

PRE-PLANT STORAGE AND HANDLING OF DIFFICULT TO TRANSPLANT ASH
ALTERNATIVES

By

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ABSTRACT

PRE-PLANT STORAGE AND HANDLING OF DIFFICULT TO TRANSPLANT ASH ALTERNATIVES

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The loss of ash (*Fraxinus* spp.) trees in the midwestern United States due to emerald ash borer has emphasized the need for more diversity in urban and community forests. However, nursery growers report that many *Fraxinus* spp. alternatives are difficult to transplant. Understanding the physiological reasons why one tree species has superior transplantability over another is an important factor in increasing production of *Fraxinus* spp. replacements. In this study, we examined five difficult to transplant ash alternatives; *Celtis occidentalis*, *Quercus bicolor*, *Quercus ellipsoidalis*, *Quercus rubra*, *Taxodium distichum*, and *Fraxinus americana*. To mimic nursery grower practices, trees were assigned at random to one of four treatments: 1) control, 2) heeled-in, 3) cold storage, or 4) sweating. After treatment, trees were assigned at random to one of three evaluations: root growth potential (RGP) test, container planting or field planting. Root growth potential was very low for *Quercus* spp. trees and varied by treatment for *C. occidentalis*. *Taxodium distichum* trees had a relatively high root growth potential in all treatments. Stem water potential measured immediately before and after pre-plant treatments suggest that *Quercus* spp. trees and *T. distichum* trees were able to rehydrate during sweating while water stress levels in *C. occidentalis* trees remained high. The results suggest that poor transplanting success reported for *Quercus* spp. trees may be related to low root growth potential, whereas poor success of *C. occidentalis* trees may be due to shoot desiccation during storage and handling.

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INTRODUCTION

Improving our understanding of factors that contribute to poor transplanting success of important ash alternatives will have direct impacts on landscape nurseries in Michigan and surrounding states and on landscapers and urban foresters.

Improving the diversity of species available for urban tree planting is critical in many areas of Michigan. In order to meet the demand to replace trees lost to emerald ash borer, urban and community foresters must rely on currently available species, many of which are already over-represented in these communities. Continued over-reliance on a few species increases the potential for catastrophic tree losses due to future exotic pest introductions.

The goal of this project was to improve the overall availability and success of important ash alternative species in production nurseries to facilitate a more diverse urban landscape.

The objectives of this study was to determine the effect of pre-plant handling on initial survival and growth of ash alternatives and to determine the rehydration, root growth potential and carbohydrate response of ash alternatives following pre-plant handling. Pre-plant treatments act to overcome dormancy, promoting rapid bud break and subsequent leaf area development. They also promote rapid resumption of root growth resulting in greater root-soil contact and minimizing overall tree moisture stress following transplanting. A secondary objective of this project is to determine the utility of gravel culture as a system to evaluate tree root growth potential.

The results of this study will aid urban and community foresters in their goal of working toward a widely diverse landscape. Tree diversity in community and urban forests can help mitigate crises that are caused by exotic pests and diseases, like the emerald ash borer.

CHAPTER ONE
LITERATURE REVIEW

Importance of the problem

The scope and value of shade tree nurseries and hardwood forest nurseries in the United States is preeminent. Tree production and care services were valued at \$14.55 billion in 2002, with a total output impact of \$21.02 billion, and creating over 250,000 jobs (Hall et al., 2005). Also stated in the study, well landscaped homes with suitable tree canopy have a 7 to 11 percent premium in value compared to similar properties without such additions. In 2006, deciduous shade trees were the third ranking category of nursery production crops with total sales of \$584 million, up 14 percent from 2003 (United States Department of Agriculture, 2007). Landscape trees in the urban community are not only important for economic advantages but for environmental improvements. Other studies have shown the importance of other non-monetary economic and environmental impacts, including energy savings for building heating and cooling, reduction of atmospheric CO₂, enhanced air quality, reduced runoff from stormwater, and other aesthetic benefits (Hall et al., 2005).

Renewed interest in diversity

Tree diversity in community and urban forests can help mitigate crises that are caused by exotic pests and diseases, like the emerald ash borer and Dutch elm disease. These devastating losses seen in communities may have been avoided by creating a diverse landscape to spread the risk of an exotic pest or disease from destroying an entire community. Monoculture had been the main reason why Dutch elm disease overwhelmed towns and cities killing a significant portion of urban forests. After the devastation caused by the Dutch elm disease over a 40-year period, attention was given to the dangers of planting a single species or only a few species in the landscape

(Bassuk, 1990). However, history seemed to repeat itself with the over abundance of ash (*Fraxinus* spp.) trees planted in urban communities and the invasion of the emerald ash borer in the early 1990s, detected in 2002. Many community forests were infected in mid-western states, most prevalently in Michigan. There are efforts to slow the progression of the EAB spread throughout the Midwest and beyond, by using a program called SLAM (SLOw Ash Mortality) (Collins & Mccullough, 2012). This program, a collaborative effort between universities and government agencies, is to impede and decrease the expansion of ash mortality by diminishing populations of the beetle in newly-infested sites, outside of known EAB infestations (Collins & Mccullough, 2012). Even with the efforts to slow down the spread of the EAB, Kovacs et al. (2011) project the spread of EAB based on the current rate of spread to infest roughly 30 states by 2020 (Figure A1.1).

The reduction in the beetle population and rate of spread is important, however, creating a diverse landscape is also important. Biological diversity is regarded as the basis for ecological stability (Cleland, 2011), consequently creating a diverse urban landscape is essential. Also, street trees are generally the main focus for many community forestry programs and the resources for establishment, maintenance, and removal of these trees consist of the majority of many urban forestry budgets (Kielbaso et al. 1988). Diversity in community and urban forests is important so depredation of entire communities can be prevented.

Bareroot production cycle

The journey of a bareroot landscape tree from liner production to the landscape is a long and arduous one. The venture begins at the liner/propagation nursery where

the bareroot liners are lifted from the field in early to mid-winter and placed in storage. The root system is, more often than not, compromised during harvest, handling, storage and shipping, and root pruning before transplanting (Struve, 1996). The storage area may be inside a barn with low light, in a refrigerated barn with low light, or outdoors in substrate, like sawdust or woodchips (Englert et al., 1993). They remain in this storage facility until processed for delivery. The delivery process begins by loading an order onto a refrigerated truck. Depending on where the trees are being delivered and how many different orders are being filled, the trees could remain in the truck for up to three weeks. Once the trees are delivered, the nurseries will typically employ storage techniques or treatments; heeling-in, cold storage, sweating, and misting. The techniques or treatments include burying the roots in a mulch trench outdoors (heeling-in), storing them in a shed or barn where there are low light and cool temperature conditions (cold storage), storing them in a warm and humid greenhouse under moist burlap (sweating), or submerging the roots in a pond or spraying the tree with water (misting). The storage techniques or treatments will commonly last for a few weeks until the ambient temperatures are adequate for transplanting. The wholesale nursery will typically grow the trees on for 1 to 3 years and then sell them to retail nurseries or landscape companies.

During the period of time that the bareroot trees are handled and stored, they can become desiccated. The nurseries will typically employ those storage techniques or treatments; heeling-in, cold storage, sweating, and misting, to aid in rehydration of the roots and shoots, overcome dormancy, and initiate bud break. Different species, however, react differently to these treatments. For example, Norway maple (*Acer*

platanoides L.) is a desiccation-tolerant species that can operate under low water status conditions and survive, while Washington hawthorn (*Crataegus phaenopyrum* Med.) is a desiccation-intolerant species that does not succeed under low water status conditions (Murakami et al., 1990). These treatments, regardless of species, are meant to improve the survivability of the trees after transplanting.

Potential causes of the problem

Regardless of the high probable mortality rate due to transplant shock, bareroot whips or lightly branched bareroot liners are the most commonly used because the ease of handling and shipping (Struve, 1996). The high mortality is generally due to the lifting and the amount of root pruning done to the liners when harvested (Struve, 1996). The reestablishment of the root system after transplanting is an important factor for tree survival. A consequence of a disproportion in the shoot surface to the root surface can result in water stress for bareroot seedlings (Baldwin & Barney, 1976). Factors affecting root regeneration and bud break are carbohydrate status and water status in the plant. The initiation of new roots by bareroot trees depend on the amount of carbohydrates stored in the stem and root tissues (Webb & Lakes, 1980). There is a seasonal carbohydrate flux in trees that affects the amount stored for reserves. Trees undergo an autumn buildup of carbohydrates to store for overwintering and to be used for spring establishment (Kobe, 1997). These stored carbohydrates are essential for the re-growth of roots and initiation of bud break at planting. Tree species that have episodic shoot growth during the season will retain less reserves in the fall than tree species that have an initial burst of shoot growth in the early growing season (Canham et al., 1999).

Carbohydrates are important for the trees ability to overcome stresses imposed during the production cycle. In the most collective terms, carbohydrate reserves seemingly play a crucial role in the reestablishment and revival of seedlings from a wide range of agents of stress and physical damage, encompassing from herbivory to frost heaving (Canham et al., 1999). Carbohydrate status is affected by respiration, temperature, and duration of cold storage (Ritchie, 1982). If an insufficient amount of carbohydrates is available the tree will be stressed upon transplanting. Davis & Jacobs (2005) explain that root carbohydrate content may possibly forecast seedling survival and growth when transplanted, most notably when seedlings are not photosynthesizing. They also state that the root carbohydrate content could be an indication of the internal reserves and could prove useful in ameliorating the survival of species that are likely to have dieback (i.e., *Quercus* spp.) (Davis & Jacobs, 2005). In storage situations, moderate temperatures and light deprivation over a continual period will accelerate the rate at which stored carbohydrates are used and it may contribute to the reduced root initiation and growth needed from the stored carbohydrates (McKay, 1996). A recent study by Cabral & O'Reilly (2008) showed that warm storage before planting did not benefit bareroot trees. Some nurseries may store the bareroot trees in an environment which may be warmer than the storage conditions the trees previously experienced. The study explains that trees placed in warm storage experienced delayed bud break and shoot growth, reduced height and diameter growth, stem quality, total biomass, and root growth. The longer the trees were stored in the warm environment the more negative affects were observed (Cabral & O'Reilly, 2008).

Root growth potential (RGP) is the ability of a tree to initiate and lengthen roots when placed into an environment favorable for root growth (Ritchie, 1985). Characteristics to quantify root growth include number of new roots, roots greater than a specific length, total root lengths, and regenerated roots (Struve, 1990). These characteristics are used to predict field performance and survival. The degree of RGP is often associated with survival and growth. Struve (1990) explains that root morphology and the rate of root regeneration are factors in the ease of transplanting. Coarse-rooted species (Hallgren & Tauer, 1989) regenerate new roots from adventitious roots, while fibrous-root species regenerate roots primarily through elongation of existing roots and subsequently from adventitious roots (Struve, 1990). Important factors that can implement control of RGP are date of lifting and duration of cold storage (Ritchie, 1985). Root growth potential or actual root growth are affected by temperature, water content, carbohydrate status, storage, and handling. Root growth potential varies seasonally, with the highest potential occurring mid-winter (Ritchie, 1985). The handling or mishandling of bareroot trees also affects the RGP when trees are lifted and/or stored (Simpson & Ritchie, 1996).

Water status of bareroot trees is another important factor in a successful transplant. Desiccation stress imposed at harvesting, storage, shipping, and transplanting is thought to be one of the major causes of re-growth failure (Murakami et al., 1990). There is a period during the dormant season when trees have the highest desiccation tolerance. The peak time and range of desiccation tolerance differs between species. Many of the trees have their peak desiccation tolerance in January or February (Englert et al., 1993). Trees should be harvested during their desiccation

tolerance peak to aid in reducing water and carbohydrate stresses. With a lower tolerance to desiccation the trees may become dried out and have issues with establishing their root system and shoot and root dieback after transplant (Englert et al., 1993).

The coarseness of a root system may explain why some species are more tolerant to desiccation than others. In a study by Englert et al. (1993) red oak trees were the most desiccation-tolerant and also had the coarsest root system of the three species in the study. They describe that the larger roots would have a “lower surface area to volume ratio,” which may have contributed to reduced water loss and able to withstand longer desiccation periods. Exposure of the roots system to the atmosphere after harvest can greatly reduce tree water status. Trees, if not protected, may lose water resulting in desiccation in roots and shoots (Bates et al., 1994), which greatly limits the reestablishment of roots and may cause significant dieback in shoots. Different species may react differently to conditions during the pre-plant processes. However, Murakami et al., (1990) found that in spite of the dramatic difference in desiccation tolerance between species and cultivars, the rate of water loss from either whole plants or root and stem tissues was similar.

Approach to dealing with transplant stress

The pre-plant storage and handling of bareroot trees are known to impair the rate of root growth, carbohydrate status, or water status and will decrease the transplanted trees survival (Watson & Himelick, 1982a). The treatments that nurseries employ help to lessen or eliminate the stress on transplanted trees. These methods assist the

transplanted tree to break dormancy, increase root growth potential, and effectively utilize carbohydrate reserves.

Heeling-in is a storage treatment used to aid in improved water status and root regeneration. Heeling-in consists of placing the roots of the trees in a trenched section of substrate or mulch, covering, and watering them in. Heeling-in has also been described as “high-density planting in a furrow” (Strange et al., 2002). The treatment reduces transplant shock and supports root regeneration (Starbuck et al., 2005).

Allowing the trees to sit in moist mulch for roughly one to four weeks can help the survival rate of the trees after they are transplanted. In a study done by Struve (2009), heeling the trees in pea gravel resulted in better root growth than in wood chips.

However, the trees in this study were left in the media for roughly six months. Strange et al. (2002) states that leaving heeled-in trees for more than one season will increase the difficulty of transplanting and decreases survivability. Concurring with Strange et al., (2002), Scianna et al., (2005) states that long-term storage of heeled-in plants will increase stresses on the plants.

Storing bareroot trees in a cooler is another common nursery practice. If the trees arrive from the wholesale nursery too early in the season to be planted, placing them in cold-storage is beneficial. Some of the benefits include increased RGP after transplanting, the tree’s ability to withstand colder temperatures, thus improving cold hardiness, and increased growth rates after transplanting (Lundberg et al., 1990).

However, cold-storage has some drawbacks. These include drying out of the roots and shoots, sub-optimal temperatures, loss of dormancy due to inappropriate temperatures, and disease if not handled properly (Dozic, 2009). As cited by Murakami et al. (1990),

Webb and von Althen (1980) showed that moisture loss by broadleaf trees during cold storage negatively affected their RGP and, subsequently, their survival and vigor. Venator (1985) conducted a study on the effect storage had on shortleaf pine seedlings. His results showed that the longer seedlings were in the storage treatments the lower the survival rates were after planting. Lifting time of bareroot trees is also believed to have an impact on cold-stored trees. Kozlowski & Davies (1975) found that lifting seedlings in early fall and placing them in cold storage reduced RGP but increased it when the seedlings were lifted in early winter. Similarly, Murakami et al. (1990) stated that plants harvested during the dormant season, in general, were better able to withstand postharvest handling and cold storage conditions. Also favorable for cold storage, several studies also illustrate the need to cover the roots and/or the shoots (Bates et al., 1994; Bates & Niemiera, 1996, 1997; Webb & Lakes, 1980) during cold storage, which helps to reduce desiccation. Along with research that documents reduced desiccation by covering roots and/or shoots, Bates & Niemiera, (1996) found that coating shoots with wax prior to cold-storage significantly decreased desiccation. This practice is commonly used for bareroot rose cranes. All of these beneficial treatments proposed for cold-stored bareroot trees can increase their chances of survival.

Sweating is another beneficial practice that forces bud break and active growth. The main objectives are to rehydrate the root system by increasing the humidity and temperature surrounding the plants (Halcomb & Fulcher, 2004). Common knowledge regarding sweating seems to be that implementing it on many bareroot tree species is beneficial but some trees are so deep in dormancy that they need to be sweated before

they can be transplanted (Bismarck Plant Materials Center, 2007). Desiccation-intolerant species, including hackberry and Washington hawthorn, may benefit from the breaking of dormancy and root rehydration (Murakami et al., 1990) that sweating promotes increasing its survivability.

Misting is another technique used to rehydrate roots and shoots before and after transplanting. The common nursery practice is to continuously spray with a water source. Misting the trees is thought to increase the water potential of the tree. Bates & Niemiera (1994) found that the water potential of desiccation-tolerant, Norway maple (*Acer platanoides* L.) and desiccation-intolerant, Yoshino cherry (*Prunus x yedoensis* Matsum.), increased when they were misted. The non-misted cherry trees were deemed unmarketable. The misting treatment could be another beneficial technique to improve the survivability of transplanted trees.

The pre-plant storage and handling of bareroot trees is important to the overall success and survivability after transplanting. There is a connection between root growth potential, water status, and carbohydrate status in the trees. Techniques used to decrease root and shoot desiccation, increase root regeneration, and reduce stress of transplant shock are widely used in the nursery industry. There is little published scientific research on the physiological reasons why these treatments work to increase survivability of bareroot landscape trees after they are transplanted.

APPENDIX 1

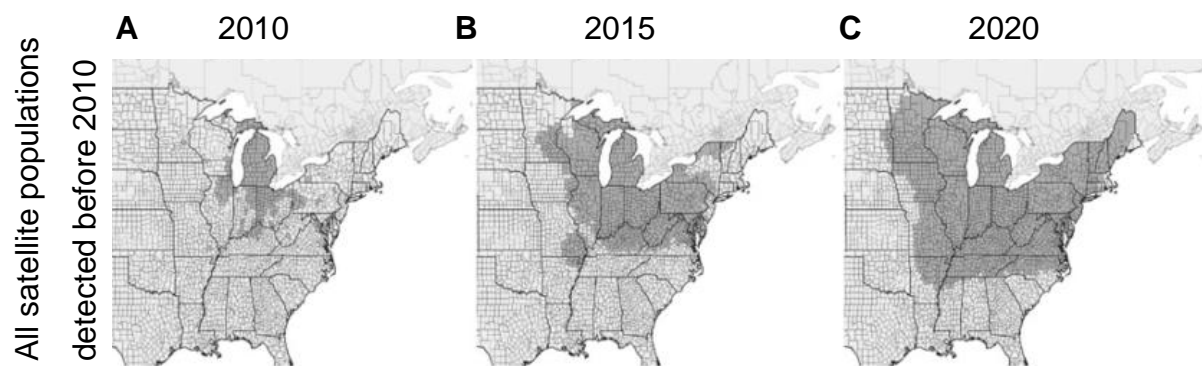


Figure A1.1. Simulation of the emerald ash borer spread in counties from March 2010 to March 2020 (base case). The maps represent the rounded average of 200 stochastic simulations (Kovacs et al., 2011).

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CHAPTER TWO

PRE-PLANT STORAGE AND HANDLING OF DIFFICULT TO TRANSPLANT ASH
ALTERNATIVES

Abstract

The loss of ash (*Fraxinus* spp.) trees in the midwestern United States due to emerald ash borer has emphasized the need for more diversity in urban and community forests. However, nursery growers report that many *Fraxinus* spp. alternatives are difficult to transplant. Understanding the physiological reasons why one tree species has superior transplantability over another is an important factor in increasing production of *Fraxinus* spp. replacements. In this study, we examined five difficult to transplant ash alternatives; *Celtis occidentalis*, *Quercus bicolor*, *Quercus ellipsoidalis*, *Quercus rubra*, *Taxodium distichum*, and *Fraxinus americana*. To mimic nursery grower practices, trees were assigned at random to one of four treatments: 1) control, 2) heeled-in, 3) cold storage, or 4) sweating. After treatment, trees were assigned at random to one of three evaluations: root growth potential (RGP) test, container planting or field planting. Root growth potential was very low for *Quercus* spp. trees and varied by treatment for *C. occidentalis*. *Taxodium distichum* trees had a relatively high root growth potential in all treatments. Stem water potential measured immediately before and after pre-plant treatments suggest that *Quercus* spp. trees and *T. distichum* trees were able to rehydrate during sweating while water stress levels in *C. occidentalis* trees remained high. The results suggest that poor transplanting success reported for *Quercus* spp. trees may be related to low root growth potential, whereas poor success of *C. occidentalis* trees may be due to shoot desiccation during storage and handling.

Introduction

Outbreak of exotic invasive pests such as emerald ash borer (EAB; *Agrilus planipennis* Fairmaire) and Dutch elm disease (*Ophiostoma ulmi* (Buisman) Nannf.) highlight the need for improved diversity in urban and community forests. EAB has had a major impact on landscapes of the midwestern United States. It is an invasive insect that affects all species of the genus *Fraxinus* including popular landscape trees such as green ash (*F. pennsylvanica* Marsh.) and white ash (*F. americana*). It was identified in Detroit, Michigan in 2002 and thought to have arrived ten years earlier in cargo shipping materials from Asia (Poland & Mccullough, 2006). EAB has devastated *Fraxinus* tree populations killing tens of millions of trees in Michigan alone, with similar numbers in the surrounding states (“www.emeraldashborer.info,” n.d.) An estimated cost for the removal of these infested trees in the United States is between \$20-\$60 billion (Poland & Mccullough, 2006). Ash trees were popular as landscape trees because of ease of transplant and site adaptability. Ironically, ash trees were widely planted to replace elm trees lost due to Dutch elm disease.

As Midwestern communities develop tree planting programs to replace tree cover lost to EAB, university extension services and others have proposed viable ash alternatives for nursery growers to reference (Cregg & Schutzki, 2004; Iles, 2012; Jull, n.d.). Increasing diversity in urban and community landscape is important to prevent a catastrophic loss like with the ash trees. Many urban and community forestry programs cite the “10% rule” or “10-20-30 rule”; the rule states that no more than ten percent of a given landscape should be planted with any one species (Santamour, 1990). Moreover, the rule states that no more than 20% of any genus or 30% of any family be planted to

create a diverse community (Hanson, 1990). Many diseases and pests tend to choose trees by family, where somewhat resistant species may harbor pests that could be damaging to other species in the same family (Hanson, 1990). To accurately apply the ten percent rule, full-stocking or planting all available tree planting sites of urban landscapes must be applied not just 10% of what is currently planted (Ball et al., 2007).

Although the benefits of promoting species diversity in urban and community forests is widely recognized, landscape nursery managers frequently note that many ash alternatives are difficult to transplant or suffer some dieback in nursery production. Widely recommended ash alternatives include oaks (*Quercus spp.*), hackberry (*Celtis occidentalis* L.), baldcypress (*Taxodium distichum* (L.) Rich. var. *distichum*), maples (*Acer spp.*), and lindens (*Tilia spp.*).

Dieback due to desiccation and poor root regeneration are major concerns for nursery growers when trying to generate a marketable product. It is known that the physiology of trees is affected when trees are transplanted. These physiological effects are commonly referred to as 'transplant shock' or 'transplant stress' and are usually evident in delayed bud-break, crown die-back, poor growth, or mortality. Transplant stress may be caused by a variety of factors but is often linked to several interrelated morpho-physiological characteristics including root growth potential, starch and carbohydrate reserves, overall root-shoot allocation, dormancy status, and fine root:coarse root ratios (Burdett, 1990; Davis & Jacobs, 2005; Haase & Rose, 1993; McKay, 1996). Root growth potential is the ability of plants to initiate and elongate their roots in a given environment (Ritchie, 1985). A low root growth potential is detrimental to a transplanted tree because the rate at which the root regenerate may not be fast

enough to offset the transplant stress it will encounter (Watson & Himelick, 1982b). Carbohydrate reserves are also important to counterbalance transplant stress. Ritchie (1982) explains that a change stored plants undergo is a gradual respiratory depletion of reserve sugars and starches. The loss of such reserves has been implicated in poor tree survival and poor root growth potential (Ritchie, 1982).

Nursery growers have had limited success addressing transplanting problems associated with many ash alternatives. In the midwestern United States, nurseries commonly receive bareroot shade tree liners (small trees, five feet in height to two inches in caliper) from propagation nurseries in Oregon in mid-March to mid-April. Trees are often heeled-in mulch or held in cold storage prior to planting. Another common practice is sweating which is thought to promote bud break and active growth. The main objectives of sweating are to rehydrate the roots and to induce the tree to break dormancy (Halcomb & Fulcher, 2004). Sweating the trees is believed to initiate growth and is widely recommended. Dozic (2009), on the other hand, found that the sweating method had no significant effects on either desiccation-tolerant or -intolerant tree species. Little is known, however, about the physiology underlying this process. The physiological basis for poor initial growth of common ash alternatives may be related to lack of rehydration, inadequate carbohydrate reserves, or poor initial root growth following transport and handling.

In this project we examined the effects of pre-plant handling on transplant stress of five ash alternative species in a typical nursery production system. The goal of this project was to improve the overall availability and success of important ash alternative species in production nurseries to facilitate a more diverse urban landscape. The

objectives of this study were to: 1) determine the effect of pre-plant handling on initial survival and growth of common ash alternatives and 2) determine the physiological responses to pre-plant handling.

Materials and Methods

The project was conducted as two related studies; one conducted in 2010 and one conducted in 2011. Experimental procedures are described for the 2010 study and highlight changes for 2011.

2010 Season

Plant material and treatments

In April 2010, we received 256 bareroot liners (5-6' whips/lightly branched liners) via refrigerated truck from J. Frank Schmidt & Son Co. nursery (Boring, OR). The shipment included 64 trees from each of four species: hackberry (*C. occidentalis*), swamp white oak (*Quercus bicolor* Willd.; *Q. bicolor*), red oak (*Quercus rubra* L.; *Q. rubra*), and baldcypress (*T. distichum*). Each tree was immediately tagged and assigned at random to one of four treatments: 1) control, 2) heeling-in, 3) cold storage, or 4) sweating (Table A2.1). All treatments, except control, were maintained for three weeks. The control trees were assigned, directly off the truck, to three evaluations; field planting, container planting, and root growth potential (RGP) test. The *heeled-in* treatment was designed to simulate a common nursery practice of storing trees outdoors prior to planting. We stood the trees up and covered the roots with container substrate mix of pine bark-peat moss (80:20; v:v). Trees were watered with an overhead sprinkler once a day. Trees in *cold storage* were placed in a 4°C (40°F) walk-in cooler at the MSU Horticulture Teaching and Research Center (HTRC: Holt, MI). Trees were situated upright in large wooden crates with their roots covered with straw and watered as needed to prevent desiccation, approximately 4 times/wk. The *sweating* treatment was designed to replicate a common nursery practice used to

stimulate growth on recalcitrant trees (Halcomb & Fulcher, 2004). Trees were placed on the floor of a hoop greenhouse with double-walled poly (4 mil) at the MSU Forestry Tree Research Center (TRC). Trees were alternated with layers of burlap and then covered with a large sheet of white plastic. Burlap was checked daily and watered as needed to prevent desiccation, approximately 4 times/wk. After treatments were applied for 3 wks, trees were assigned at random to one of three evaluations: field planting, container planting, and root growth potential test. We measured shoot water potential using a pressure chamber (PMS Instrument Company, Albany, OR) immediately before and after pre-plant treatments (Table A2.2).

Root Growth Potential (RGP) test

Root growth potential was evaluated by planting trees in a gravel culture system for three weeks and determining new root growth. Control trees were placed in the gravel culture system immediately upon receipt; trees in the three pre-plant treatments were placed in the gravel culture system after 3 wks of treatment (Table A2.4). Trees were planted in 25-gallon (95-liter) containers filled with clean pea-gravel in a hoop greenhouse with double-walled poly (4 mil) at the TRC. Four trees, one from each treatment x species combination, were planted in each container with eight replications. Root systems were kept moist using a micro-sprinkler system controlled by an automatic timer. Irrigation was set to run for 8 s every 16 min. Initial tree height and stem diameter were measured at planting. Stem diameter was measured with a caliper 3" above the graft union on the *C. occidentalis*, *Q. bicolor*, and *Q. rubra* and 6" above the root collar on the *T. distichum*. New shoot growth or stem dieback was determined

at the end of the RGP test. Stem dieback was estimated as the distance from the tip of the terminal shoot to the highest living bud. After 3 wks the trees were removed from the gravel and new root growth was assessed by counting the number of new white roots and measuring root lengths of a random sample of ten new roots. Total new root length was estimated as number of new roots x average new root length. All new roots were harvested and dried in an oven at ~75°C for approximately 1 wk. The dried roots were then weighed.

Field and container planting

We planted four trees from each species x treatment combination, including control trees, in a field block at the Sandhill farm section of the HTRC. Trees were planted in two rows, 3' (0.91 m) on center (Table A2.4). Soil type at the site was sandy loam. We planted an additional four trees per species x treatment combination, including control trees, in 15-gallon (57-liter) container filled with a mixture of pine bark and peat moss (80:20, v:v). Trees were watered by hand approximately 2 times/wk.

Growth and physiology

Height and stem diameter were measured at initial planting and at the end of the growing season of field-grown, and container-grown trees. Stem diameter was measured with a caliper 3" above the graft union on the *C. occidentalis*, *Q. bicolor*, and *Q. rubra* and 6" above the root collar on the *T. distichum*. Stem dieback was estimated as the distance from the top-most portion of the terminal leader to the point where current season growth initiated.

We measured pre-dawn and mid-day stem water potential using a pressure chamber on field and container-grown trees. We measured chlorophyll fluorescence (ratio of variable fluorescence to maximal fluorescence; F_v/F_m) on the leaves using an OS-30p Chlorophyll Fluorometer (Opti-Science, Hudson, NH). Leaves were dark acclimated for 10 min prior to measurement. All growth and physiological measurement dates are listed in Table A2.2.

2011 Season

Plant material and treatments

In April 2011, we received 316 bareroot liners (5-6' whips/lightly branched liners) via refrigerated truck from J. Frank Schmidt & Son Co. The shipment included 64 trees from each of four species: *C. occidentalis*, *Q. rubra*, and *T. distichum*, white ash (*Fraxinus Americana* L.), and 60 trees of northern pin oak (*Quercus ellipsoidalis* E.J. Hill). *Quercus ellipsoidalis* (*Q. ellipsoidalis*) was substituted for *Q. bicolor* because of supply issues at the liner production nursery and white ash (*F. americana*), which is widely considered as easy to transplant, was included as a positive control.

We collected samples and took pressure chamber readings on trees before and after lifting, at the liner production nursery in Oregon. Pre-harvest samples were taken on February 3, 2011. Post-harvest samples were taken on March 31, 2011. Shoot and root samples that were collected on both dates for carbohydrate analysis and were packaged in a cooler with dry ice and shipped to Michigan State University.

The same pre-planting treatments were executed on the trees as in the 2010 season (Table A2.1).

RGP test

The root growth potential test of the control trees was put through the same methods as the 2010 season (Table A2.5).

Field and container planting

Field and container planting was the same as the 2010 season (Table A2.5). The field trees had an irrigation line setup to run approx. once per day for 10 min.

Growth and physiology

Height, stem diameter, dieback, water potential, gas exchange, and chlorophyll fluorescence were measured the same as the 2010 season (Table A2.3).

Carbohydrate analysis

Shoot and root samples of the trees were collected at J. Frank Schmidt & Son Co. for pre-harvest analysis on February 3 and post-harvest analysis on March 31. Total non-structural carbohydrate content was analyzed using the DuBois phenol-sulfuric acid method (DuBois et al., 1956). Dubois describes that when “phenol [is] in the presence of sulfuric acid [it] can be used for the quantitative colorimetric microdetermination of sugars and their methyl derivatives, oligosaccharides, and polysaccharides.” D-glucose was used for the standard curve.

Statistical analyses

For both years, effects of treatment, species, and interaction of species and treatment on each response variable were evaluated by Analysis of Variance. Data were analyzed as a completely randomized design using the model $y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijk}$; where y_{ijk} =response variable; α_i =species effect; β_j =treatment effect; and ε =experimental error. Where significant ($p < 0.05$) species or treatment effects were indicated, means were separated by Tukey's studentized range test.

Results

2010 Season

Pre-plant water status

The initial water potential (Ψ_w) of shoots of all the tree species was relatively low upon arrival from the Oregon nursery, ranging from -1.5 for the *T. distichum* trees to -3.7 for the *C. occidentalis* trees (Figure A2.1). The pre-plant treatments did not consistently improve water status. Trees that were cold-stored had the overall lowest Ψ_w . Heeling-in improved water status of *C. occidentalis* trees compared to the untreated controls. The two *Quercus* species showed water status recovery with the sweating treatments compared to the control treatment. *Taxodium distichum* trees had the highest Ψ_w and were unresponsive to treatments except for reduced water status during cold-storage (Figure A2.1).

Root growth potential

New root growth potential (RGP) varied by species but was not affected by pre-plant treatments. *Taxodium distichum* trees produced the highest number of new roots while both *Quercus* species showed consistently low RGP, generating less than 30 new roots per tree during the 3-week RGP test (Figure A2.2). *Celtis occidentalis* trees that were heeled-in prior to the RGP test produced an average of 180 new roots.

Shoot growth/stem dieback

Our experimental protocol provided three opportunities to evaluate shoot growth (or stem dieback) in response to pre-plant treatments: shoot growth during the RGP test, growth during and after container-planting and growth after field-planting. In general, pre-plant treatments had little effect on stem growth or dieback after planting although there were a few exceptions to this trend. In the container-planted trial, sweating increased stem dieback for *Q. bicolor*, *Q. rubra*, and especially *C. occidentalis* trees (Figure A2.3). In the field-planted trial, *C. occidentalis* and *Q. bicolor* trees experienced significant dieback at the end of the season (Figure A2.4). All of the *T. distichum* trees died in this trial due an intermediate undetected irrigation system failure. *Quercus rubra* trees showed the highest growth of any species. In the root growth potential trial, species affected shoot growth in trees of both *Quercus* species showing net growth. The interaction of species and pre-plant treatments was significant (<0.05) due to increased stem dieback for *C. occidentalis* trees that had been sweated and *T. distichum* trees that had not been subjected to any pre-plant treatments (Figure A2.5).

Post-planting physiology

Predawn and midday water potential (Ψ_w) of container-grown trees varied among species (Table A2.6). *Taxodium distichum* trees had the highest Ψ_w for both predawn and midday readings and *C. occidentalis* trees had the lowest. Pre-plant treatments had relatively little effect on Ψ_w of container-grown trees and treatments did not improve water status for any trees of any species. No differences between species or treatments were found for the predawn and midday Ψ_w evaluation of the field-planted

trees. The missing values for *T. distichum* trees are due to the Ψ_w exceeding the limits of the pressure chamber we used (Table A2.7).

Neither species nor pre-plant treatment affected ($p < 0.05$) chlorophyll fluorescence (Fv/Fm) of trees planted in containers on the early season (June 29) measurements (Table A2.8). On the late-season measurement date (Sept. 1), however, cold-storage or sweating before planting reduced Fv/Fm for trees of most species. *Quercus bicolor* and *T. distichum* trees had higher values than the other species and the cold-stored and sweated trees showed no benefit (Table A2.8). Pre-plant treatments and species did not affect Fv/Fm of field-planted on either measurement date (Table A2.9).

2011 Season

Carbohydrate analysis

Total nonstructural carbohydrates (TNC) of root and shoot tissue varied with species and time of sample collection. Nonstructural carbohydrate concentration of root tissue of most trees increased from the initial sample and the post-harvest sample. *Celtis occidentalis* and *F. americana* trees were the exception with a decrease and no change respectively (Figure A2.6). The total nonstructural carbohydrates in the shoots showed that was no change between the pre- and post-harvested trees. *Fraxinus americana* trees were the exception with a decrease in carbohydrates between the two dates (Figure A2.7). *Quercus* species had lower root TNC than trees of the other species, especially before lifting, and *Q. ellipsoidalis* trees had the lowest TNC of all trees sampled.

Pre-plant water status

Stem Ψ_w of all trees decreased ($p < 0.05$) from the time they were lifted at the liner nursery in Oregon (post-harvest) until they arrived on the truck in East Lansing (Control) (Figure A2.8). This indicates significant water loss occurred from when the trees were harvested and during storage and shipping. Species varied in their ability to re-hydrate during the pre-plant treatments. *Quercus rubra* trees showed significant Ψ_w recovery after heeling-in and sweating. In contrast, sweating reduced Ψ_w of *C. occidentalis* trees.

Root growth potential

Root growth potential (RGP) varied among species but not treatments within species. *Fraxinus americana* and *T. distichum* trees showed significantly higher root numbers throughout all the treatments than trees of the other species. Trees of both of the *Quercus* species had consistently low root regeneration across all pre-plant treatments (Figure A2.9).

Shoot growth/stem dieback

Tree growth responses to pre-plant treatments varied by species and differed among the evaluation plantings. In the container-planted trial, pre-plant treatments did not improve stem growth relative to the control for any species (Figure A2.10). *Celtis occidentalis* trees showed dieback in all treatments. Sweating trees before planting reduced growth of *F. americana* trees. The field-planted trees experienced dieback across all species excluding *F. americana* trees. *Fraxinus americana* trees showed

growth in all treatments but no pre-plant treatment showed benefits. The cold storage pre-plant treatment reduced dieback of field-planted *T. distichum* trees (Figure A2.11). In the RGP trial most trees had some net shoot growth. The two significant exceptions were the dieback of trees in the control treatment of *C. occidentalis* trees, establishing the pre-plant treatments were beneficial and the dieback of the cold storage treatment on *F. americana* trees showed evidence that it was not beneficial (Figure A2.12).

Post-planting physiology

Pre-plant treatments did not affect ($p>0.05$) predawn or midday Ψ_w of trees in either the container-planted trial or in the field-planted trials (Tables A2.10 & A2.11). Water potentials varied among species in both trials. *Quercus ellipsoidalis* trees had more negative predawn Ψ_w than the other species in the field-planted trial. By midday, however, *Q. rubra* and *F. americana* trees had similar Ψ_w levels. In the container-planted trial, *T. distichum* trees has the least negative pre-dawn Ψ_w and had relatively less negative midday Ψ_w , along with *F. americana* trees.

Pre-plant treatments did not affect chlorophyll fluorescence (F_v/F_m) of container-planted or field-planted trees (Tables A2.12 & A2.13). For container-planted trees, F_v/F_m did not vary among species. Species affected ($p<0.05$) F_v/F_m on both measurements dates in 2011 in field-planting. *Fraxinus americana* and *T. distichum* trees had relatively high F_v/F_m compared to *C. occidentalis* and *Q. ellipsoidalis* trees in June. By the end of the summer, however, F_v/F_m was lower for *F. americana* trees than for *T. distichum* trees.

Discussion

This research was conducted to evaluate methods to increase the survivability of bareroot tree liners after lifting and prior to transplanting of ash alternatives. Implementation of effective pre-plant treatments could aid in enhancing species diversity through increased survivorship and faster growth of a diverse group of landscape trees. Poor survival or extensive dieback of trees following transplanting has been linked to a variety of factors including desiccation, poor carbohydrate status, or ability to regenerate new roots (Lundberg et al., 1990; McKay, 1996; Scianna et al., 2005).

2010 Season

Results of the first-year trials showed no defined trends within species in response of the trees in response to the pre-plant treatments. There were no definite trends within the treatments. In general, the initial Ψ_w of the sweating treatment was less negative between species and the cold storage was more negative compared to the other treatments. These differences may be due to the moist environment that the sweated trees were subjected to, compared to the cold-stored trees, which were not inflicted to such an irriguous environment. There were significant differences seen between *C. occidentalis* trees and *T. distichum* trees. *Celtis occidentalis* trees had more negative Ψ_w values than *T. distichum* trees. The data reveals that *T. distichum* trees was better at maintaining a less negative Ψ_w than the other species and, therefore, was initially under less stress.

There was a high amount of dieback of the sweating treatments in both the container- and field-grown trees. The trees that were implemented with the sweating treatments showed little difficulty regenerating new roots in any of the species. *Celtis occidentalis* trees had the poorest response to the sweating treatment, which resulted in more negative Ψ_w , shoot dieback in the field and in containers, and shoot dieback in the root growth potential test. Young *C. occidentalis* trees have a fibrous root system and Struve (1990) states that “species with fibrous root systems are easier to transplant than species with coarse root systems.” Yet, we saw that *C. occidentalis* trees’ ability to transplant was one of the most difficult. This may be from the stress implemented on them during the treatments and after transplant.

Quercus rubra trees had low RGP compared to the other species, but they grew relatively well in the container, field, and RGP plantings. This may be from the larger roots and available carbohydrates stored in the roots. The initial Ψ_w of *Q. rubra* trees for all treatments was relatively high. The trees were better able to retain water in their shoots. The high Ψ_w in the shoots at the time of transplanting could also explain the growth seen in the plantings.

Quercus bicolor trees had similar Ψ_w , RGP heights, and root numbers as *Q. rubra* trees but the species did not grow as well in the container- and field-grown trees as the *Q. rubra* trees. The differences seen between these two species may be due to their specific soil type. *Quercus bicolor* grow best in moist to wet soils (wetlands) while *Q. rubra* perform best in well-drained soils (Rogers, 1990; Sander, 1990). The soil type at our field-planted location was a well-drained sandy soil and the substrate for the

container-planted was a pine bark-peat moss blend, also well-drained (as stated in the materials and methods). This soil/substrate type is not conducive to *Q. bicolor* trees' natural soil habitat possibly causing the lack in growth.

Taxodium distichum trees had the highest Ψ_w and greatest root number but showed very little growth in the container-grown test and all the trees died in the field-grown test. The complete mortality of the *T. distichum* trees in the field may be due to irrigation failure during the growing season. The irrigation failure seemed to cause all the *T. distichum* trees mortality but *C. occidentalis* trees showed significantly high shoot dieback, *Q. bicolor* trees showed a small amount of shoot dieback but no growth across all treatments, and *Q. rubra* trees showed growth across all treatments. This shows that *T. distichum* trees could not overcome drought stress, even though they had a high amount of root regeneration, while the other species did overcome the drought stress and survived.

Chlorophyll fluorescence was used as an overall stress indicator of our trees. It is considered a rapid and non-destructive method of measuring stress (Rose & Haase, 2002). The optimum value for a fluorescence reading is 0.83 Fv/Fm (Hunt, 2003) and highly stressed readings are less than 0.60 (Ritchie, 2006). The chlorophyll fluorescence of all the tree species had sub-optimal values, this means that the trees were under environmental stresses. In the container-planted trees there were no differences seen between the species and the treatments in June 2010 but in September 2010 there were variations among species and treatments. *Quercus bicolor* trees and *T. distichum* trees showed higher Fv/Fm values than the other two species, coincidentally, those two species showed the most growth. In the field-planted trees,

there were no differences seen between the treatments and the species fluorescence values for the two dates indicating all the species were exposed to the same amount of stress. As stated above, *T. distichum* trees did not survive the field planting.

Bareroot trees may have the ability to generate new roots, as all the trees did, they may not be able to rehydrate their shoots when transplanted, causing stress and dieback.

2011 Season

Total nonstructural carbohydrate (TNC) data illustrated that the pre- and post-harvest values did not change in the shoots. There were, however, species differences. *Celtis occidentalis* trees had the lowest amount of TNC, followed by *T. distichum* trees, the *Quercus* spp., and *F. americana* trees, which had the highest. Storage conditions are one of the main reasons for carbohydrate depletion (McKay, 1996). The unchanged pre- and post-harvest values of TNC in the shoots are evidence that the storage method at the wholesale level is adequate. The TNC of the roots, however, tell a different story. There was an increase of TNC from pre-harvest to post-harvest for the *Quercus* spp. and *T. distichum*. *Fraxinus americana* trees had no differences between the harvest dates and *C. occidentalis* trees had a decrease. In a study conducted by Girard et al. (1997), *Acer rubrum* bareroot seedlings did not show significant depletion of TNC after 12 days of exposure to ambient conditions. The bareroot trees in this study were stored outside in sawdust for ~50 days in late winter in Oregon. These differences may be attributed to the reallocation of TNC to the roots for an increase and respiration of the trees for a decrease.

The water potential (Ψ_w) values of the bareroot trees at post-harvest were less negative than when the trees arrived at Michigan State University (control treatment). This shows that a significant amount of water loss occurred while the trees were in pre-delivery storage and on the delivery truck. After the pre-plant treatments were imposed the water status of the trees seemed to improve to pre- and post-harvest Ψ_w values, as compared to the control group. This trend was detected in *T. distichum* trees and *Q. rubra* trees, more specifically in the heeled-in and sweated trees of *Q. rubra* trees. An observed exception was the sweated treatment which revealed the Ψ_w exceeded the limit of the pressure chamber for *C. occidentalis* trees and *Q. ellipsoidalis* trees (-4.0 MPa). The high levels of water stress may be attributed to the trees drying out or not being adequately watered during the sweated treatment period. The *Quercus* spp. and *C. occidentalis* trees in the 2010 season did not see the high levels of water stress during the sweating treatment nor did *Quercus rubra* trees in the 2011 season. These data suggest that conditions immediately before and during transport are critical to maintaining the trees water status.

All the tree species exhibited root regeneration in the root growth potential (RGP) planting. *Fraxinus americana* trees and *T. distichum* trees had the highest amount of new root growth. Despite having the highest amount of new root growth, the least negative Ψ_w , and a higher root TNC post-harvest, *T. distichum* trees had the least amount of growth in the RGP planting and the field planting. Furthermore, *T. distichum* trees had the most growth in the container planting. This may be explained by the type of environment they favor. *Taxodium distichum* does best in moist to wet soils and are

not conducive to dry or drought conditions (Elcan & Pezeshki, 2002) and they favor an organic substrate over a sandy soil (Day et al., 2006), which was our field conditions. *Taxodium distichum* trees may have not done well in the RGP planting because of the highly porous pea gravel used.

The *Quercus* spp. trees had consistently low new roots but still had significant growth in the RGP planting. *Quercus ellipsoidalis* trees in the sweated treatment that exceeded the limits of the pressure chamber with highly negative Ψ_w had significant shoot growth. This may be due to the *Quercus* spp. ability to rehydrate their shoots and their tolerance to desiccation (Englert et al., 1993).

There was growth of the container-grown trees except for *C. occidentalis* trees. *Celtis occidentalis* trees showed the ability to generate new root growth but then exhibited dieback or mortality when planted in containers or in the field. This may be due to the significant water stress the trees were under when transplanted after the pre-plant treatments especially the sweating. Also, the pre-dawn and midday Ψ_w for *C. occidentalis* trees were the most negative of any species in the container-grown trees but similar to the other species in the field planted. *Taxodium distichum* trees had similar results in the field-grown planting; the trees were able to regenerate new roots but then died in the field. However, *T. distichum* trees had the least negative Ψ_w after the treatments were implemented and for the predawn/midday Ψ_w and the container-grown trees had significantly more growth than the other species. There was erratic irrigation failure in the field but not to the extent of the 2010 season. *Taxodium distichum* trees survived in the 2011 season, unlike the 2010 season, but had significant

shoot dieback. *Taxodium distichum* trees had significant growth in the container-planting, demonstrating that drought stress had a major impact on the survivability of the trees.

The chlorophyll fluorescence of the trees in the container-planting had somewhat sub-optimal values but did not differ among species or treatments. However, the field-planting showed differences between species. *Celtis occidentalis* trees had the lowest values indicating that they were under more stress than the other tree species. The chlorophyll fluorescence data point out that the container-planted trees are less stressed than the field-planted trees.

Fraxinus americana trees, the positive control, performed as we would have expected. The trees had significant growth in all three plantings, exhibited high amount root regeneration, and had high chlorophyll fluorescence values.

Conclusion

This research demonstrated the use of pre-plant treatments on bareroot tree liners. The need for the trees to rehydrate after post-harvest is an important factor for their survivability. Our study shows that the pre-plant treatments did help to rehydrate the shoots and induce root regeneration.

Species behave differently, and different pre-plant treatments could benefit them differently. The sweating treatment showed no benefit to the survivability of the species but aided in the regeneration of new roots. *Taxodium distichum* trees exhibited a high amount of root regeneration but when exposed to drought conditions showed severe dieback and death. *Fraxinus americana* had similar amounts of root regeneration but when exposed to the same drought condition still showed growth across all treatments. Other pre-plant treatments, such as misting/soaking or applying a wax coating to the bareroot trees, could also improve the growth and survival. Further research is needed to document the effect of the pre-plant treatments on ash alternative species.

APPENDIX 2

Table A2.1. Overview of plant material, pre-plant treatments and evaluations of 2010-2011 pre-plant storage and handling tests.

Plant Material	
<u>2010</u>	<u>2011</u>
<i>C. occidentalis</i>	<i>C. occidentalis</i>
<i>Q. rubra</i>	<i>Q. rubra</i>
<i>Q. bicolor</i>	<i>Q. ellipsoidalis</i>
<i>T. distichum</i>	<i>T. distichum</i>
	<i>F. americana</i>
Pre-plant treatment	
Control	
Heeled-in	
Cold storage	
Sweat	
Evaluation	
Field-planted (4 trees/spp)	
Container-planted (4 trees/spp)	
Root growth potential (8 trees/spp)	

Table A2.2. Growth and physiology measurement dates 2010.

	Unassigned Planting	Field Planting	Container Planting
Pre-treatment water potential	April 23		
Post-treatment water potential	May 19		
Height measurement		May 20 & August 13	May 20 & August 13
Pre-dawn water potential		July 23	July 23
Mid-day water potential		July 23	July 23
Gas exchange		July 14 & August 13	July 14 & August 13
Chlorophyll Fluorescence		June 29 & September 1	June 29 & September 1

Table A2.3. Growth and physiology measurement dates 2011.

	Unassigned Planting	Field Planting	Container Planting
Pre-treatment water potential	April 18		
Post-treatment water potential	May 11		
Height measurement		May 13 & November 4	May 13 & November 4
Pre-dawn water potential		July 21	July 21
Mid-day water potential		July 21	July 21
Gas exchange		June 29 & July 25	June 17 & August 5
Chlorophyll Fluorescence		June 16 & August 24	June 16 & August 24

Table A2.4. Schedule of treatments and evaluations for pre-plant handling trial 2010.

Treatment	Evaluation test		Date In		Date Out
Control	RGP Test		April 24		May 14
	Field-planting		April 23		
	Container-planting		April 23		
Heeled-in	Placed in TRMT		April 22		
	RGP Test		May 18		June 11
	Field-planting		May 17		
	Container-planting		May 17		
Cold storage	Placed in TRMT		April 22		
	RGP Test		May 18		June 11
	Field-planting		May 17		
	Container-planting		May 17		
Sweating	Placed in TRMT		April 22		
	RGP Test		May 18		June 11
	Field-planting		May 17		
	Container-planting		May 17		

Table A2.5. Schedule of treatments and evaluation tests for pre-plant handling trial 2011.

Treatment	Evaluation test		Date In		Date Out
Control	RGP Test		April 13		May 9
	Field-planting		April 14		
	Container-planting		April 15		
Heeled-in	Placed in TRMT		April 13		
	RGP Test		May 11		June 7
	Field-planting		May 6		
	Container-planting		May 4		
Cold storage	Placed in TRMT		April 12		
	RGP Test		May 11		June 7
	Field-planting		May 6		
	Container-planting		May 4		
Sweating	Placed in TRMT		April 12		
	RGP Test		May 11		June 7
	Field-planting		May 5		
	Container-planting		May 6		

Table A2.6. Mean predawn and midday water potential (Ψ_w) of container-grown bareroot tree liners subjected to four pre-plant treatments. July 23, 2010.

Treatment	Predawn shoot Ψ_w (MPa)			
	<i>C. occidentalis</i>	<i>Q. bicolor</i>	<i>Q. rubra</i>	<i>T. distichum</i>
Control	-0.28a	-0.53a	-0.39a	-0.13a
Heel	-0.50b	-0.50a	-0.93a	-0.29a
Store	-0.25a	-0.96a	-0.43a	-0.25a
Sweat	na	-1.09a	-0.83a	-0.23a
	AB	B	AB	A

Treatment	Midday shoot Ψ_w (MPa)			
	<i>C. occidentalis</i>	<i>Q. bicolor</i>	<i>Q. rubra</i>	<i>T. distichum</i>
Control	-1.90a	-1.20a	-0.79a	-0.95a
Heel	-1.89a	-1.45a	-1.94b	-1.16a
Store	-1.94a	-1.40a	-1.15ab	-1.10a
Sweat	na	-2.15a	-1.89b	-1.23a
	C	B	B	A

Note: means within a column followed by the same lowercase letter are not different ($p < 0.05$). Species mean within a row followed by the same uppercase letter are not different ($p < 0.05$). Mean separation by Tukey's studentized range test.

Table A2.7. Mean predawn and midday water potential (Ψ_w) of field-grown bareroot tree liners subjected to four pre-plant treatments. July 23, 2010.

Treatment	Predawn shoot Ψ_w (MPa)			
	<i>C. occidentalis</i>	<i>Q. bicolor</i>	<i>Q. rubra</i>	<i>T. distichum</i>
Control	-0.83a	-1.25a	-1.43a	na
Heel	-0.65a	-0.73a	-2.75a	na
Store	-0.80a	-1.78a	-2.71a	-0.90a
Sweat	-0.80a	na	-1.40a	na
	A	A	A	A

Treatment	Midday shoot Ψ_w (MPa)			
	<i>C. occidentalis</i>	<i>Q. bicolor</i>	<i>Q. rubra</i>	<i>T. distichum</i>
Control	-2.17a	-1.88a	-2.50a	na
Heel	-2.63a	-2.58a	-3.33a	na
Store	-1.95a	-1.80a	-2.63a	-1.90a
Sweat	-2.35a	-3.75a	-2.60a	na
	A	A	A	A

Note: means within a column followed by the same lowercase letter are not different ($p < 0.05$). Species mean within a row followed by the same uppercase letter are not different ($p < 0.05$). Mean separation by Tukey's studentized range test.

Table A2.8. Mean chlorophyll fluorescence of container-grown bareroot tree liners subjected to four pre-plant treatments for two dates; June 29, 2010 and September 1, 2010.

Treatment	Maximum quantum efficiency (Fv/Fm)			
	<i>C. occidentalis</i>	<i>Q. bicolor</i>	<i>Q. rubra</i>	<i>T. distichum</i>
Control	0.735a	0.744a	0.709a	0.723a
Heel	0.731a	0.684b	0.739a	0.710a
Store	0.730a	0.740a	0.736a	0.698a
Sweat	0.762a	0.729a	na	0.726a
	A	A	A	A

Treatment	Maximum quantum efficiency (Fv/Fm)			
	<i>C. occidentalis</i>	<i>Q. bicolor</i>	<i>Q. rubra</i>	<i>T. distichum</i>
Control	0.738a	0.788a	0.766a	0.808a
Heel	0.747a	0.767a	0.717ab	0.780ab
Store	0.648b	0.732ab	0.678ab	0.741b
Sweat	0.710ab	0.680b	0.652b	0.742b
	B	A	B	A

Note: means within a column followed by the same lowercase letter are not different ($p < 0.05$). Species mean within a row followed by the same uppercase letter are not different ($p < 0.05$). Mean separation by Tukey's studentized range test.

Table A2.9. Mean chlorophyll fluorescence of field-grown bareroot tree liners subjected to four pre-plant treatments for two dates; June 29, 2010 and September 1, 2010.

Treatment	Maximum quantum efficiency (Fv/Fm)			
	<i>C. occidentalis</i>	<i>Q. bicolor</i>	<i>Q. rubra</i>	<i>T. distichum</i>
Control	0.714a	0.666a	0.690a	0.679a
Heel	0.746a	0.714a	0.719a	0.680a
Store	0.722a	0.709a	0.712a	0.761a
Sweat	na	0.662a	0.716a	na
	A	A	A	A

Treatment	Maximum quantum efficiency (Fv/Fm)			
	<i>C. occidentalis</i>	<i>Q. bicolor</i>	<i>Q. rubra</i>	<i>T. distichum</i>
Control	0.769a	0.799a	0.755a	na
Heel	0.780a	0.781a	0.673a	na
Store	0.763a	0.774a	0.708a	na
Sweat	0.769a	0.769a	0.710a	na
	A	A	A	na

Note: means within a column followed by the same lowercase letter are not different ($p < 0.05$). Species mean within a row followed by the same uppercase letter are not different ($p < 0.05$). Mean separation by Tukey's studentized range test. The missing values for *T. distichum* are due to the irrigation issues and zero survival of the trees.

Table A2.10. Mean predawn and midday water potential (Ψ_w) of container-grown bareroot tree liners subjected to four pre-plant treatments. July 21, 2011.

Treatment	Predawn shoot Ψ_w (MPa)				
	<i>C. occidentalis</i>	<i>F. americana</i>	<i>Q. ellipsoidalis</i>	<i>Q. rubra</i>	<i>T. distichum</i>
Control	-1.18a	-1.24a	-1.19a	-1.01a	-0.63a
Heel	-1.51a	-1.31a	-1.12a	-0.79a	-0.75a
Store	-0.98a	-1.31a	-0.93a	-0.91a	-0.66a
Sweat	-1.03a	-1.23a	-1.08a	-1.12a	-0.72a
	C	C	BC	B	A

Treatment	Midday shoot Ψ_w (MPa)				
	<i>C. occidentalis</i>	<i>F. americana</i>	<i>Q. ellipsoidalis</i>	<i>Q. rubra</i>	<i>T. distichum</i>
Control	-2.35a	-1.66a	-2.49a	-2.09a	-1.72a
Heel	-2.18a	-2.09a	-2.64a	-2.28a	-1.88a
Store	-2.07a	-1.88a	-2.15a	-2.06a	-1.81a
Sweat	-2.23a	-1.95a	-2.75a	-2.41a	-1.77a
	BC	AB	C	BC	A

Note: means within a column followed by the same lowercase letter are not different ($p < 0.05$). Species mean within a row followed by the same uppercase letter are not different ($p < 0.05$). Mean separation by Tukey's studentized range test.

Table A2.11. Mean predawn and midday water potential (Ψ_w) of field-grown bareroot tree liners subjected to four pre-plant treatments. July 21, 2011.

Treatment	Predawn shoot Ψ_w (MPa)				
	<i>C. occidentalis</i>	<i>F. americana</i>	<i>Q. ellipsoidalis</i>	<i>Q. rubra</i>	<i>T. distichum</i>
Control	-1.25a	-1.18a	-2.05a	-2.00a	-0.83a
Heel	-1.48a	-1.30a	-2.10a	-1.13a	na
Store	-1.40a	-1.37a	-4.00a	-1.50a	na
Sweat	-1.34a	-1.29a	-2.60a	-1.50a	na
	A	A	B	A	A

Treatment	Midday shoot Ψ_w (MPa)				
	<i>C. occidentalis</i>	<i>F. americana</i>	<i>Q. ellipsoidalis</i>	<i>Q. rubra</i>	<i>T. distichum</i>
Control	na	-2.52a	-3.53a	-3.15a	-2.58a
Heel	-1.38a	-2.40a	-2.63a	-2.55a	-3.90a
Store	-2.13a	-2.48a	-2.85a	-2.80a	na
Sweat	-1.68a	-2.35a	-2.20a	-3.02a	na
	A	AB	B	B	B

Note: means within a column followed by the same lowercase letter are not different ($p < 0.05$). Species mean within a row followed by the same uppercase letter are not different ($p < 0.05$). Mean separation by Tukey's studentized range test.

Table A2.12. Mean chlorophyll fluorescence of container-grown bareroot tree liners subjected to four pre-plant treatments. June 16, 2011.

Treatment	Maximum quantum efficiency (Fv/Fm)				
	<i>C. occidentalis</i>	<i>F. americana</i>	<i>Q. ellipsoidalis</i>	<i>Q. rubra</i>	<i>T. distichum</i>
Control	0.741a	0.759a	0.753a	0.760a	0.770a
Heel	0.738a	0.761a	0.758a	0.731a	0.712a
Store	0.709a	0.766a	0.767a	0.717a	0.768a
Sweat	0.745a	0.735a	0.745a	0.737a	0.774a
	A	A	A	A	A

Note: means within a column followed by the same lowercase letter are not different ($p < 0.05$). Species mean within a row followed by the same uppercase letter are not different ($p < 0.05$). Mean separation by Tukey's studentized range test.

Table A2.13. Mean chlorophyll fluorescence of field-grown bareroot tree liners subjected to four pre-plant treatments for two dates; June 16, 2011 and August 24, 2011.

Treatment	Maximum quantum efficiency (Fv/Fm)				
	<i>C. occidentalis</i>	<i>F. americana</i>	<i>Q. ellipsoidalis</i>	<i>Q. rubra</i>	<i>T. distichum</i>
Control	0.717a	0.764a	0.746a	0.719a	0.765a
Heel	0.690a	0.747a	0.727a	0.692a	0.763a
Store	0.687a	0.731a	0.740a	0.726a	0.766a
Sweat	0.635a	0.766a	0.730a	0.691a	0.768a
	C	A	AB	BC	A

Treatment	Maximum quantum efficiency (Fv/Fm)				
	<i>C. occidentalis</i>	<i>F. americana</i>	<i>Q. ellipsoidalis</i>	<i>Q. rubra</i>	<i>T. distichum</i>
Control	0.772a	0.697a	0.780a	0.737a	na
Heel	0.752a	0.718a	0.744a	0.748a	na
Store	0.749a	0.719a	na	0.724a	0.786a
Sweat	0.736a	0.745a	0.727a	0.757a	0.779a
	AB	B	AB	AB	A

Note: means within a column followed by the same lowercase letter are not different ($p < 0.05$). Species mean within a row followed by the same uppercase letter are not different ($p < 0.05$). Mean separation by Tukey's studentized range test.

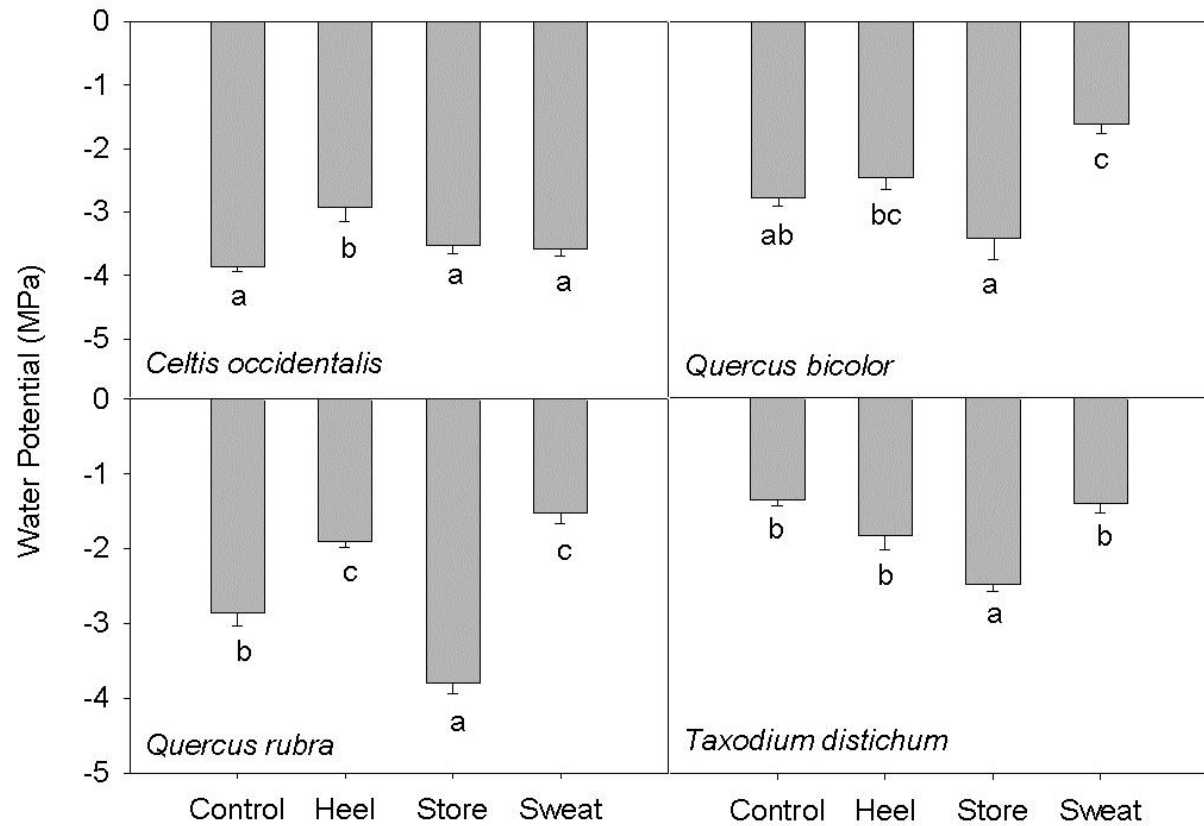


Figure A2.1. Mean initial water potential of bareroot tree liners subjected to four pre-plant treatments prior to planting in 2010.

Note: spp: $p < 0.001$, trmt: $p < 0.05$, spp x trmt: $p < 0.05$

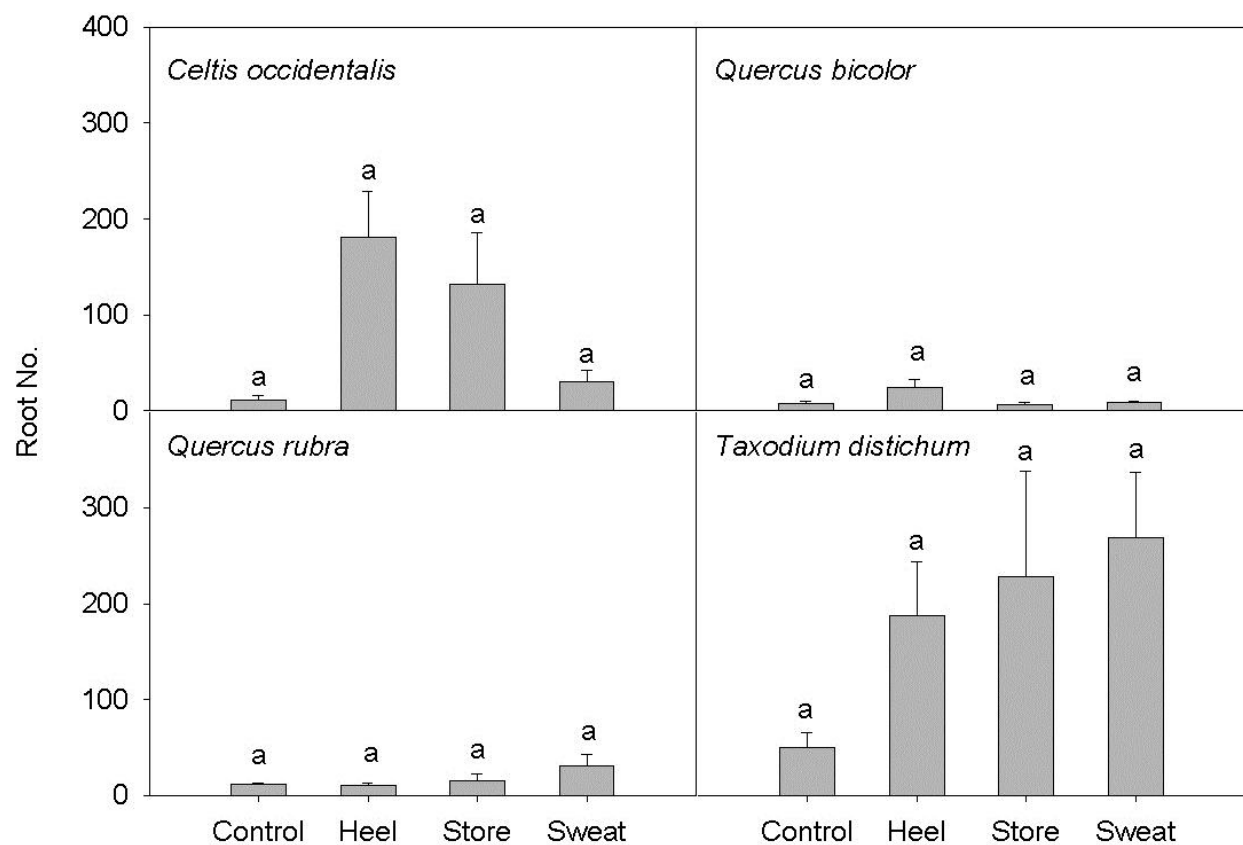


Figure A2.2. Mean root number of root growth potential-grown bareroot tree liners subjected to four pre-plant treatments in 2010.

Note: spp: $p < 0.001$; trmt: ns; spp x trmt: ns

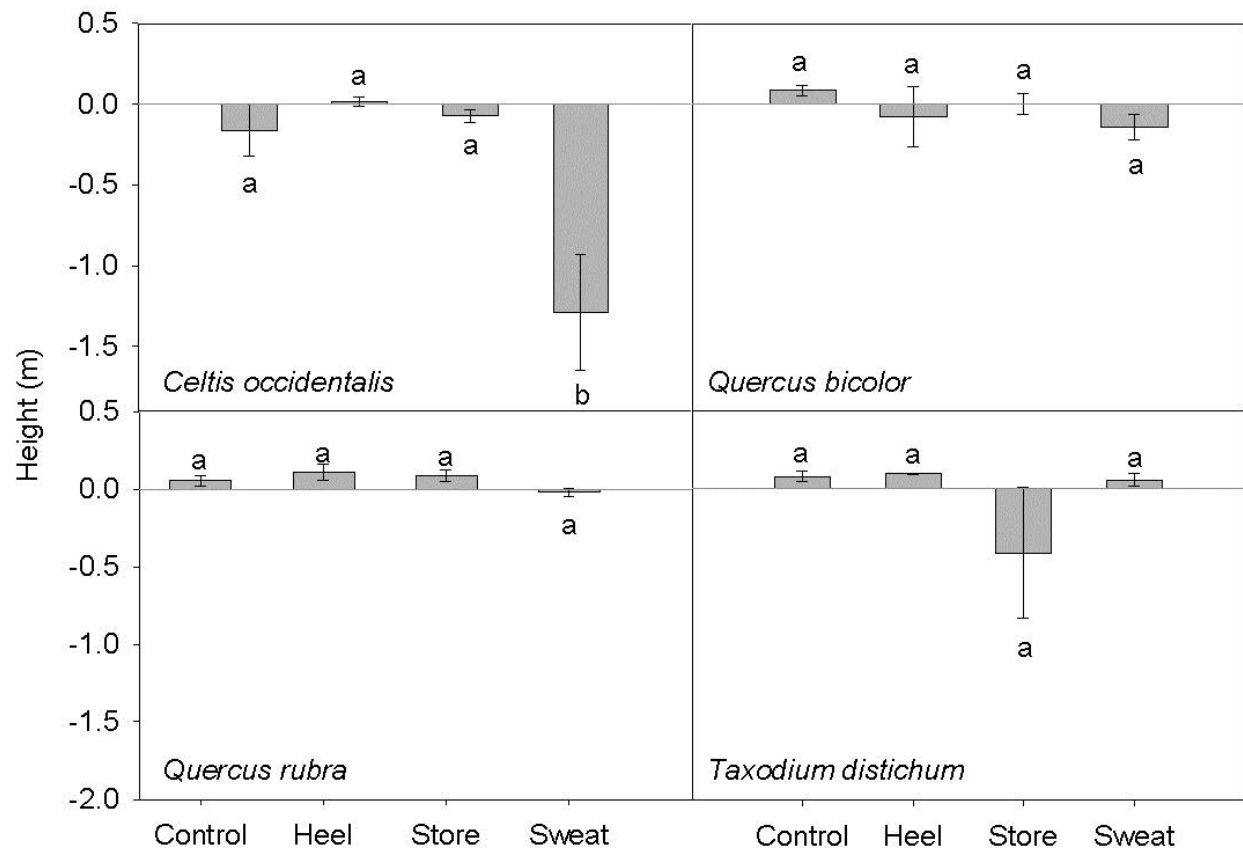


Figure A2.3. Mean growth/dieback of container-grown bareroot tree liners subjected to four pre-plant treatments in 2010.

Note: spp: $p < 0.001$, trmt: $p < 0.05$, spp x trmt: $p < 0.05$

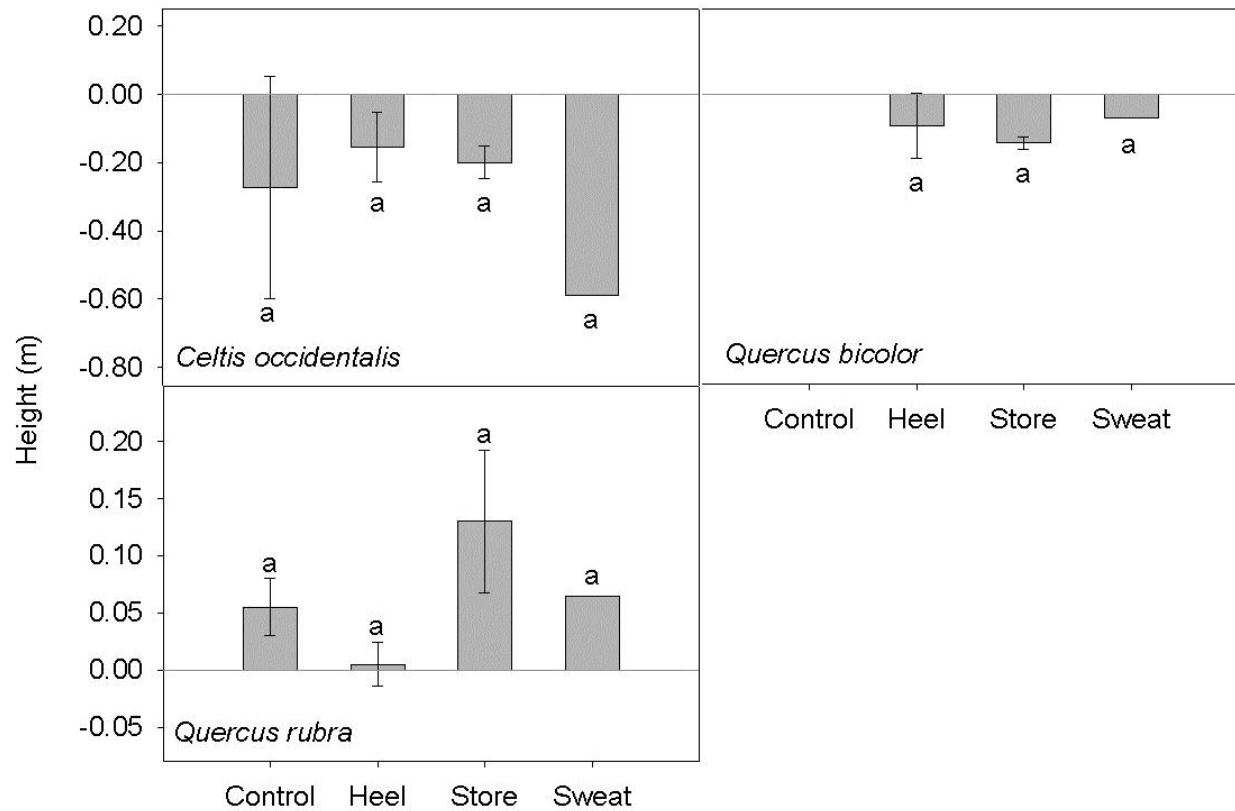


Figure A2.4. Mean growth/dieback of field-grown bareroot tree liners subjected to four pre-plant treatments in 2010.
 Note: Baldcypress data not shown due to death of all trees. spp: $p < 0.001$; trmt: $p < 0.05$; spp x trmt: $p < 0.05$

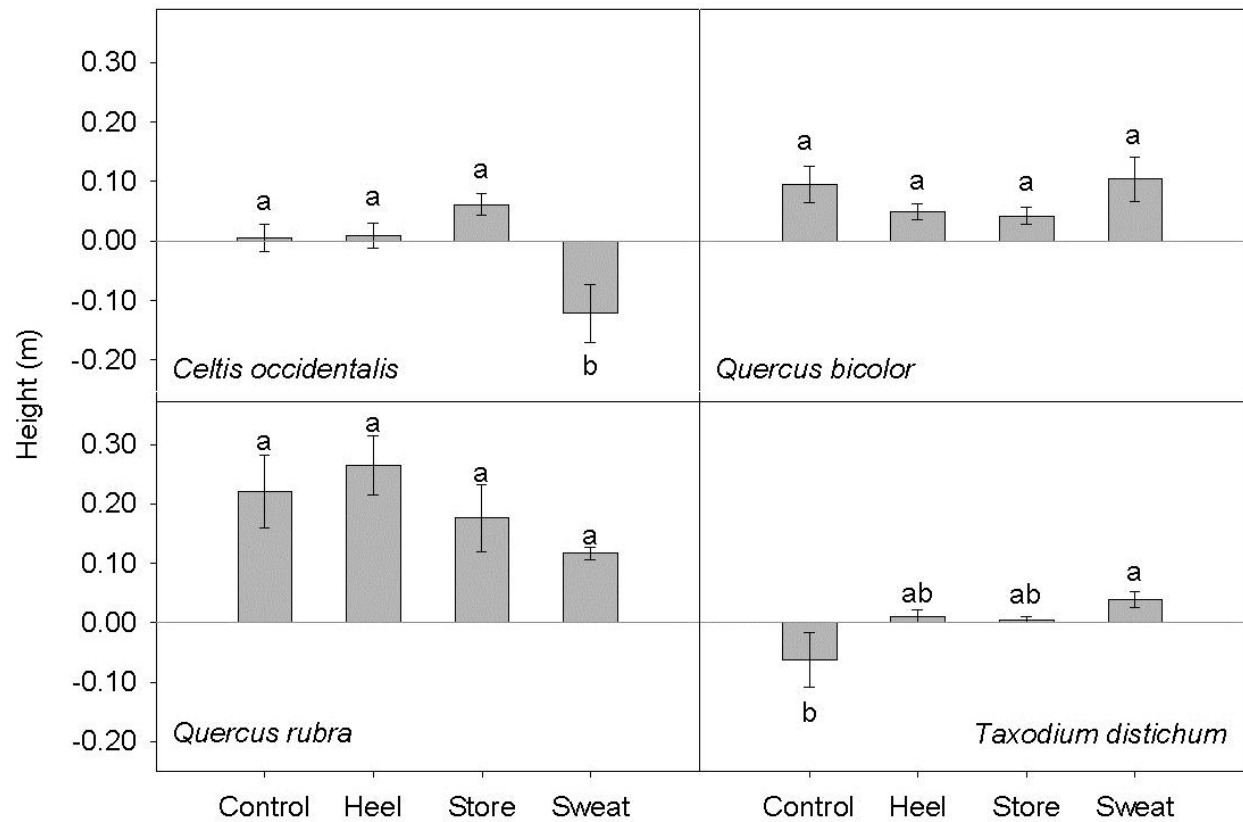


Figure A2.5. Mean growth/dieback of root growth potential-grown bareroot tree liners subjected to four pre-plant treatments in 2010.
 Note: spp: $p < 0.001$; trmt: ns; spp x trmt: $p < 0.05$

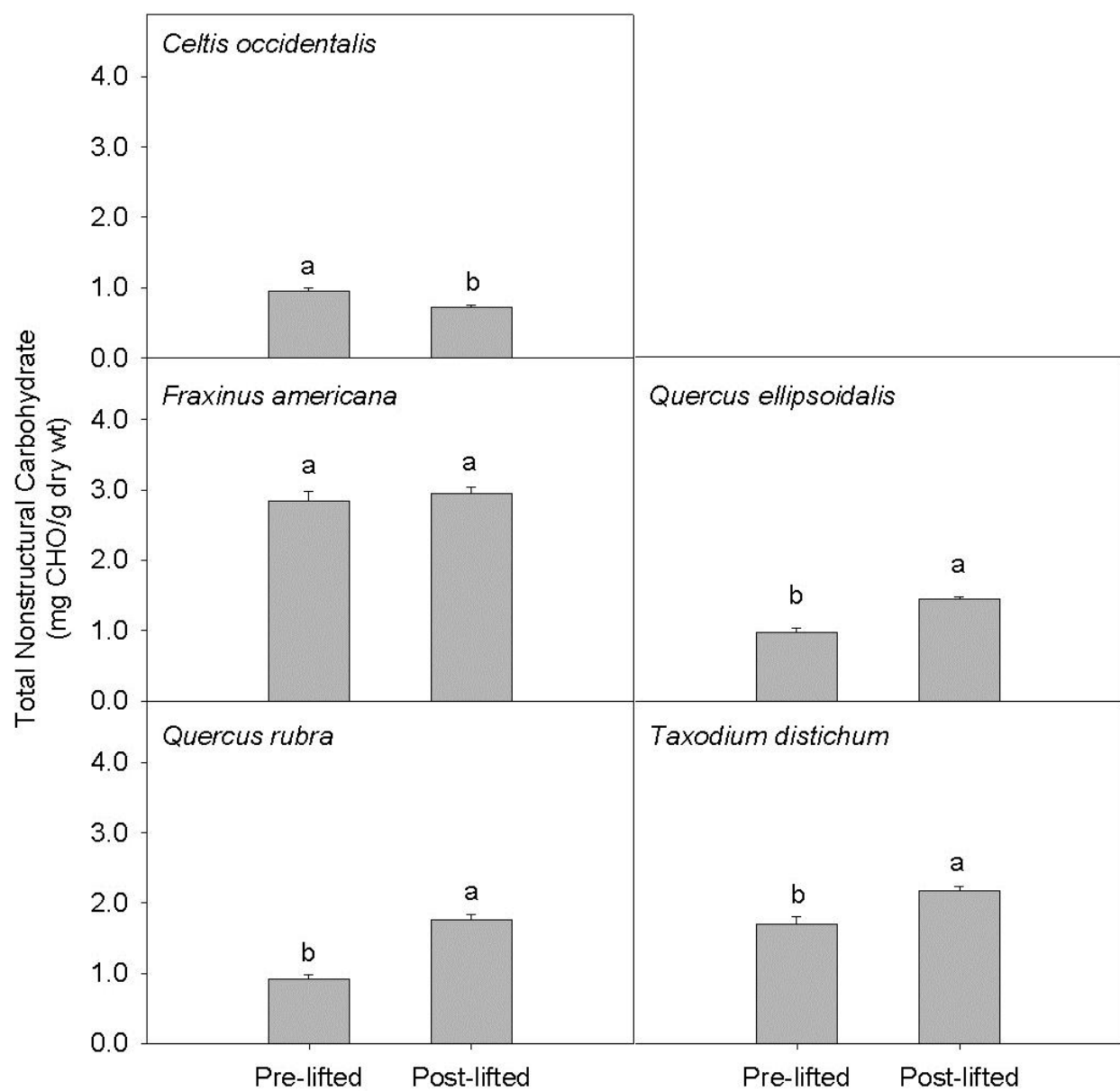


Figure A2.6. Mean total non-structural carbohydrate content of roots of bareroot tree liners collected pre- and post-lifted in 2011.

Note: spp: $p < 0.001$; time: $p < 0.0001$

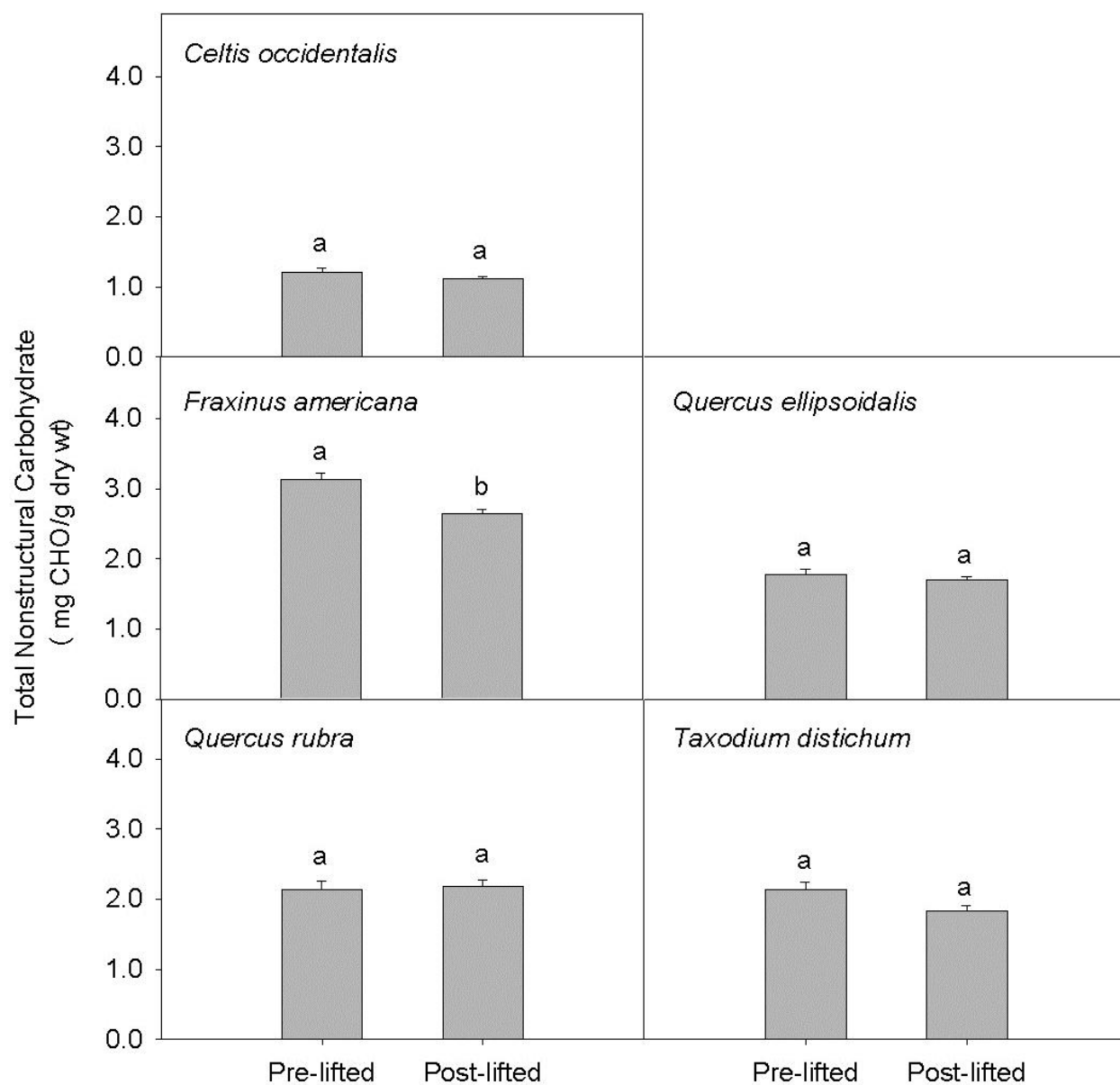


Figure A2.7. Mean total non-structural carbohydrate content of shoots of bareroot tree liners collected pre- and post-harvest in 2011.

Note: spp: $p < 0.001$; time: $p < 0.05$

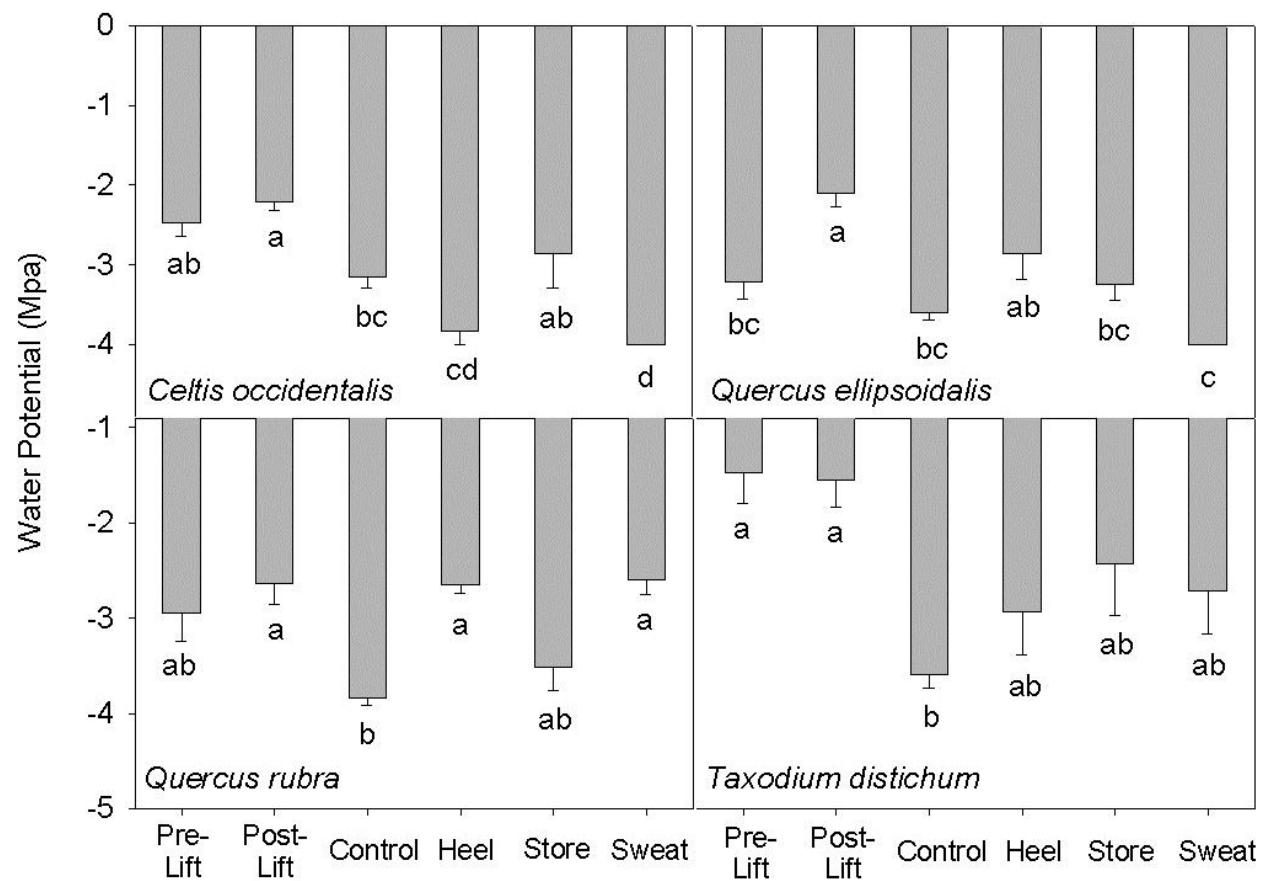


Figure A2.8. Mean water potential of bareroot tree liners prior to lifting (pre-lift), after lifting (post-lift), and four pre-plant treatments prior to planting in 2011.

Note: spp: $p < 0.001$; trmt: $p < 0.05$; spp x trmt: $p < 0.05$

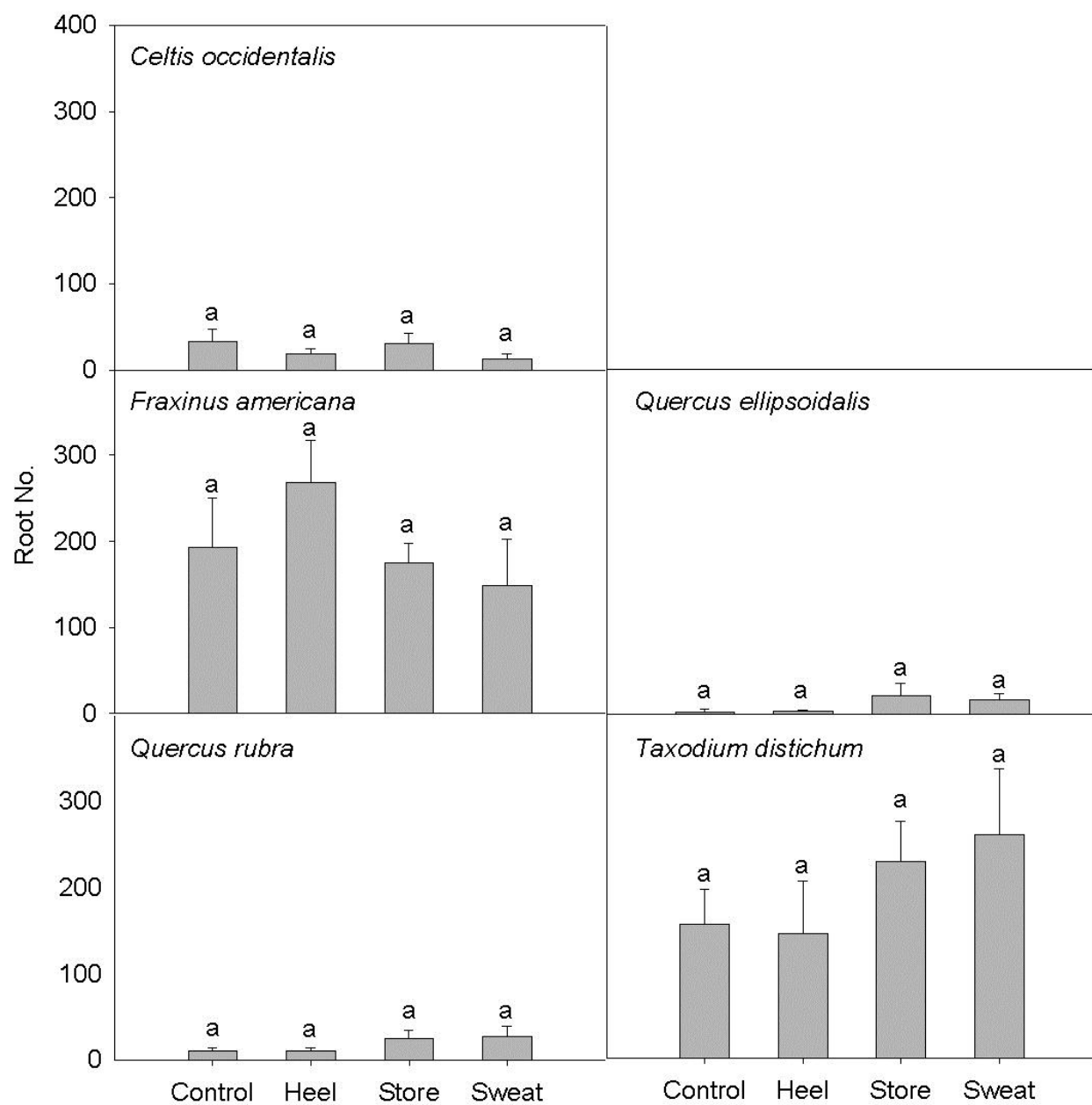


Figure A2.9. Mean root number of root growth potential-grown bareroot tree liners subjected to four pre-plant treatments in 2011.

Note: spp: $p < 0.001$; trmt: ns; trmt x spp: ns

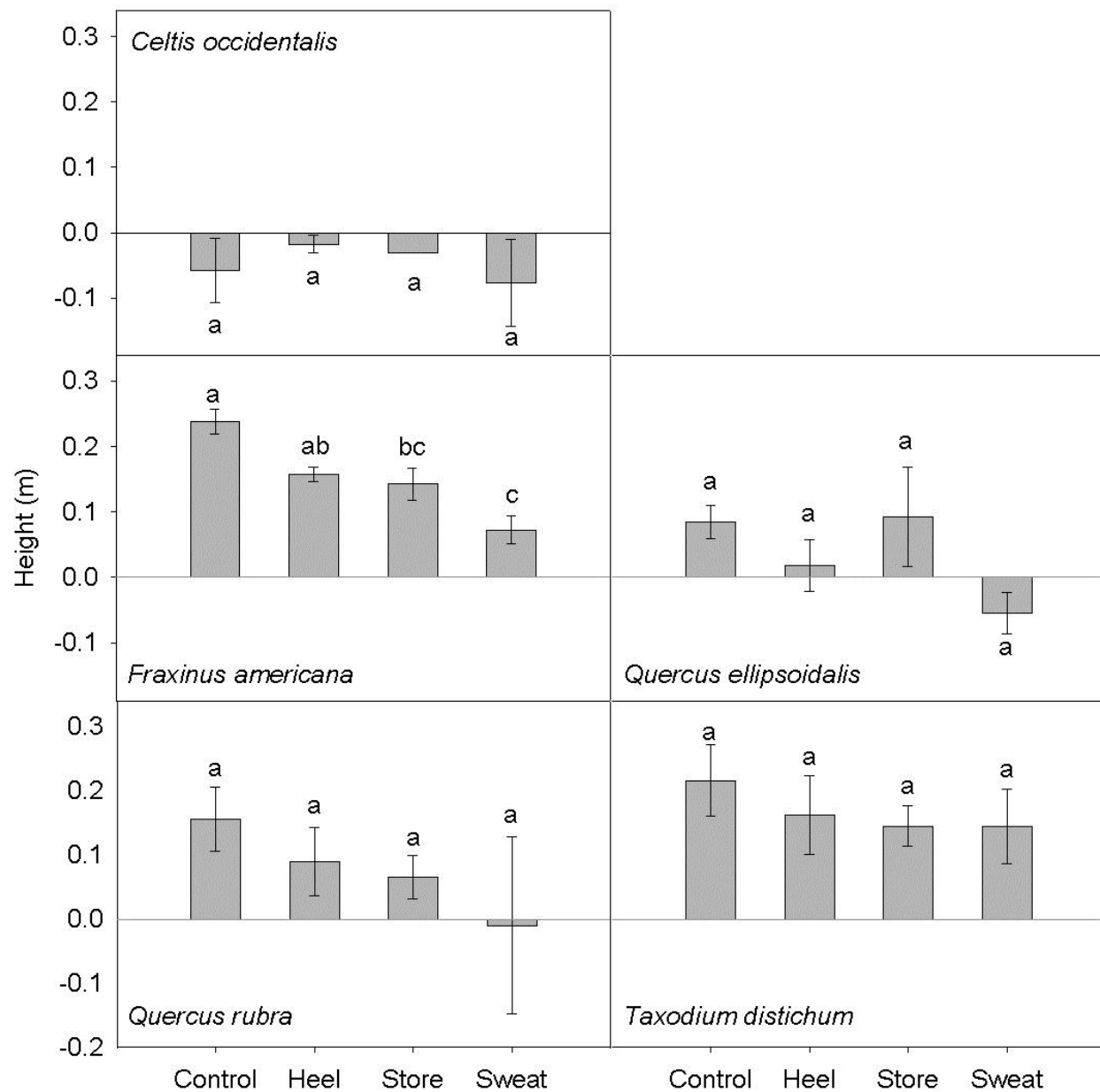


Figure A2.10. Mean growth/dieback of container-grown bareroot tree liners subjected to four pre-plant treatments in 2011.

Note: spp: $p < 0.001$; trmt: $p < 0.05$; spp x trmt: ns

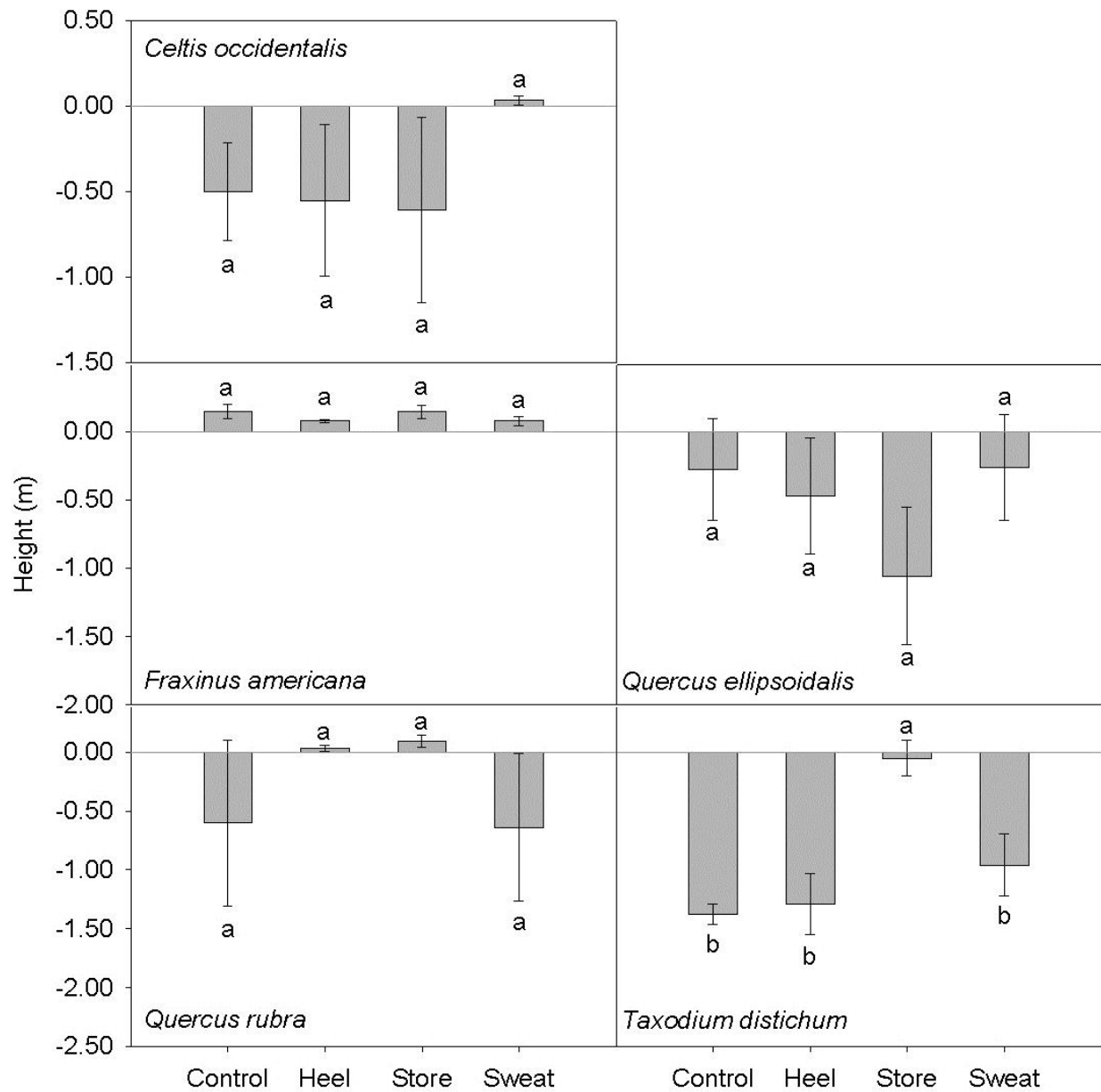


Figure A2.11. Mean growth/dieback of field-grown bareroot tree liners subjected to four pre-plant treatments in 2011.

Note: spp: $p < 0.05$; trmt: ns; spp x trmt: ns

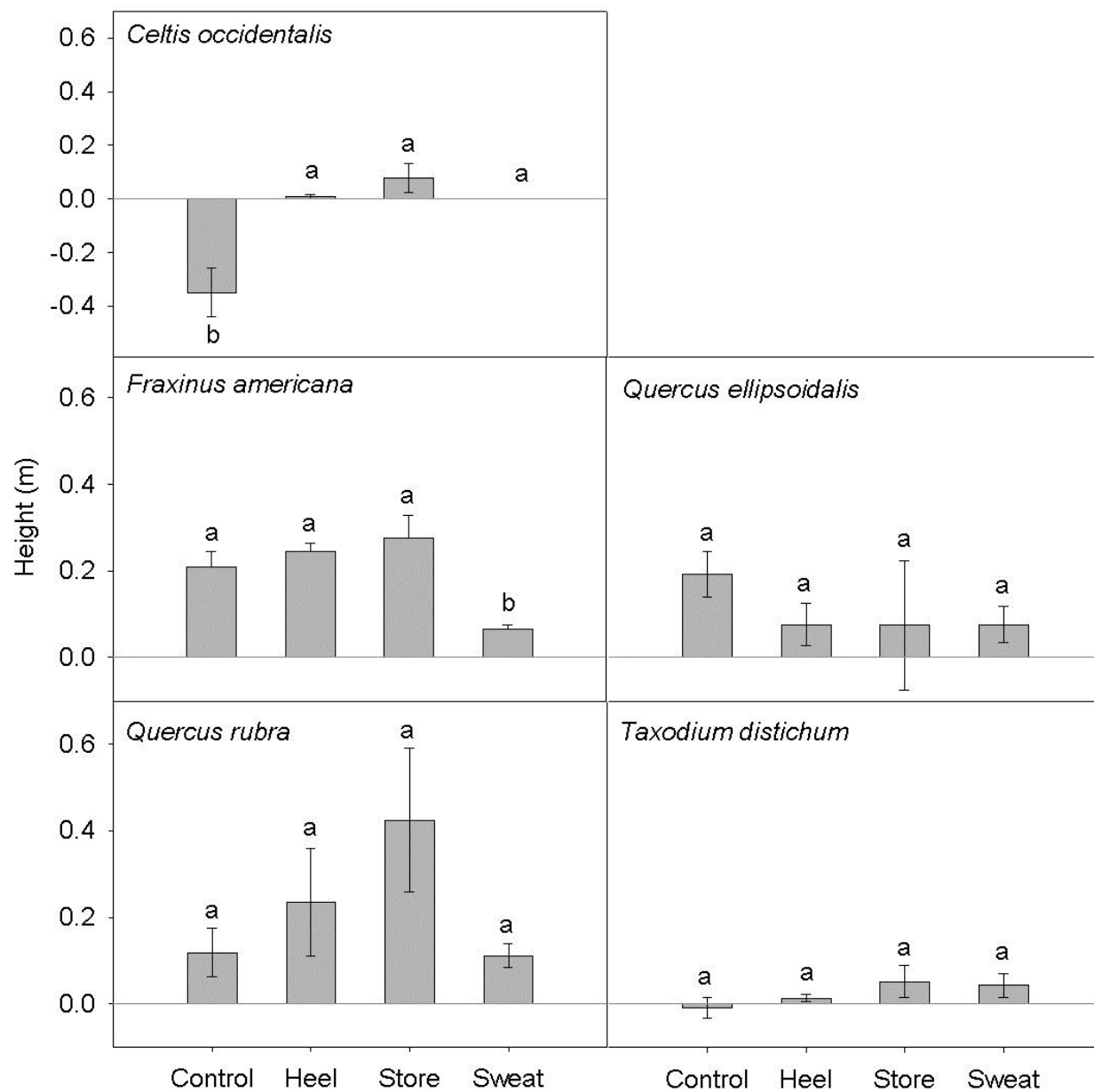


Figure A2.12. Mean growth/dieback of root growth potential-grown bareroot tree liners subjected to four pre-plant treatments in 2011.

Note: spp: $p < 0.001$; trmt: $p < 0.05$; trmt x spp: $p < 0.05$

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