Ecophysiological responses of Preconditioning of Forest Species. A Review

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Received 10 April 2016; Accepted 23 May 2016

Abstract

Ecosystem-specific prevailing unfavorable climatic conditions pose obstacles to a successful regeneration. For the Mediterranean ecosystems those adverse growth conditions occur especially during summer when drought phenomena are more intense and frequent. Forest nurseries need to produce the best quality of transplanting material in order to achieve greater seedling survival. The aim of this paper is to review research conducted on seedling preconditioning of forest seedlings prior to regeneration, with emphasis on drought, in order to enhance the transplanting success. Other types of preconditioning, such as cold and mycorrhizae, are also analyzed. The hypothesis is that seedlings that have undergone a degree of stress enhance seedling characteristics that enable the seedlings to overcome transplanting stress. Studies showed that under drought preconditioning seedlings tent to decrease their height and shoot/root ratio indicating greater dry mass accumulation to the below ground seedling parts. Also, it was suggested that acclimation occurred mostly through the reduced level of stomata conductance and transpiration rates. Overall, this review will provide a better understanding on the responses of seedlings that experience preconditioning prior to transplant.

Keywords: drought preconditioning, Ecological implications, rehabilitation, restoration, semi-arid ecosystems

1. Introduction

The frequency and intensity of disturbance in Mediterranean ecosystems is very high mainly due to extreme natural disasters (e.g. fires) [1] in close relation to the anthropogenic interferences (e.g. alteration in land uses). For those ecosystems, the success of the regeneration efforts troubles many scientists, since the prevailing summer drought conditions is one of their main limiting factors that negatively affects seedling survival [2, 3, 4, 5, 6]. The periodic highly intense drought phenomena pose obstacles to regeneration efforts that are associated to intense climatic alterations [7, 8].

Forest nurseries try to produce a substantial number of seedlings in order to respond to the regeneration needs of a specific site. They have to produce the best quality of transplanting material in order to achieve greater seedling survival after reforestation efforts. The ability to produce adequate number of seedlings, accompanied with the “best” quality to tolerate adverse environmental conditions that Mediterranean ecosystems pose, is a great challenge.

In addition, the severity and duration of those drought periods will increase due to predicted warmer temperatures should increase [9, 4]. Further, abnormal growth patterns might induce events, such as late-spring frosts that can result in increased seedling mortality. Those climatic conditions, particularly during the first period of seedling establishment after the transplant are very substantial for their survival and further growth [7].

Foresters understand that it is hard to reduce the transplanting shock that seedlings undergo during the period of establishment. Nonetheless, research has indicated that there are techniques that can be used as tools to help reduce the transplanting shock and assist with the further growth and establishment of the seedlings. Preconditioning is one of those tools that help seedlings to get better equipped to overcome those potentially adverse growth conditions and successfully establish on the site. “Preconditioning” also known as ‘hardening” is a method that is exposing seedlings under fixed growth conditions in order to physiologically and morphologically enhance functional traits that should prepare the seedlings to tolerate and resist the potential adverse field conditions. The idea is that seedlings that have undergone a degree of stress, such as lack of water that mimics drought growth periods, so seedlings after their biomass allocation by enhancing traits that help them sustain viability under adverse conditions at the regenerated sites. One of those traits is increased ability to produce new roots. Research has indicated that characteristics such as vigorous root systems enable the seedlings to withstand and tolerate post-planting drought conditions [10, 11, 12, 13]. Hence, the aim of this review is to explore work that has been conducted on preconditioning forest species and present results that reflect morphological as well as physiological alterations of the seedlings. Emphasis will be given on the drought preconditioning, since this is the main concern for the Mediterranean ecosystems.
2. Drought preconditioning

Drought-preconditioning is a technique that is usually performed by the nurseries prior to seedling transplant in order to reduce the transplant shock [14, 15]. This technique has been widely applied in boreal or temperate habitats. It can be applied by either by reducing the irrigation frequency or quantity, usually during the last weeks or right before transplanting occurs [16, 17].

Drought preconditioning has been performed for a while for forested species. Early work showed that xeric ecotype seedlings of *Pseudotsuga menziesii* (douglas-fir) when exposed to drought preconditioning decreased their transpiration rates for water conservation when compared to mesic ecotype seedlings [18]. Additional research for the same species showed reduced mortality levels during the storage of the seedlings after they were preconditioned to mild moisture stress [19]. Further, those seedlings were also associated with decreased height and shoot/root ratio with greater accumulation of dry matter to the roots. Seedlings of *Picea mariana* Mill. BSP (black spruce) showed tolerance during water stress by maintaining lower osmotic, water potentials and stomatal conductance, while turgo increased [20]. Further, drought preconditioning of *Picea mariana* (black spruce) seedlings derived from upland and lowland areas, had greater values on net photosynthesis, stomatal conductance and transpiration rates, while there were no differences in water potential and total sugar content, with acclimation occurring mostly through the stomatal and photosynthetic adjustments [21]. Preconditioned seedlings of *Pinus ponderosa* survived 14 days longer under additional imposed drought conditions than well watered seedlings, with needle morphology and biomass allocation patterns being better surrogates in determining drought tolerance than gas exchange patterns [22]. One-year-old *Pinus halepensis* seedlings that were subjected for two months under four water regimes, indicated that root growth was significantly reduced for seedlings that experienced the greater water stressed conditioned [23]. Drought-preconditioning seedlings of *Cedrus brevifolia* Henry, *C. libani* Loudon and *C. atlantica* Manetti decreased the quantum yield of PSII at higher temperatures levels (3 to 4°C), while maintaining high net CO₂ assimilation levels (80%) when compared to well-watered plants [24]. This indicated that drought-preconditioned seedlings were thermo-tolerance; a substantial attribute of the species that are exposed during their growth to increased temperature conditions. Drought preconditioning of *Juniperus oxycedrus* survive 12 days longer than non-preconditioned seedlings, possibly due to water-conserving strategy associated to stomata closure at relatively high water potentials, while they did not show any alterations to root/shoot, as well as chlorophyll fluorescence, leaf capacitance, cell water relationships and transpiration rates [17].

For the broadleaved species of *Q. coccifera* and *J. oxycedrus* it has been found that drought preconditioned seedlings showed similar response in terms of chlorophyll fluorescence, leaf capacitance, cell water relationships and transpiration rates, while *P. lentiscus* preconditioned seedlings maintained higher water content that allowed higher net CO₂ assimilation rates [17]. For the species of *Pistacia lentiscus* L., it was also indicated that under low water availability resulted to the overall reduction of seedling biomass [25]. The ratio of the leaf area to the roots surface area was greater for seedlings that received the least water. In addition, the sapwood area that actively contributed to water uptake was correlated primarily with the leaf area and the surface area of the newly developed fine roots. They suggested that this was an opportunistic behavior of the species that benefits from periods of high water availability by investing in the growth of new roots. This might imply that this species might not respond well under prolonged water scarcity conditions. For the species of *Arbutus unedo* reduced water availability induced increase in the root collar diameter and the overall above ground seedling accumulation while decreasing the below ground seedling growth levels [26]. Specifically, the total dry weight, leaf dry weight and leaf weight ratio were lower for the drought preconditioned seedlings, while they had greater means for the root weight and root/shoot ratio. For *Prunus armeniaca* L. cv. Búlida seedlings it has been indicated that a daily irrigation at 25% field capacity induced plant hardening that enabled plants to endure drought growth conditions better, due to their greater osmotic adjustment that prevented plants from dehydration and leaf abscission [27].

Drought preconditioning was also conducted for the highly demanding in water availability species of *Betula papyrifera* Marsh. and *Populus balsamifera* L. [28]. The results indicated for both species decreased shoot biomass and increased the root/shoot ratio. However, the two species responded differently in terms of assimilation and water utilization rates, with *Betula papyrifera* following a water-conserving strategy as revealed by lower net assimilation rates and water utilization compared to *Populus balsamifera*. Further, for *Salix* cultivars that were subjected to drought acclimation had decreased stomatal conductance and osmotic potential [29]. Drought preconditioning has also conducted for *Eucalyptus globules* that indicated significant alterations in both morphological and physiological characteristics [30]. Specifically, preconditioned seedlings were more stunted in their height, root collar diameter and leaf area, while they also had greater stomata conductance, water potential and predawn relative water content compared to non-preconditioned seedlings. Further, the reduced leaf area and the osmotic adjustment helped the acclimation of preconditioned seedlings that were also resulted to lower mortality rates when compared to non-preconditioned. Guarnaschelli et al. [31] also found similar responses to drought preconditioning seedlings for *Eucalyptus globulus* subsp. *bicostata* with stunted growth levels as expressed with reduced growth levels such as leaf area and overall seedling size.

3. Other preconditioning types

**Cold preconditioning (cold hardiness):** One of the nursery practices prior to transplant is preconditioning seedlings to cold temperatures in order to increase their tolerance to early frost conditions. The idea is that plants that occur in cold regions undergo a hardening process in...
the fall that enables them to survive lower temperatures [32]. Particularly coniferous species depend on their freezing ability to overcome winter and survive under low temperatures that reach values way below 0°C, as well as to overcome cycles of freezing and thaw [33, 34]. Many scientists have used frost preconditioning, especially for conifer species since their foliage (needless) is frequently exposed to temperatures bellow 0°C. Cold-hardiness has an impact on the ability of forest seedlings to withstand lifting, storage as well as transplanting stresses. The level of cold-hardiness can be used to induce stress at the time of lifting that ultimately increases the resistance and tolerance of the seedlings to be stored and transplanted with increased levels of frost tolerance. So, many plants increase their freezing tolerance at the end of summer and fall upon exposure to low non-freezing temperatures and short days, a phenomenon known as cold acclimation. At the beginning of spring, when temperatures are rising, this process is reversed. That is more applicable to northern areas (ex. Canada) for conifer species where low temperatures dominate the ecosystems. However, early and late frost events that could severely kill all plants, usually occur in Mediterranean ecosystems that might predispose seedlings to adverse growth conditions and could severely harm them causing even mortality.

Several processes involved in drought tolerance confirm also an increase in cold tolerance [35, 36]. It has been observed that drought resistant genotypes of *E. globulus* showed greater cold tolerance than drought susceptible ones [37]. This implies that the application of drought hardening treatments may also increase their cold tolerance. The accumulation of solutes that commonly occurs under water stress, decreases the osmotic potential and may cryoprotect freezing cell structures. Coopman et al. [38] applied two drought hardening treatments at different genotypes of *E. globules* subsp. *globulus* under nursery conditions that resulted to increased drought tolerance as well as their freezing tolerance. They observed that freezing tolerance varied within the genotypes and the level of drought preconditioning.

Research investigated the genetic effect on fall and spring cold hardiness in two western Oregon breeding populations (Coast and Cascade mountains) of *Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco (Douglas-fir) that were also subjected to two drought preconditioning “well-watered” and “mild drought” regimes [39]. Results indicated that cold hardness needs to be managed as two traits of fall and spring cold hardiness. Further, seedlings grown under summer drought sustained significantly less cold injury in the fall than those that were well-watered. For Douglas-fir mild water stress (-5 to -10 bars) introduced during late July until the end of August improved the cold hardness of the seedlings, but it also increased the root weight and decreased levels on the seedlings’ height [19].

**Nutrient preconditioning:** Nutrient preconditioning may also be used to help nursery practices related to application of fertilization levels to produce suitable seedlings for field transplant. For the species of *Quercus* spp., fertilization at 1.68 g of nitrogen per seedling, with nitrogen content of 40% for *Quercus rubra* and 35% for *Quercus alba* [40]. Also, experiments that were conducted under greenhouse conditions indicated that increased fertilization levels resulted in increased heights, shoot/root mass ratio and root nitrogen content [41].

Research has also been conducted on the combined effect of drought preconditioning and nutrient availability. Specifically, the effect of high nitrogen levels on the growth of drought preconditioned was studied of the Mediterranean species of *Pinus pinea* L. [42]. The results indicated that drought preconditioning enhanced the tolerance of the plants, while nitrogen preconditioning levels reduced their tolerance, with no effect on the drought acclimation ability of the seedlings. Under field conditions, plants with increased nitrogen fertilization levels under drought conditioning indicated decreased growth, while under mesic conditions their growth was better. The results revealed that plants that were grown under increased nitrogen levels were more susceptible to frost damage with decreased plasmalemma stability to dehydration. Also, those seedlings despite the fact that were more vigorous with greater stomata conductance, new root growth capacity and respiration, they also showed increased levels of frost damage. Only seedlings that were exposed to drought preconditioning indicated decreased frost damage, stomatal conductance and transpiration. Overall, while drought preconditioning increased the overall tolerance of the seedlings, increased nitrogen levels reduced it and yet had no effect on the drought acclimation of the seedlings.

Similar research on nitrogen and drought preconditioning for *Pinus contorta* var. *latifolia* Engelm. seedlings indicated that the starch concentration in the leaves increased when nitrogen or water were limited [43]. Also, preconditioning under low nitrogen levels increased subsequent root weight ratio and reduced the concentration of sugars that is an asset of the seedlings to drought resistance. Harvey & van den Driessche [44] observed that increased nitrogen supply under dry conditions increased leaf loss and decrease water potential in *Populus trichocarpa*, making them more vulnerable to cavitation. The responses of *Pseudotsuga menziesii*, *Pinus contorta* and *Picea glauca* were analyzed by van Den Driessche [14] for seedlings that were grown in containers under greenhouse conditions and subjected to two nitrogen-, three potassium- and three drought treatments. The research indicated that increased nitrogen levels increased shoot growth, drought resistance and survival for *P. contorta* and *P. glauca*, while *P. menziesii* showed an interaction between drought and nitrogen treatment with small response in survival and dry weight to potassium (K) application.

Further, research on nutrient preconditioning of seedlings in the nursery showed a strong potential in improving the morphology of *Quercus suber* seedlings [45]. However, the relationship between these changes and seedling survival were not clearly determined. Further, research indicated that a year after transplanting survival was negatively correlated with the plant size for the species of *Pistacia lentiscus*, *Quercus coccifera*, *Rhamnus lycioides*, *Rhamnus alaternus* and *Tetraclinis articulata* [46]. Seedlings that were grown under nutrient deficit conditions had greater ability to establish under semi-arid environments than well-fertilized seedlings.
**Mycorrhizae preconditioning:** The increased ability of nutrients uptake has been attempted to be enhanced by inducing the symbiotic association of mycorrhizae. This is another potential cultivation practice for forest nurseries that can be achieved through the inoculation of ectomycorrhizae. Research has indicated that the presence of ectomycorrhizae for the species of *Quercus suber* L. resulted in increased levels of leaf area, seedling dry weights and increased water use efficiency [47]. Further, they had greater nitrogen content, photosynthetic pigments as well as photosynthetic capacity. More importantly, the research showed that inoculated plants with ectomycorrhizae had increased growth and survival after a year of field transplant. Similar results were obtained also for *Quercus ilex* [48].

The effect of mycorrhizae has also been determined in relation to drought preconditioning for the species of *Arbutus unedo*, a species of high production value [49]. This was a study that was conducted under nursery conditions and indicated a superiority of drought preconditioned and mycorrhiza inoculated plants in relation to growth patterns that resulted to increased root dry weight, leaf water potential values, stomatal conductance and photosynthesis.

**Preconditioning:** Preconditioning in regard to tolerance to flood has also been applied prior to seedling transplant for forest species that usually occur and grow in riparian areas. Specifically, experiments related to flood-preconditioning have been conducted for the species of *Taxodium distichum*, *Quercus nuttallii* and *Quercus michauxii* [50]. They showed that the net photosynthesis substantially decreased for preconditioned seedlings of both the oak species with no affect on the overall growth and their physiological responses. On contrary, for the *Taxodium distichum* the net photosynthesis was not affected, while the stomata conductance increased when compared with seedlings that were not subjected to flood-preconditioning.

**4. Conclusions**

Overall, preconditioning can be used to enhance the functional traits that promote the resistance of forest seedlings to tolerate adverse environmental growth conditions. Mediterranean ecosystems are usually characterized by summer droughts, while frost damage might also be an issue for the welfare of the seedlings. Based on the reviewed literature, drought preconditioning is a promising forest practice that could help the transplanted seedlings to overcome both summer droughts and spring frost conditions.

*This paper was presented at International Conference titled "Frontiers in Environmental and Water Management", that took place March 19-21st 2015, at Kavala Greece.*

**Acknowledgment**

This research project is funded under the Project ‘Research & Technology Development Innovation Projects’-AgroETAK, MIS 453350, in the framework of the Operational Program ‘Human Resources Development’. It is co-funded by the European Social Fund through the National Strategic Reference Framework (Research Funding Program 2007-2013) coordinated by the Hellenic Agricultural Organization – DEMETER (Institute: Forest Research Institute/ Scientific supervisor: George Chalyvopoulos).

**References**

32. J. Levitt, Minneapolis: Burgess (1941).
44. H.P. Harvey, and R. van den Driessche, Tree Physiology. 19, 943 (1999).