021). This reduction of nonstructural carbohydrates caused by blackout can be aggravated due to subsequent long-term storage of seedlings (Puttonen, 1986; Ritchie, 1982), a frequent practice in seedlings subjected to blackout.

The aridity of the planting field sites did not influence the effect of blackout on growth, likely due to small aridity differences across studies. Similarly, no differences were found between angiosperms and gymnosperms. However, because we had a limited number of study cases for angiosperms, these results should be interpreted with caution (Fig. 5.8).

Finally, species π_{tlp} positively affected the effect size, with more drought-tolerant species (lower π_{tlp} values) showing negative effects of blackout on growth, while low drought-tolerant species (higher π_{tlp} values) had positive effects (Fig. 5.9). Bearing in mind that lower osmotic potential is often linked to increased tissue soluble carbohydrate concentration (Martínez-Vilalta et al., 2016), it is possible that blackout reduced tissue carbohydrate concentration (Luo et al., 2021) mediated by decreased net photosynthesis. A decrease in tissue soluble carbohydrate concentration may hinder seedling outplanting growth more in species that have a higher demand for soluble sugars to keep the π_{tlp} low.

In summary, the main findings on hardening are that drought hardening applied at the end of cultivation in the nursery can increase field performance with increasing aridity of planting sites. Drought hardening seems to be more effective in improving survival than growth, and survival of shrubs more than that of trees. On moderate and low aridity sites, drought hardening can have negative effects on field survival in species with low inherent drought tolerance, i.e., high osmotic potential values. Despite the fact that drought hardening can increase slightly outplanting survival and growth under drier conditions, we suggest using this nursery practice in synergy with other nursery and outplanting practices, such as cultivating in larger containers, more intense soil preparation, or planting as early as possible within the planting window.

Finally, while blackout is widely recognized as an effective way for enhancing seedling frost tolerance and promoting bud set, we see evidence that it can reduce field growth, particularly in species with high inherent drought tolerance (i.e., low osmotic potential). Future research should focus on developing nursery practices to mitigate negative effects on field growth.

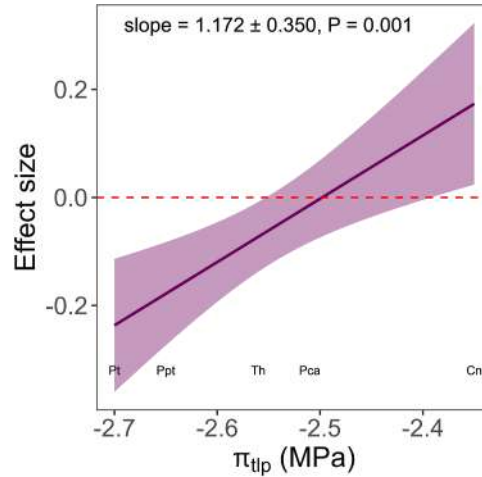


Figure 5.9 Variation of the effect size of blackout on outplanting growth measured under field conditions in relation to species osmotic potential. Cn = *Chamaecyparis nootkatensis*, Pca = *Picea abies*, Ppt = *Populus tremuloides*, Pt = *Pinus tabuliformis*, Th = *Tsuga heterophylla*.

Container properties on seedling survival

Introduction and challenges

Container selection is a critical decision for nursery growers, significantly influencing seedling growth, nursery management, and cultivation practices. Container characteristics affect the root and shoot morphology and physiology of seedlings in the nursery (Benamirouche et al., 2020; Dixit and Burney, 2024; Grossnickle and El-Kassaby, 2016; Haase et al., 2021a, 2021b; Landis et al., 2010a, 2010b) with significant consequences for field establishment (Davis and Jacobs, 2005; Grossnickle, 2012; Grossnickle and Ivetić, 2022; Tsakalimi et al., 2005). The primary function of a container is to provide an environment for seed germination and seedling growth (Landis et al., 1990). In addition, a container should hold an adequate amount of growing medium to supply nutrients, air, water, microorganisms, and physical support for the seedling, while meeting the operational and economic needs of the nursery (Landis et al., 1990).

Several container characteristics strongly affect nursery and planting operations, such as automation, costs, durability, handling, shipping, and storage, making them critical considerations for nursery managers when selecting containers (Landis et al., 1990). Although these factors often weigh heavily in the decision-making process, the ultimate choice should be based on the quality of the final product. The success of a forest restoration project depends on the functional attributes of the seedlings (Grossnickle and MacDonald, 2018a, 2018b; Stanturf et al., 2014). Therefore understanding how container characteristics affect these functional attributes is essential for the success of plantings.

The primary characteristic by which the container (the cell, caveat, or pot) is identified is its size, commonly expressed in terms of volume. Container volume results from various combinations of width and depth (Landis et al., 1990; Wilkinson et al., 2014), which also leads to more elongated or flattened shapes. The effect of volume on seedling functional attributes, especially morphology, has been widely studied for many species and growing conditions (Climent et al., 2011; Dominguez-Lerena et al., 2006; Mariotti et al., 2015a, 2015b; Oliet et al., 2012; Puértolas et al., 2012; South et al., 2005; Sutherland and Day, 1988; Tsakaldimi et al., 2005). Generally, increased container volume enhances seedling nursery shoot and root growth, with some differences due to the specific study case. The effect of container volume on plant growth was highlighted in a meta-analysis by Poorter et al. (2012), which reported that doubling pot size increased plant biomass by 43%. This growth enhancement with rooting volume appears to be associated with an increase in photosynthesis rate, likely linked to enhanced nutrient and water uptake rather than changes in leaf morphology or biomass allocation.

Depth is another characteristic of containers that can influence seedling properties, though to a lesser extent than volume. To our knowledge, the impact of other container characteristics (i.e., width and shape) has not been extensively studied in experimental trials on forest species. Although depth is correlated with container volume, container depth might be more important for species with strong taproots. For instance, in *Quercus* species, a positive relationship has been observed between container depth (>18 cm) and shoot and root system biomass, height, root system depth, and taproot length (Chirino et al., 2008; Mariotti et al., 2015b, 2015a). Seedlings grown in deeper containers reach deeper soil layers faster after planting, showing higher root hydraulic conductance and stomatal conductance under water stress (Chirino et al., 2008). The container shape and its aeration and drainage conditions are also an important issue that highly correlates with seedling quality and growth (Grossnickle and Ivetić, 2022).

Cultivation density, i.e., seedlings m^{-2} , is another critical property of containers that strongly affects seedling growth, and it is negatively related to container size (Landis et al., 1990). Higher density increases competition among seedlings for light and affects microenvironmental growing conditions, such as water penetration into the growing medium, irrigation spatial homogeneity, air and growing medium temperature, and air humidity (Landis et al., 1990). These microclimatic growing conditions can also influence disease spread. High density generally reduces seedling mass, while its effect on height is less clear, depending more on species' shade tolerance and growth rate. For instance, in *Pseudotsuga menziesii* and *Pinus contorta*, higher growing densities resulted in taller seedlings with smaller root collar diameter, and reduced root, shoot, and leaf mass (Simpson, 1994). Similar results were observed in other conifers such as *Pinus pinea* (Dominguez-Lerena et al., 2006) and *Larix occidentalis*, where increased growing density led to lower root biomass following a simulated outplanting (Aghai et al., 2014). In broadleaved species, a lower density increased growth in *Quercus robur* (Banach et al.,

2023) and higher photosynthetic rate, water-use efficiency, stomatal conductance, and chlorophyll concentration in *Zelkova serrata*, *Fraxinus rhynchophylla*, and *Quercus serrata* (Seok Cho et al., 2017).

The importance of nursery practice on plant quality and outplanting success must be validated by assessing at least short-term survival and growth under field conditions (Davis and Pinto, 2021; Grossnickle and MacDonald, 2018a, 2018b) (see also Chapter 8 of this book). Many studies on containers have evaluated the morphological and physiological characteristics of seedlings at the end of nursery cultivation without conducting field trials. In addition, most research has focused on specific aspects, such as species comparisons, container types, and sizes, without a comprehensive quantitative review, except for Poorter et al. (2012), which focused on rooting volume. This lack of comprehensive reviews complicates providing informed recommendations on appropriate container choice for specific planting purposes, particularly for nursery managers working with diverse species, planting contexts, and objectives.

A PEN-CAFoRR COST Action task group has compiled a global database of published studies and gray literature to assess the relationship among container characteristics, seedling morphology, and growth at the end of nursery cultivation, as well as outplanting performance. In this section, we use part of this database to conduct a quantitative analysis on the effects of container characteristics on the outplanting survival of seedlings. Our analysis focuses on oaks (*Quercus* spp.) and pines (*Pinus* spp.) as model groups, as they represent ecologically and functionally distinct angiosperms and gymnosperms, respectively. Both genera are widely distributed and used in forestry. Oaks show strong taproots, high root mass allocation, larger leaves, and greater gas-exchange capacity compared to pine species (Baquedano and Castillo, 2006; Kolb and Stone, 2000; Sánchez-Gómez et al., 2006). Species ecology determines resource needs, growth rate, and biomass allocation patterns, which can influence the response of seedlings to variation in container properties (Climent et al., 2011). Providing informed recommendations on container characteristics could be facilitated if different response patterns are observed among plant functional groups. Specifically, we address the following questions: (1) Do changes in container volume, depth, and cultivation density affect seedling outplanting survival? (2) Do container properties affect differently the survival of oaks and pines?

The database included 12 study cases in oak species and 21 for pine species. The effect size metric used was the RR, the same metric used in Section 4 on the effect of hardening on survival. The calculation of RR is explained in Puértolas et al. (2024). Specifically, for each study, we calculated the pair-wise Ln of the ratio between the survival rate for a specific container volume (or depth) treatment and the survival of the smallest (or shallowest). For calculating the RR of cultivation density, the control was the highest density value. Thus positive effect sizes (RR) indicate that survival was greater in seedlings cultivated in larger containers and with lower cultivation densities. In the

analyses, we included the difference in volume between each pair-wise comparison as a covariate. We restricted the case studies to those with a maximum volume difference of 1200 mL to exclude case studies that used abnormally large containers not used at the operational level in forest restoration.

Results on the effects of container properties on oak and pine seedling survival

Outplanting survival of oaks and pines showed different responses to variations in container volume and cultivation density (Fig. 5.10). While an increase in container volume related to the control treatment (i.e., the smallest container) significantly increased the survival in pines (slope = 0.0603; $P < 0.0001$), in oaks, volume increase showed no significant effect on survival (slope = 0.0093, $P > 0.05$). In contrast, the increase in cultivation density difference strongly reduced oak survival (i.e., strong negative variation in the effect size) (slope = -0.60; $P < 0.001$) while variation in cultivation density had no effect on pine survival (slope = -0.0404, $P > 0.05$). Oak survival significantly increased from a density reduction of more than 100 seedlings m^{-2} . In

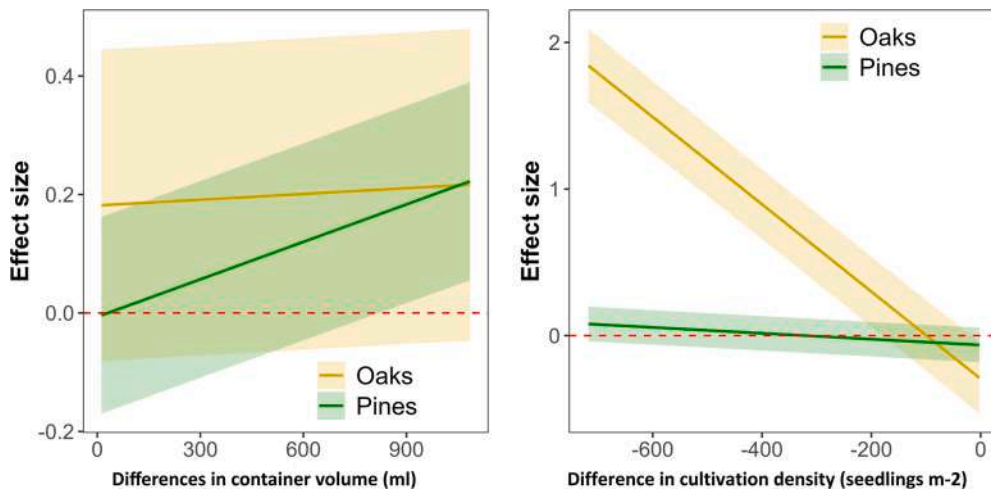


Figure 5.10 Linear relation of the predicted effect size on outplanting survival (Ln rate ratio) with the differences in container volume (left panel) and cultivation density (right panel) in oaks (*Quercus* spp.) and pine (*Pinus* spp.) species. Difference in container volume and cultivation density represents, for each case study, the difference between a specific container volume or a cultivation density and the smallest container volume or the highest density, respectively (control treatments). In the left panel, a positive effect size means that the survival is greater for the large volume container than for the small container, while a negative value indicates the opposite. In the right panel, a positive effect size means that survival is greater for the low cultivation density than for the high cultivation density, while a negative value indicates the opposite. Bands for the fitted model are 95% confidence intervals.

summary, to enhance oak survival, increasing cultivation space between plants is more effective than increasing container volume, while pines benefit more from increasing container volume. The differences between the plant groups might be related to the size of the seedling canopy. Compared to pine species, most oaks usually have larger leaves, so the canopy occupies more space and greater spacing is required if no foliage overlap and competition is wanted. A common issue with many broadleaf species is avoiding heterogeneous fertigation due to canopy overlapping when cultivated at densities similar to those used for conifers (Schmal et al., 2011; Wilkinson et al., 2014). As a result, some seedlings might receive significantly more water and nutrients than others, increasing seedling size heterogeneity in the crop. A fraction of the plants may show poor development in the nursery and subsequently low field establishment.

In both oaks and pines, the highest survival was achieved when differences in container depth and volume were maximized simultaneously (Fig. 5.11). For both species, the negative effects on survival as a result of a reduction in container volume difference can be counterbalanced by an increase in container depth difference. Very interestingly, the survival of pines was more responsive to container depth differences than that of oaks. For a specific container volume, oaks require greater container depth to maximize survival than pine species do. This finding was unexpected, given that the development of a long taproot is a trait often regarded as crucial for oak establishment (Moore, 1985; Mucha et al., 2018; Osonubi and Davies, 1981).

Based on our results, the design of low to moderate volume containers (<400 mL) for oaks should prioritize reducing spacing between plants rather than increasing depth. In contrast, for pine species, increasing container volume by increasing depth is more effective than reducing spacing. This suggests that container shape must be tailored to the target species.

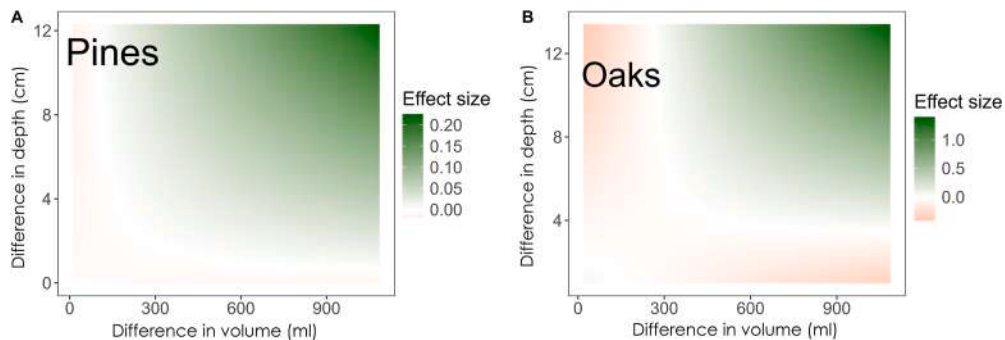


Figure 5.11 Predicted effect size on outplanting survival (Ln rate ratio) in relation to the variation in differences in container depth and volume for pine (*Pinus* spp.) and oaks (*Quercus* spp.) species.

Outplanting survival effectiveness of alternative and innovative growing media

Introduction and challenges

The production of seedlings in containers using soilless media has increased during the last 50 years globally (Grossnickle and El-Kassaby, 2016; Schmilewski, 2009). Growing media need to be cost-effective (Grafiadellis et al., 2000), making nursery cultivation more efficient in terms of shorter production cycles, increased stock uniformity, frequently superior field performance (Barnett, 1986; Wilson and Jacobs, 2006), and increasing water and nutrient use efficiency (Van Os, 1999). Initially, growing media were based on inexpensive materials obtained close to the nursery area. In many cases, these were soils from land clearings or simply the natural soils around the nursery. These materials were very heterogeneous, and it was difficult to control their quality from year to year. The use of materials with well-defined hydrological characteristics improved and homogenized the cultivation of forest species. In this context, the availability of effective growing media (hereafter GM) is one of the key points in nursery cultivation to grow target seedlings for different production purposes, such as ornamental, agricultural, and forestry.

Sphagnum peat moss (*Sphagnum* spp.), generally known as peat, is commonly used worldwide in plant propagation due to the following reasons: (a) it has good biological, technical, and economic performance; (b) it is highly reliable, uniform, and reproducible as their properties keep constant across years; and (c) it is widely available at competitive prices. However, the exploitation of peat is associated with several ecological concerns, such as the fragility of peatland ecosystems, their function as a C sink, CO₂ emissions related to its extraction and processing, and the nonsustainable rotation length of peat production (Alexandrov et al., 2020; UN Climate Change, 2021). Environmental concerns in Europe promoted actions to preserve peatlands as an ecosystem as well as to reduce CO₂ emissions (Council Directive 92/43/EEC, 1992; Schmilewski, 2008; Wallace et al., 2010). Moreover, pressures on the nursery sector have increased the demand for alternative, renewable, sustainable, and reliable growing media (Barrett et al., 2016; Ceglie et al., 2015; Gruda, 2019). Currently, one of the major challenges for nursery owners is replacing peat with more sustainable materials that may also represent a worthy strategy to recycle organic waste. The reclamation of dredged sediments or the use of substrates enriched with industrial waste could offer alternative solutions such as growing media for forestry species (Tognetti et al., 2004; Ugolini et al., 2018).

According to the TPC (Davis and Pinto, 2021), the characteristics of a quality seedling can change widely with species and site. Nursery environment and practices, including the selected GM, strongly affect seedling morpho-physiological traits (Grossnickle, 2012; Guehl et al., 1989; Mariotti et al., 2015b; van den Driessche, 1991b). An effective GM should have a balance between air porosity and water holding capacity to

promote root development and water uptake. Additionally, GM must have a high cation exchange capacity to ensure fertility and be economically feasible. Many environmental (e.g., nursery location), biological (e.g., selected species and quality and size of the reproductive material), and socioeconomic (e.g., budget and availability of raw material for GM) factors have to be considered to select the best GM, and frequently the decision is made considering trade-offs among these factors (Landis and Morgan, 2009).

Worldwide, the performance of commonly used growing media such as peat or pine bark is known. However, the performance of innovative and/or sustainable GM alternatives to the common ones has been specifically studied concerning species, seedling type, or nursery at a local scale (i.e., country and region), and the studied materials are frequently related to local recycling chains. Consequently, to promote new policies to guide the nursery sector in achieving higher sustainability levels, it is crucial to identify GM that have been tested, and that could be sustainable, effective, available, innovative, and marketable to grow forest seedlings to represent reliable alternatives to peat (Mariotti et al., 2023).

In a world of increasing natural resources scarcity and climatic crisis, the use of sustainable alternative GM has much to offer in a truly green industry as part of the circular economy, utilizing sustainable resources, and minimizing waste materials while improving the productivity and efficiency of container seedling production and forestation. A recent study (Mariotti et al., 2023) published the first global review on innovative and sustainable materials that can be used in GM as an alternative to peat (hereafter referred to as alternative material or AM). In this work, a global database was compiled according to defined criteria and information (for details, see Mariotti et al., 2023). This database included study cases from experimental trials reported in 866 articles, where AMs were tested for growing seedlings in nurseries and, where applicable, for evaluating the outplanting performance of the seedlings. The review identified 11 AMs: bark, biochar, wood chips, coconut fiber (coir), compost, manure, rice, sawdust, sludge, wood fiber, and other organic waste that included all other material not included in the previous categories. The results reported information about the origin and the use of the AMs worldwide, but the most innovative information was related to the first attempt to study the effectiveness of the studied AMs. The effectiveness of each case study in the AM database was classified at three levels as: (a) *promising*, when the GM physico-chemical characteristics (i.e., pH and porosity) were in a range considered suitable for a good GM according to Landis et al. (1990); (b) *effective*, when the growth in the nursery of the seedlings cultivated with the AM was at least as good as the growth of the seedlings cultivated with peat; and (c) *most effective*, when the field performance of seedlings cultivated in the nursery with the AM was at least as good as the field performance of the seedlings cultivated with peat.

Mariotti et al. (2023) reported that the wide availability of alternative GM highlights the global interest in this innovation and that there is no unique solution, an ideal AM, or

a perfect GM mixture suitable for growing forest seedlings worldwide. In this paper, the authors did not analyze in depth the studies that evaluated the effect of AM on outplanting performance. One limitation for such analysis is the limited number of studies reporting outplanting performance, the acid test for the effectiveness of nursery cultivation treatments of container seedlings raised in GM, composed of AM.

In this section, we focused the analysis on the “most effective” study cases of the database collected by [Mariotti et al. \(2023\)](#). Results based on outplanting performance in relation to the environment and the aim of the planting project are crucial for providing informed recommendations on nursery practices and seedling quality ([Grossnickle and MacDonald, 2018a, 2018b](#); [Mataruga et al., 2023](#)).

The specific questions addressed in this section were: (1) Which AM or AM mixtures most effectively enhanced outplanting performance? Namely, we focused on the efficiency of using one or more AMs, the amount of AM in the GM, and any traditional materials included in the AM mixtures; (2) Is there a minimum proportion for each AM in the GM to be effective? (3) Do AMs affect the outplanting performance of angiosperms and gymnosperms differently? (4) Does the aridity of planting sites affect the effectiveness of AM?

To address these questions, the database created by [Mariotti et al. \(2023\)](#) was filtered into 82 study cases that showed outplanting performance results. These study cases encompassed 17 species (13 angiosperms and 4 gymnosperms) and all continents, but with a strong representation of cases in South America (Africa 3.8%, Asia 17.5%, Europe 15.0%, North America 8.8%, Oceania 2.5%, South America 52.5%).

Results

Most of the AMs were tested individually as the sole component in the GM (single AM in [Table 5.1](#)), while in other cases the AM was mixed with traditional materials. For manure and sawdust, all study cases consisted of mixtures with traditional materials. We found a statistically significant difference in the distribution of effective and ineffective cases between the “single AM” and “mixed with other AM” categories (i.e., when the AM was combined with other AMs in lower proportions in the GM); for coir ($\chi^2 = 4.25$, $P = 0.046$) and sludge ($\chi^2 = 5.15$, $P = 0.040$). Both materials were mostly used as single AMs in the GM, and both were mostly ineffective when mixed with other AMs. Compost and rice were generally ineffective, whether used as a single AM or mixed with other AM ([Table 5.1](#)).

In most studies involving bark and other organic waste, these materials were used mixed with other AMs ([Table 5.1](#)). Bark was generally ineffective in promoting seedling performance when used in mixtures, but when used alone, it had a positive effect on outplanting performance. All study cases involving “other organic wastes” mixed with AMs were effective. The most effective AM combinations were coir with rice, sludge

Table 5.1 Proportion (%) of study cases where each alternative material (AM) was studied as a single AM and with other AM in the GM, and the effectiveness (%) of each combination in increasing outplanting performance. Effectiveness was calculated per material out of the total of “single AM” or “mixed with other AM” study cases.

Alternative material	Mixed with other AM	Single AM
Bark (n = 14)	57.1	42.9
Effective cases	33.3	75.0
Coir (n = 7)	28.6	71.4
Effective cases	0	66.7
Compost (n = 13)	7.7	92.3
Effective cases	9.1	0
Manure (n = 3)	100	0
Effective cases	100	0
Organic Waste (n = 8)	87.5	12.5
Effective cases	100	50.0
Rice (n = 8)	15.8	84.2
Effective cases	7.7	33.3
Sawdust (n = 1)	100	0
Effective cases	100	0
Sludge (n = 15)	33.3	66.7
Effective cases	11.1	66.7

with bark, wood fiber with organic waste, compost with organic waste, and bark with sludge.

The proportion (%) of AM in the GM was categorized into five classes (1–20, 21–40, 41–60, 80–100; Table 5.2). Manure and sawdust were used exclusively in the 41%–60% and 61%–80% classes, respectively, with 100% of cases showing effective results in promoting the seedling field performance. Other AMs showed a broader range of proportions in the GM. Notably, bark and sludge used in a 41%–60% proportion were mostly ineffective when mixed with other AM, whereas coir and compost in the same proportion showed a higher frequency of effective results when used as a single AM. The effectiveness of other organic wastes was distributed relatively evenly across the 21%–40%, 41%–60%, and 61%–80% classes when mixed with other AMs. Rice was most effective as a single AM, with its success falling into the 21%–60% range of the GM.

The traditional materials studied by Mariotti et al. (2023) included peat, soil, and other materials such as vermiculite and perlite. A portion of the studies did not use any traditional materials. Peat was the traditional material most frequently mixed with the AMs in the GM (36% of all cases), while in 36% of the cases, the GM was made only with an AM. Both peat and soil were effective in most study cases where they were tested (Fig. 5.12). Other traditional materials were largely effective only when combined with sawdust. Manure was tested only with soil, and sawdust with other traditional materials, with both combinations being 100% effective. Compost and other

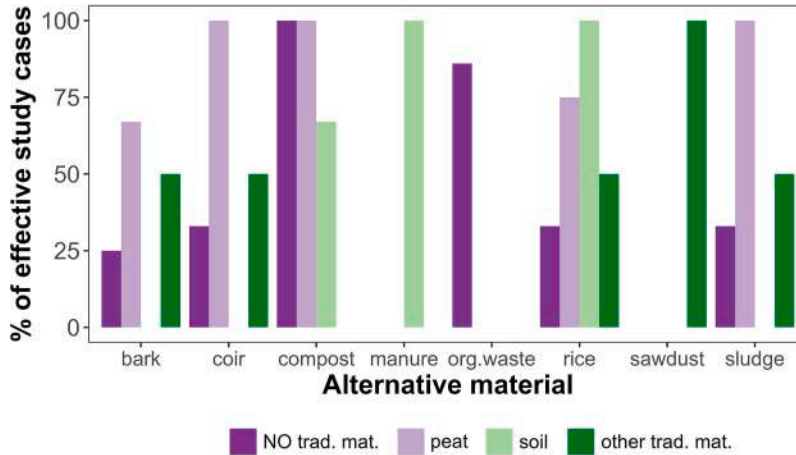


Figure 5.12 Proportion (%) of effective study cases per AM and per type of traditional material (peat, soil, other traditional materials), and pure AM (absence of any traditional material), indicated as NO traditional material in the graph. Proportions have been calculated out of the total of study cases per AM and per traditional material. No effective case studies were found for the following combinations: bark, coir, and sludge with soil; manure with no traditional material, peat, and other traditional material; organic waste with peat, soil, and sawdust with no traditional material, peat, and soil. In other cases, the absence of a bar in the graph indicates that 0% of effective study cases were found.

organic wastes were notably effective when used without any traditional material, whereas the opposite was true for bark, coir, rice, and sludge.

To assess a minimum effective proportion for each AM, we merged the proportion classes shown in [Table 5.2](#) in the GM and applied a chi-square test to compare effective and ineffective cases across two new classes: above or below 40%, and above or below 60%. Due to the limited number of study cases, only sludge showed significant results with a threshold of 60% ($\chi^2 = 4.203$, $P = 0.046$): a proportion of sludge greater than 60% resulted in 100% positive cases. For other AMs, no significant thresholds or clear trends were identified, so the results in [Table 5.2](#) should be considered as a preliminary indication of the effective proportions of these alternative materials.

Regarding the effect of AM on the field performance in angiosperms and gymnosperms ([Fig. 5.13](#)), compost, sawdust, and sludge promoted effective results in gymnosperms. Sawdust showed 100% positive cases, but it was based on a single study. Excluding sawdust, all other AMs showed at least some effectiveness in angiosperms, with over 65% positive outcomes for manure, organic waste, and rice, while sludge showed < 10% positive results.

To evaluate if planting site aridity modulated the effectiveness of each AM, we classified sites into subhumid and arid/semiarid categories based on their aridity index using their location coordinates and following the same method reported in the other sections of this chapter. Humid sites had an aridity index >0.5, while the dry sites had

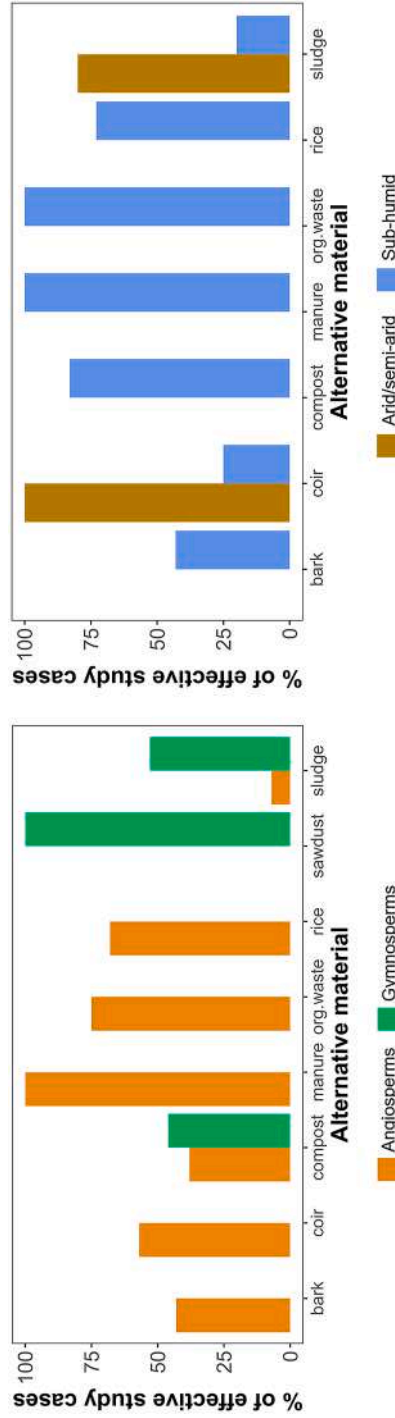


Figure 5.13 Proportion (%) of effective study cases for each AM separated into angiosperms and gymnosperms (left panel) and climate aridity (right panel). No effective case studies were found for angiosperms in sawdust and for gymnosperms in bark, coir, manure, organic waste, or rice. In dry climates, no effective cases were found for bark, compost, manure, or rice. In other cases, the absence of a bar in the graph indicates that 0% of effective study cases were found.

values < 0.5 . The effectiveness of rice and sludge depended on the aridity of planting sites ($\chi^2 = 4.054$, $P = 0.048$ for rice; $\chi^2 = 5.146$, $P = 0.040$ for sludge). Rice performed better on humid sites, while sludge performed better on dry sites. Although the coir showed a higher proportion of positive cases in dry climates, the difference was not statistically significant. For compost, manure, and organic wastes, no significant climate-related differences were observed, although there was a generally higher frequency of effective cases on humid sites (Fig. 5.13).

We conclude that there is potential for alternatives to traditional materials, especially to peat. Most AMs come from local circular bioeconomy models, meaning that utilizing local products or byproducts would greatly contribute to global sustainability in the forest nursery sector. The effectiveness of alternative GM materials depends on local ecological and economic conditions, which emphasizes the need to explore effective AM combinations tailored to the local context. Additionally, some alternative materials, like coir, require refinement for nursery use or are sourced from distant countries, raising sustainability concerns. These factors should be carefully considered in future efforts toward enduring sustainability.

Recommendations for nursery cultivation and field planting

Our quantitative review indicates that, in general, increasing the size of plants used in forest plantings enhances survival in the short term. However, there are situations in which using large plants may offer limited or no advantage. This is important when making recommendations. Specifically, the stocktype, particularly if BR, the intensity of soil preparation, and the functional traits of the species influence how variations in plant size and S/R affect survival. These effects are also frequently influenced by the aridity of planting sites. Generally, any factor or condition that potentially reduces transplanting seedling shock, such as intensive soil preparation, enhances the advantage of using larger plants. Accordingly, our recommendations differentiate plantings in dry climates with an aridity index ≤ 0.5 (Fig. 5.14 and Table 5.3) from those in humid climates with aridity index values > 0.5 (Fig. 5.15 and Table 5.4).

Functional traits are key to making specific recommendations for the cultivation in the nursery of a wide range of species. Understanding functional traits and their link to the ecological capacity of plants should be an essential component of the training for stakeholders involved in forestation. Traits such as SLA of a species can be used as an indicator to determine the most suitable seedling morphology for forestation under specific climatic conditions, as well as to optimize cultivation practices. For example, knowing that a species has high SLA values and low wood density indicates inherent rapid growth capacity and consequently high resource demand (Chave et al., 2009; Reich, 2014; Wright et al., 2004). These seedlings can potentially achieve substantial size in the nursery within a short period under favorable environmental conditions and resource availability. In this case, nursery managers should implement cultivation

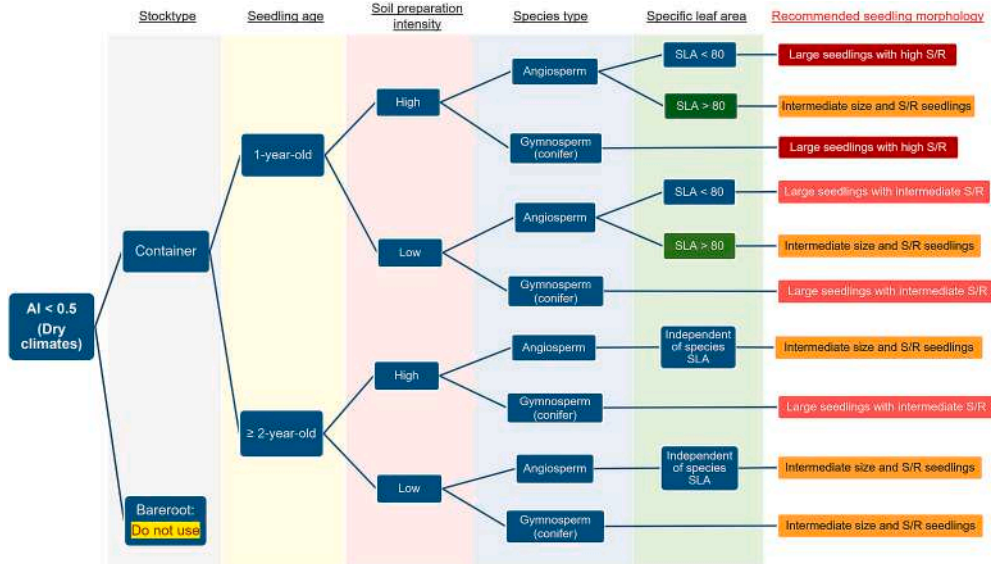


Figure 5.14 Decision diagram of the recommended morphology for planted seedlings in dry climates (aridity index ≤ 0.5) based on stocktype, soil preparation intensity, species type, and specific leaf area (SLA). Red boxes indicate large and high S/R seedlings. Light red indicate large seedlings with intermediate S/R, and orange boxes indicate intermediate size and S/R seedlings.

protocols that prevent the production of excessively large seedlings or those with a pronounced imbalance in the S/R, particularly when grown in small-volume containers. Conversely, species with low SLA and high WD typically grow slowly, requiring extended cultivation periods, and maximizing growth can be achieved through early sowing and intensive fertilization. Data on SLA and other functional traits for numerous plant species worldwide are available in the TRY Plant Trait Database (<https://www.try-db.org/TryWeb/Home.php>).

In our experience, a large seedling ready for planting is characterized by substantial biomass in the stem, roots, and, in the case of evergreen species, abundant foliage. For a given species and age, the morphology of seedlings varies depending on cultivation conditions, provenance, and seed size. This book includes a compendium of cultivation practices and plant quality characteristics (Chapter 11), detailing size ranges for the most common seedling types across numerous European forest species. We propose defining a large seedling as one with biomass above the 75th percentile of the species' size distribution. Seedlings of intermediate size would fall around the mean, while the use of seedlings below the mean size is generally discouraged due to their limited vigor (Grossnickle, 2012; Villar-Salvador et al., 2012).

Because biomass is not a practical metric for operational-scale evaluations, seedling size is typically assessed using root collar diameter and shoot height. Many regions

Table 5.3 Recommendations of the type of plant phenotype according to its morphology for forest plantings in dry climates—aridity index ≤ 0.5 —and main cultivation and planting hints to achieve these phenotypes and maximize outplanting performance, respectively. Shaded rows are specific hints for bareroot stock.

1		Avoid using bareroot plants except in wet habitats such as river forests or wetlands	
Angiosperms			
	Plantation context	Seedling characteristics	Cultivation and planting hints
2	<ul style="list-style-type: none"> • Container stock • Species SLA $< 80 \text{ cm}^2 \text{ g}^{-1}$, mainly evergreen • 1-year-old seedling (for species easily cultivated in one growing season) • Intense soil preparation at planting 	Large with high (but not strongly unbalanced) S/R	Early sowing at the end of winter to ensure a longer growing period (Cuesta et al., 2010a, 2010b). Cultivate in 300–400 mL containers. A larger container can increase field performance but reduce cost–benefit (Puertolas et al. 2012). Cultivation density should be adjusted to the species' leaf size and growth rate to minimize competition for light and irregular watering. Most species should not be shaded during cultivation; if shading is necessary to avoid excessive evapotranspiration, it should be moderate and limited to the warmest periods of the year. For species with osmotic potential at turgor loss $< \sim 2.3 \text{ MPa}$, moderate drought hardening can help to reduce S/R (Toca et al., 2022) and increase field survival. A medium to high fertilization rate is recommended during the rapid growth phase (Luis et al., 2009; Oliet et al., 2004, 2009a, 2009b; Villar-Salvador et al., 2004a; Villar-Salvador et al., 2013b, 2008), depending on if seedlings need to be large or of intermediate size. Pay attention to autumn fertilization. This practice does not alter seedling morphology but promotes nutrient loading and frost resistance, which benefits plant establishment (Andivia et al., 2011; Oliet et al., 2013; Rikala et al., 2004). Early planting in the wet season is highly advised (Andivia et al., 2011; Palacios et al., 2009).
3	<ul style="list-style-type: none"> • Container stock • Species SLA $< 80 \text{ cm}^2 \text{ g}^{-1}$, mainly evergreen • 1-year-old plant (for species easily cultivated in one growing season) • Low-intensity soil preparation at planting 	Large with intermediate S/R	
4	<ul style="list-style-type: none"> • Container stock • Species SLA $< 80 \text{ cm}^2 \text{ g}^{-1}$, mainly evergreen • ≥ 2-year-old plant (for species that can be cultivated within 1 growing season) • Independent of soil preparation intensity 	Intermediate size and S/R	

5	<ul style="list-style-type: none"> ● Container plant ● Species $SLA > 80 \text{ cm}^2 \text{ g}^{-1}$, mainly deciduous ● 1-year-old and older seedlings ● Independent of soil preparation intensity 	Intermediate size and S/R	Seed later in the nursery to shorten the growing season, combined with a medium fertilization rate during the rapid growth stage to develop intermediate size and S/R seedlings. If economically possible, use containers of $> 200 \text{ mL}$, preferably $\geq 300 \text{ mL}$, without shading or only moderately restricted to the warmest periods and times of the day of the crop. Drought hardening is not advised. We recommend autumn fertilization before foliage senescence. If it is a deciduous species, we recommend outplanting at the end of winter, as its roots do not grow in winter (Lyr and Hoffmann, 1967; Riedacker, 1976).
Gymnosperms (conifers)			
6	<ul style="list-style-type: none"> ● Container plant ● 1-year-old and older plants ● Intense soil preparation at planting 	Large with high (but not strongly unbalanced) S/R	Follow the same hints of planting context 2–4. If cultivation with older plants must continue to a second growing season, then the fertilization regime during the second vegetative period should be slightly lower than the first one to slow growth and reduce S/R. Fall fertilization is highly desirable.
7	<ul style="list-style-type: none"> ● Container plant ● 1-year-old and older container seedlings ● Low-intensity soil preparation at planting 	Intermediate size and S/R	Follow the same hints of planting context 2–4. If cultivation with older plants must continue to a second growing season, then the fertilization regime during the second vegetative period should be significantly lower than the first one to significantly slow growth and reduce S/R. Fall fertilization is highly desirable.

Table 5.4 Recommendations of the type of plant phenotype according to its morphology for forest plantings in humid climates (aridity index > 0.5) and main cultivation and planting hints to achieve these phenotypes and maximize outplanting performance, respectively. Shaded rows are specific hints for bareroot stock.

1			
Avoid using bareroot stock if soil preparation intensity is low			
Angiosperms			
Plantation context	Seedling characteristics	Cultivation and plantation hints	
2	<ul style="list-style-type: none"> ● Container stock ● Irrespective of species SLA, seedling age, and soil preparation intensity 	<p>Large with high (but not strongly unbalanced) S/R</p>	<p>If economically feasible, use containers > 200 mL, especially for broadleaves. Adjust cultivation density to species leaf size and growth rate to minimize competition for light and irregular watering. Drought hardening is not advised. A high fertilization rate is recommended during the rapid growth phase. Pay attention to autumn fertilization as it promotes nutrient loading.</p> <p>Adjust cultivation density to species leaf size and growth rate to minimize light competition. Most species should not be shaded during cultivation; if shading is necessary, it should be moderate and limited to the hottest weeks of the year and day. Drought hardening is not advised. A medium fertilization rate is recommended during the rapid growth phase. Perform autumn fertilization as it promotes nutrient loading.</p>
3	<ul style="list-style-type: none"> ● Bareroot stock ● Irrespective of species, SLA, and seedling age ● Intense soil preparation at planting 	<p>Intermediate size with large (but not strongly unbalanced) S/R</p>	
Gymnosperms (conifers)			
4	<ul style="list-style-type: none"> ● Container stock ● Species SLA > 60 cm² g⁻¹ ● Irrespective of soil preparation intensity 	<p>Intermediate size and S/R</p>	<p>Seed slightly later than in planting context 2.</p> <p>The rest of the nursery practices can follow the same hints of recommendations 3.</p>
5	<ul style="list-style-type: none"> ● Container stock ● Species SLA < 60 cm² g⁻¹ ● Irrespective of soil preparation intensity 	<p>Large with high (but not strongly unbalanced) S/R</p>	<p>Follow the same hints of the planting context 2.</p>
6	<ul style="list-style-type: none"> ● Bareroot stock ● Species SLA > 60 cm² g⁻¹ ● Intense soil preparation 	<p>Intermediate size and S/R</p>	<p>Follow the same hints of the planting context 3, but consider using drought hardening in the last cultivation stages to reduce S/R.</p>
7	<ul style="list-style-type: none"> ● Bareroot stock ● Species SLA < 60 cm² g⁻¹ ● Intense soil preparation 	<p>Large with high (but not strongly unbalanced) S/R</p>	<p>Follow the same hints of the planting context 3, but use high fertilization rates.</p>

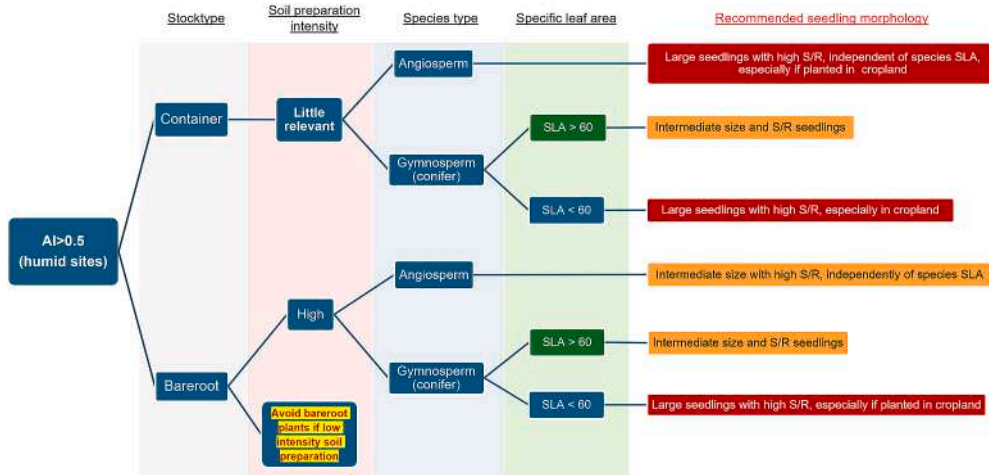


Figure 5.15 Decision diagram of the recommended morphology for planted seedlings in humid climates (aridity index > 0.5) based on stocktype, soil preparation intensity, species type, and specific leaf area (SLA). Red boxes indicate large and high S/R seedlings while orange boxes indicate intermediate size and S/R seedlings.

and countries have established quality standards based on these metrics (Mataruga et al., 2023). We recommend prioritizing the root collar diameter as a seedling size indicator, as shoot height is sometimes less reliable in predicting outplanting performance (Bayala et al., 2009; Grossnickle, 2012; Mexal and Landis, 1990; South and South, 1987), especially in seedlings grown at high densities or under prolonged shading (Puértolas et al., 2009). In such conditions, shoot elongation may not correlate well with increased biomass. Seedlings grown at high density or under shade often exhibit high height-to-root collar diameter ratios, resulting in slender phenotypes that should not be considered as “large seedlings.”

The recommendation to use large seedlings for a specific species should be evaluated based on the cultivation age. It is not advisable to use older seedlings to achieve larger sizes. For instance, if it is common to use 1 + 0 container seedlings for a species because this stocktype produces well-consolidated plugs and vigorous plants within one growth season, switching to 2 + 0 seedlings to increase size is not recommended. There are two reasons for this. First, our results indicate that increasing seedling age diminishes the positive effect of plant size on survival in dry sites (Fig. 5.1). Second, older seedlings are more likely to develop a pronounced imbalance between shoot and roots, which can negatively impact survival in arid areas. Marked shoot-to-root imbalances are expected to be more common in fast-growing species and when small-volume containers are used. This does not imply that 2 + 0 seedlings, which might be needed for operational reasons (e.g., 1 + 0 stocks that cannot be planted for any reason and remain in the

nursery), are unsuitable in scenarios where 1 + 0 plants are typically used. For species commonly grown in 1 + 0 formats, nursery practices should be adjusted to achieve appropriate size and S/R values for the 2 + 0 stocktype. For instance, reducing the fertilization rate in the second growing season or starting the cultivation in larger containers. Some species, such as *Abies alba*, *Pinus cembra*, and *Taxus baccata*, show slow juvenile growth, making it difficult to produce high-quality seedlings within the first growing season. For these species, seedlings at least two years old are typically used, and cultivation periods may extend up to four years. In such cases, the optimal planting size should be referenced to the values corresponding to each age.

Based on the data from our database, S/R imbalances do not appear to be a widespread issue, except in BR seedlings planted in dry areas and with plants older than one year. This suggests that, under standard cultivation protocols, plants do not develop high, unbalanced S/R that compromise their establishment. It is not feasible to establish a universal optimal S/R value, as this varies significantly between species, even among those that are phylogenetically and functionally related (see [Matías et al., 2017](#); [Villar-Salvador et al., 2013b](#)). For instance, in Mediterranean oaks (*Quercus* spp.), the optimal S/R for 1 + 0 seedlings is typically < 1 ([Villar-Salvador et al., 2013b](#)), whereas in Mediterranean pines, such as *Pinus halepensis* or *P. pinea*, the S/R is usually > 1.5 ([del Campo et al., 2011](#); [Dominguez-Lerena et al., 2006](#)). Some practitioners consider plants to have a balanced S/R when the length of the shoot and the container depth are the same. In container stock, this is not a suitable criterion, as for most species, shoot height typically exceeds container length during a single growing season in plants that have good field performance. Moreover, within the same container type, larger plants generally exhibit higher S/R values ([Oliet et al., 2009a, 2009b](#)).

Regarding the recommendation for alternative growing media, our results align with [Mariotti et al. \(2023\)](#), confirming that no single, universal formula for producing innovative and sustainable growing media exists for forest nurseries. However, some key recommendations can be derived from the most effective combinations tested, providing a starting point for further trials and guidelines for nursery use. All alternative materials were most effective at 40%–60% of the GM volume. This was the optimal range for compost and rice hulls, while bark was effective only at $\geq 40\%$. Coir, organic waste, and sludge were the most adaptable to different mixtures, exhibiting positive results across a broader range of proportions. For gymnosperms, the range of tested alternative media remains unfortunately limited, although promising results have been observed with sludge and sawdust. In angiosperms, compost, organic waste, and rice show the most potential without the need for mixing with traditional materials such as peat. Coir and sludge have been particularly effective in dry climates, performing better than in wet climates, which is important given the vulnerability of forestation efforts in arid areas. These differences related to climate might reflect that different species were tested, which potentially can show different responses to the same growing

medium. Most alternative materials are readily available, although their transportation from distant regions may pose environmental sustainability concerns. Globally, materials such as compost, coir, sludge, and wood-derived products are widely produced and commercially accessible, while materials such as rice hulls or manure are more relevant locally, particularly in rice-growing regions or less developed countries where manure is a valuable resource.

Further research on alternative GM, with an emphasis on outplanting performance, is needed to guide practitioners. Most GM studies focus solely on nursery cultivation, with no field validation, which limits concluding their effectiveness in forestation projects.

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