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Use of plastic bottles as an alternative container type for propagation of forest tree seedlings in restoration programs

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USE OF PLASTIC BOTTLES AS AN ALTERNATIVE CONTAINER TYPE FOR PROPAGATION OF FOREST TREE SEEDLINGS IN RESTORATION PROGRAMS

A Thesis
Submitted to the Faculty
of
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by
Safiullah Khurram

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of
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I dedicate this thesis to my parents whose always support me

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ABSTRACT

Khurram, Safiullah. M.S., Purdue University, May 2015. Use of Plastic Bottles as an Alternative Container Type for Propagation of Forest Tree Seedlings in Restoration Programs. Major Professor: Douglass F. Jacobs.

Deforestation and forest degradation is a global issue, especially in poor and developing regions of the world. In order to combat deforestation it is critical to enhance the productivity of forest restoration operations, which often involve planting of nursery-grown forest tree seedlings. Production of low quality stock types with deformed and spiraled root systems is a significant issue hindering successful restoration programs. Polybags (i.e., small plastic bags) are a common container type for seedling propagation in developing countries. However, polybags produce seedlings with spiraled and deformed root systems that reduce outplanting survival and performance. Use of discarded plastic water bottles could be a feasible alternative as a container type for seedling propagation in restoration programs. The overall objective of this study was to develop technology for repurposing discarded plastic beverage bottles to grow quality native plants, trees and shrubs to benefit agroforestry, reforestation, restoration, and conservation programs. Specific objectives for this study were accomplished in two separate experiments (CHAPTER 2): 1) Container Comparison Experiment – to compare root and shoot development of seedlings grown in plastic bottles, modern nursery containers, and polybags; and 2) Bottle Modification Experiment – to examine the effects of root spiraling control techniques and container opacity on seedling morphological attributes.

In the Container Comparison experiment, seedlings of two species, Afghan pine (*Pinus eldarica* Medw.) and Arizona walnut (*Juglans major* [Toor.] Heller), were grown in four container types; Coca-Cola® beverage bottle (Coke), modern container Deepot™ D27 (D27), Polyethylene polybag (polybag), and Sam's Club® water bottle (Sams). At the first sampling period in August, Arizona walnut seedling shoot height, shoot dry biomass, and root dry biomass were all significantly greater in D27 containers compared to Coke bottles and polybags, while Sams bottles did not differ among treatments. Afghan pine seedling shoot height was significantly greater for seedlings grown in the Sams bottles compared to polybags, while Coke bottles and D27 did not differ among treatments. Root fibrosity was greater for seedlings grown in both Coke and Sams bottles compared to D27 and polybags. Similarly, the number of lateral roots was greater in Coke bottles compared to D27 and polybag containers. At the final measurement period (November), significant differences among treatments were found for all root morphological responses; for both species, seedlings grown in plastic bottles and modern containers had significantly less spiraled roots compared to the polybag. Seedling shoot and root development in plastic bottles at the end of the growing season was equal to or greater than that of the modern container. First year field height and diameter of Arizona walnut and Afghan pine were similar among containers. Similarly, first year field survival of both species was not affected by container type and was 100% for both species.

In the Bottle Modification experiment, Afghan pine seedlings were grown in Coca-Cola® beverage bottle with three opacity levels (green, black, and clear) and three spiraling control methods (side-slits, internal-ridges, and control). There were no significant interactions between spiral prevention and opacity treatments except for algae growth inside the container walls; black containers with either of the spiral control treatments produced lower algae fresh weight compared to clear and green containers. Spiral control treatments had significant impacts on Afghan pine RCD; Side-slit containers produced greater RCD compare to control and internal ridge containers. Side-slit and internal-ridge containers produced significantly lower numbers of spiraled roots compared to control (solid-wall) containers. At the beginning of the growing season, container opacity had significant impacts on seedling shoot height; green and clear containers produced significantly taller shoots compared to black. At the end of the growing season, black containers produced seedlings with significantly more fibrous roots compared to green containers, but no differences were detected in comparison to clear bottles. There were no significant interactions between spiral prevention and opacity treatments for first year field height and diameter growth. Individually, both spiral prevention and opacity treatments had no significant influences on Afghan pine field height and diameter excepting opacity for height growth. Green containers produced seedlings with significantly greater field diameter than black, while clear was not different among them.

Based on this research, plastic bottle containers may provide an effective alternative for production of high quality seedlings; use of side-slits represents a feasible way to prevent root spiraling. Future research should examine alternative media types from locally available resources and the growth of a variety of native species in these bottle container types.

CHAPTER 1. A REVIEW OF SEEDLING ROOT SYSTEM MORPHOLOGY AND PROPERTIES OF FOREST NURSERY CONTAINERS– THEIR ROLE IN OUTPLANTING SURVIVAL AND GROWTH

Introduction

According to the FAO Global Forest Resources Assessment (GFRA) report (2010), 10 million hectares of land was afforested and reforested per year between 1998 and 2007 throughout the world, and the total area of world plantation forest was estimated around 264 million ha, approximately 6.6 percent of the global forest area. Therefore, across the world billions of forest tree seedlings have been produced in nurseries annually. To enhance plantation productivity, the production of high quality nursery stock is vital. Establishment of successful trees and forests can be met by planting quality seedlings with targeted morphological and physiological characteristics to meet outplanting site conditions associated with satisfactory survival and growth (Rose and Haase 1995; Landis 2003).

A quality seedling is one with superior survival and growth (Duryea 1985; Mattsson 1997) available at a reasonable cost (Davis and Jacobs 2005a). Generally, seedling quality is associated with genetic, physical superiority and growing practices from nursery to outplanting site (Davis and Jacobs 2005a; Davis and Jacobs 2005b; Mexal and Landis 1990; Jaenicke 1999; Wightman et al 2001). Production and selection of good quality stock-types are the basis for successful tree planting. Poor quality seedlings will result in poor quality trees even if they planted in fertile and well-prepared sites. Seedling quality is important to resist stressful activities such as handling, lifting, grading and planting, but the most critical entity is their performance in the field (Sutton 1979). Previously there was less emphasis on root system quality, and many of the research papers and reviews have focused on whole plant physiological status and aboveground morphology (Sutton 1979; Jaramillo 1980; Ritchie 1984; Duryea 1985; Grossnickle and Folk 1993; Mattsson 1997; Mohammed et al. 1997; Puttonen 1997; Mattson 1997; Wilson and Jacobs 2006).

Currently, in most developed countries of world, forest regeneration and restoration programs attempt to use quality plant materials through the implementation of the target seedling concept (Ciccarese 2005). According to this concept, not all seedling types are suitable for all kinds of environmental and edaphic conditions (Mexal and landis 1990; Dumroese et al. 2005). According to Landis (2011) it’s unknown when the “target seedling” term was used for the first time, but it has been a standard for nursery and reforestation practices for many years. Landis (2011) indicated that the target seedling concept has been developed in three chronological stages. First, the evaluation of nursery stocks based on morphological parameters such as: height, RCD, oven-dry masses, and root/shoot ratio. Secondly, use of physiological research (examination of plant tissue nutrients contents, carbohydrates reserves or plant tissue water pressure) for seedling quality assessment. Lastly, to achieve the target seedling concept it’s important to use native plant species for restoration of degraded sites. IUFRO defined seedling quality as “fitness for purpose” in its workshop entitled “Evaluation of Planting Stock Quality” (Lavender et al. 1980). Consequently, the target seedling concept is the outcome of communication between producers and customers or people who plant trees in the field. Nurserymen communicate with tree planters and ask about the outplanting site ecological conditions and produce seedlings in correspondence with those circumstances (Landis 2003; Dumroese et al. 2005).

In most developing countries of world, seedling production is of low quality typically consisting of deformed root systems which has a serious hindrance on forest regeneration and restoration programs (Nixon et al. 2000; Gregorio et al. 2005; Harrington et al. 2012; Takoutsing et al. 2014). Forest nurseries' customers are less knowledgeable about seedling quality. Thus, shoot height is a common indicator that has been used for seedling quality assessment (Degrande et al. 2013; Grossnickle 1992). Furthermore, low quality seedlings have been supplied at reduced costs, thereby discouraging low-income buyers from purchasing high quality seedlings (Takoutsing et al. 2014). Additionally, bareroot nurseries are more common suppliers than container nurseries for regeneration and restoration activities in developing countries; and inferior practices (e.g., compacted soils with low nutrient reserves and lack of root culturing practices) used in these regions may result in seedlings with poor root architecture and morphology (Groninger 2005; Harrington et al. 2012; Takoutsing et al. 2014). In the few instances of container nurseries, most use polyethylene plastic bags as the container type (Harrington et al. 2012; Takoutsing et al. 2014; Gregorio et al. 2005). However, polybags often produce seedlings with deformed and spiraled (Sharma 1987; Aldrete et al. 2002; Gregrio et al. 2008) root systems that lead to root girdling and weak performance after outplanting (Sharma 1987). Poor drainage (Mexal 1996) and root egression into the soil surface below (Dumroese and Wenny 1997) from drainage holes are other common limitations of polybags increasing the difficulty of removing seedlings from bags at planting. These trends indicate a need to develop cost-efficient and effective systems of propagating forest tree seedlings in container nurseries in developing countries.

In this Chapter, I review some of the main indices used to evaluate nursery seedling quality for reforestation and restoration programs, discuss specific needs for improving propagation systems in container nurseries in developing countries related mainly to container attributes and resulting effects on seedling quality, and outline specific objectives and hypotheses for the research undertaken in this M.S. Thesis.

Shoot Height and Diameter

Shoot height and root-collar diameter (RCD) have been most commonly used as morphological parameters for forest tree seedling quality assessment (Sutton 1979; Chavasse 1980; Jaramillo 1980; Davis and Jacobs 2005a; Haase 2007). Major advantages are that these measurements are non-destructive, simple and easy to implement (Ritchie 1984; Thompson 1985; Racey 1985) and are good indicators of field performance (Dey and Parker 1997). Many research studies show a close correlation between seedling initial RCD and height to outplanting survival and growth (Mullin and Svaton 1972; Smith 1975; Pawsey 1972; Cleary et al. 1978; Matsuda 1989; Bayley and Kietzka 1997; Jacobs et al. 2006). However, RCD tends to be the better predictor of outplanting survival and growth; ecological conditions of outplanting sites often influence how seedling initial height impacts outplanting performance (Mexal and Landis 1990). For instance, seedlings with greater initial height performed vigorously in moist and highly competitive sites and were able to compete with existing vegetation better than smaller seedlings (Cleary et al.

1978). Another study, however, reported that seedlings with larger shoot/root ratio (large shoots) had greater transpirational and photosynthetic area and increased potential for water loss in dry sites (Carlson and Miller 1990). In contrast, shorter seedlings performed well in dry and less competitive locations because of reduced transpiration area. Schmidt-Vogt (1981) reported that shorter seedling outplanting survival was better than taller, while subsequent growth of taller was superior to smaller seedlings. Another disadvantage of taller seedlings is wind damage due to higher shoot/root ratio and weak support of root system (Ritchie 1984).

Puttonen (1989) argued that the role of initial height in outplanting performance is a confounded issue. Chavasse (1977) indicated that growth and performance of Radiata pine and Douglas-fir in outplanting sites were not correlated to seedling initial height over the period of a few years; however, survival and total dry mass of konara oak (*Q. serrata* Thunb.) were positively correlated to initial height five years after outplanting (Matsuda 1989). Therefore, seedling height can have adverse effects on survival during initial years following outplanting, but subsequent survival of smaller and larger seedlings is indistinguishable. Rose et al. (1997) found that survival of small size (1+0) ponderosa pine seedlings was greater than large size (2+0) seedlings in different outplanting sites at the first two growing seasons while during the end of third growing season survival of small and large sizes seedlings was identical. In the same study they observed that initial height of seedlings was a good indicator of ensuing height growth in the field. Additionally, after outplanting height growth was correlated to the initial height; 2+0 seedlings height was greater than 1+0 at all sites during the first two growing seasons except one dry and harsh climate site. Therefore, these results suggest that taller seedlings perform well in moderate environmental conditions.

Mexal and Landis (1990) indicated that stem diameter is a good predictor of outplanting survival and growth because seedlings with larger root-collar diameter have greater nutrient reserves and root volume, both of which are good indicators of outplanting survival and performance. Haase and Rose (1993) found that larger seedlings with increased stem diameter and dry weight had higher nutrient contents and concentration and performed vigorously after outplanting. There was a strong correlation between initial RCD and first year survival (Black et al. 1989) and height growth (Omi et al. 1986) for Douglas-fir seedlings. In another study, South et al. (1988) observed that increased tree wood volume of loblolly pine (*Pinus taeda* L.) after 30 years of growth was strongly correlated to initial seedling diameter when they were outplanted. South et al. (2005) also observed that field performance of longleaf pine (*Pinus palustris* Mill.) was positively correlated to increasing seedling RCD.

Root Morphology and Seedling Performance

Roots are responsible for providing access to vital nutrients and water for growth as well as anchoring a plant in a growing medium (Drew and Lynch 1980). Bigger, healthier root systems often yield superior plants (Sillick and Jacobi 2009). Previously, there was little knowledge about root morphology and architecture, and researchers have only supported the generalization that seedlings with larger root volume perform vigorously after outplanting (Rose et al. 1991, 1997; Long and Carrier 1993). Researchers have since developed the idea that adequate root morphology and architecture are also significant for outplanting establishment and growth (Lynch 1995; Jacobs and Seifert 2004; Davis and Jacobs 2005a; Thompson 1985). The stabilization and establishment of forest trees is critical for successful forest regeneration in restoration programs; therefore, the architecture of root systems of planting stock should not be malformed by inadequate operations and tools or improper handling in the nursery or at outplanting sites. A weakly anchored and deformed root system can have serious impact on mechanical stability of forest trees (Lindstrom and Rune 1999).

The above ground morphological parameters of the plant can easily be examined by visual assessments. Evaluation of below-ground part, however, is time consuming and destructive. Despite visual evaluation of above ground morphology it is critical to have below ground root system assessment for accurate and precise quality examination (Davis and Jacobs 2005a). Therefore, including an assessment of the root system in the overall seedling quality evaluation will better assist with determining the seedlings performance after outplanting (Jacobs et al. 2003; Wilson and Jacobs 2003).

Root and Shoot Volume

Root and shoot volumes are good indicators of seedling quality assessment for long term outplanting performance (Harrington 1994; Rose et al. 1997). They are indicative of root and shoot fresh mass from RCD down to the root tip and from RCD up to the shoot tip, respectively. Volumes are typically measured non-destructively through the water displacement method (Burdett 1979; Harrington 1994). Rose et al. (1997) concluded that ponderosa pine and Douglas-fir seedlings with greater root volume had increased survival, diameter, height, and fresh weight compared to small root volume seedlings. A study by Jacobs et al. (2005) found that initial seedling height, stem diameter, and fresh mass of three hardwood species (white oak, northern red oak, and black cherry) increased with increasing root volume. First and second year height and stem diameter of seedlings with larger root volume and more first order lateral roots (FOLRs) were significantly greater than those with smaller root volume and fewer FOLRs. They also found that initial root volume of oak species was a better predictor than the number of FOLRs for field height and stem diameter growth. A drawback to use only root volume as an indicator for morphological quality assessment is that it is not indicative of seedling root system fibrosity (Thompson 1985) or architecture because the water displacement volume of many fine and few large roots or spiraled and non-spiraled roots would be the same.

First Order Lateral Roots

Similar to other morphological parameters, FOLRs also play fundamental role in nursery and outplanting survival and growth. These roots are significantly important for the initiation of new roots and water and nutrient uptake after outplanting (Struve 1990). Ruehle and Kormanik (1986) concluded that there was close correlation between number of initial first order lateral roots and nursery and field performance. In this study, northern red oak seedlings with a greater number of FOLRs increased nursery performance as well as increased height, RCD, shoot and root dry mass after outplanting. Higher rates of survival have also been attributed to a greater number of FOLR (Sander 1977; Hobbs 1984; Thompson and Schultz 1995). Adequate lateral root morphology and the presence of permanent first order lateral roots were important for early establishment and growth of seedlings in outplanting sites (Schultz and Thompson 1989).

Root Fibrosity

A fibrous root system is an attribute of higher quality stock and it helps determine water and nutrient uptake capacity of plants. Root volume alone is not a good indicator to determine seedling root system quality because it does not have the ability to distinguish greater number lateral fine roots from a single large taproot (Thompson 1985). Root system fibrosity is also used to determine the ability of seedlings to establish after outplanting. Root system fibrosity played a prominent role in root growth potential (the ability to produce new roots) of transplants and field establishment of seedlings (Stone et al. 1962; Burdett 1976; Rowan 1983; Duryea 1985; DeWald and Feret 1987; Kainer and Duryea 1990). Researchers have used various approaches to determine root system fibrosity such as the number of higher order lateral roots per seedling (Deans et al. 1990), the number of active root tips (Kainer and Duryea 1990) and the percentage of root dry mass indicated by lateral roots (Tanaka et al. 1976). A review of the literature clearly shows that there is not a standardized method to assess root system fibrosity (Davis and Jacobs 2005a). Likewise, determination of root system fibrosity is also a time consuming and tedious process.

Morphological Indices of Seedling Quality Assessment

Root to shoot ratio, sturdiness quotient (SQ), and Dickson’s quality index (DQI) have been prevalently used to predict seedling quality and outplanting performance

(Deans et al. 1989; Jacobs et al. 2005; Zida et al. 2008). Root to shoot ratio is usually given as the ratio of root dry mass over the dry mass of the seedling shoot. It is one of the chief parameters used to determine the capacity of seedlings root system to fulfill above-ground (shoot) nutrient and water requirements. The ecological conditions of the outplanting site determine the optimal root/shoot ratio of nursery seedlings. For instance, seedlings produced for dry and nutrient poor environments must have larger root to shoot ratio; conversely, seedlings for moist and competitive environments should be produced with relatively optimal root/shoot ratio. There is not a standard value for root/shoot ratio; however, researchers suggested the value between one and two as optimal for different environments and tree species (Jaenicke 1999). Takoutsing et al. (2014) reported that seedlings with greater root/shoot ratio performed better than seedlings with smaller ratios in dry field conditions. Many research studies supported the idea that root/shoot ratio is the best indicator to match seedlings with environmental conditions of the outplanting site (McDonald 1991; Barnett and McGilvary 1993; South et al. 2005; Gregorio et al. 2005).

Sturdiness quotient refers to the proportion of seedling height over root collar diameter and has been used to express seedling vigor and robustness (Thompson 1985). Reduced value of SQ is indicative of greater physical strength and demonstrates that the seedling shoot is enough strong to withstand conditions in the outplanting site. However, higher values of SQ designate that seedlings are not physically strong enough to endure conditions in the field. Black spruce (*Picea mariana*) seedlings with higher value of SQ were very vulnerable to frost, wind damage, and drought (Roller 1977). The optimal value for seedling sturdiness is proposed to be less than six (Jaenicke 1999). Dickson’s quality index integrates shoot/root ratio and sturdiness quotient and is a useful method for seedling quality examination. Bayala et al. (2009) reported that DQI was a major indicator for predicating field performance of five semi-arid tree species seedlings. The formula for calculating DQI is as follow (Bayala et al. 2009):

$$\text{Quality index} = \frac{\text{Seedling dry weight (g)}}{\frac{\text{Height (cm)}}{\text{Root collar diameter (mm)}} + \frac{\text{Shoot dry weight (g)}}{\text{Root dry weight (g)}}}$$

Root System Deformities

If the natural tendency of a seedling’s root system growth is disturbed by limited container volume or nursery manipulations, seedling vigor and root morphology may be detrimentally altered (Thompson 1985) potentially resulting in negative post-planting growth and survival. Initial root form is fundamental for subsequent root morphology and architecture in the field (Sutton 1979). Adequate root morphology and architecture are significant because soil fertility and nutrients are dispersed unevenly and the seedling root system determines their capability to achieve these resources (Lynch 1995). In many cases, root deformation due to restricted root system or other nursery operations caused subsequent seedlings toppling and reduced growth several years after outplanting (Budy and Miller 1984; Lindstrom 1990; Halter and Chanway 1993; Halter et al. 1993).

The most significant root system deformities correlated to container nursery systems are root spiraling. Usually, root restriction and deformation are associated to design and container size. The design of interior container walls has a significant impact on seedling root system architecture. For instance, smooth container walls cause roots to spiral around the whole root system and reduce root vigor in nursery as well as in the field (Landis et al. 2010a). When roots hit the smooth container wall they continue to grow around the container circumference and restrict the whole root system. Spiraled roots confine the entire root system and prevent root expansion and growth. Evidence showed that trees with poor and unstable root system toppled and collapsed several years after outplanting in the field (Stefans-son 1978; Mason 1985; Burdett et al. 1986; Schnekenburger et al. 1985). Root spiraling stops water and nutrient movement throughout the root system (Hay and woods 1978; Watson and Himelick 1997) and trees may produce poor quality wood (Rune and Warensjö 2002). Therefore, characteristics of nursery container types play a significant role in future plantation establishment and growth.

Container size also has a strong influence on seedling growth and morphology. Larger containers produced taller seedlings with larger diameter, biomass, and nutrient concentrations, including N and K (Dominguez-Lerena et al. 2006). Larger volume containers retain a greater amount of water and nutrients, along with more space for root development. Regardless of economic considerations, larger containers with better spiral control techniques have positive impacts on seedling growth (McConnughay and Bazzar 1991; Hsu et al. 1996) and survival (Ward et al. 1981) post-planting.

Merits of Container Use in Forest Nurseries

Seedling production in containers has many advantages over bare-root stock-types. The most obvious advantage of seedling production in the container is the easy manipulation of environmental conditions in greenhouses to produce high quality seedlings in short growing period (Tinus 1974; Hanover et al 1976). For instance, container loblolly and slash pine seedlings were plantable in 12-14 weeks (Barnett and Brissette 1986). Container nurseries can avoid unfavorable conditions like exposures to high and low temperatures, frosts, droughts, pests, diseases, and weeds. Container seedlings have an extended planting window (Dumroese et al. 1992; Menzies 2001) because their roots are covered and unexposed to damage by planting operations or harsh environmental conditions. They could plant yearlong at any preferred time or favored environmental situation (Luoranen et al. 2003). An extended planting window can also help foresters to protect their new plants from frosts, sunburn, or drought exposures. Compared to bare-root, container stocktypes protect the root system from unfavorable conditions during outplanting and can be stored for long periods in the field when severe weather conditions prevent planting (McKay 1997). This can help planters to plant their seedlings in more preferable conditions. Container seedlings are less exposed to planting shock in the field because of undisturbed and protected root system by their plugs (Brissette et al. 1991). Extra care and maintenance are not required for container stock during transportation and storage compared to other stock types. Container stock may be transplanted at any growing stage (dormant, active growing, hardening off) and time, even during mid-summer (Brissette et al. 1991; Luoranen et al., 2003). This characteristic gives merits to container stock type over bare-root. Due to the ease of planting, container seedlings are less exposed to poor planting such as J- or L-rooting, which is more common with bare-root stocktypes (et al. 2010). Survival of container stocktypes has been observed to be higher than bare-root as shown by Gwaze et al. (2006) who reported that eight years survival of shortleaf pine (*Pinus echinata* Mill.) container seedlings (82%) was significantly greater than bare-root stocktypes (54%). Compared to other stocktypes, container seedling attributes tend to be more uniform because of similar growing conditions in a greenhouse environment (Gulden and Barnett 1982).

Container Attributes and Seedling Growth

One possible technique to alter seedling morphology to fit the outplanting site conditions is modifying the physical properties of nursery containers. Container type, color, design, depth, diameter, volume and cavity spacing determine initial seedling morphology and ensuing performance after outplanting (Tanaka and Timmis 1974; Ingram 1981; Hunt, 1990).

Container Volume

Seedling size is directly related to container volume and it potentially determines outplanting performance (Pinto et al. 2011). Seedlings grown in larger containers may produce larger root systems with sufficient quantities of nutrient reserves. Container volume had substantial impact on *Pinus pinea* seedling size in the nursery and later in the field. Seedlings reared in large volume containers had increased height, diameter, total biomass and higher nutrient (N, K) concentrations (Dominguez-Lerena et al. 2006). Numerous studies evaluated container volume effects on seedling growth and outplanting performance for both hardwood and conifer species (Appleton and Whitcomb 1983; McConnaughay and Bazzaz 1991; Aphalo and Rikala 2003; Dominguez-Lerena et al. 2006). These studies have found that larger containers have adequate space for root growth and provide sufficient water and nutrients for producing seedlings with better morphological and physiological attributes. Poorter et al. (2012) indicated that reduced growth of seedlings in small volume containers was due to decreased photosynthesis rate. The major disadvantages of seedling production in larger containers are higher costs of containers, larger space requirements, higher costs of transportation, handling, and planting in the field, and the longer time required to grow larger seedlings (Landis et al. 1990; Landis 2010b).

Container Design

Container type and color influence seedling growth and play a significant role in outplanting field performance. Single and Single (2010) reported that container design has a strong influence on seedling root system architecture and spiraling prevention. Nowadays, root spiraling is not a big problem for nursery production in developed countries because almost every type of container has a means for root spiraling prevention.

Choice of container type for propagation of a particular tree species depends on root system morphology, targeted criteria, and economics (Luna et al. 2009). Various containers types are available in the industry; some are reusable, while others are not. Two common modern container types are free-cell Deepots® and aggregated Stroblocks®. Deepots are made from black thick plastic with different sizes and round or square shapes. This container has interior ribs or ridges for root spiraling prevention. Usually Deepots aggregate together in hard plastic racks and most of them are reusable. Styroblock is another type of modern container with aggregated cells. This container type is reusable, light weight, and easy to shape. Styroblocks are usually white and therefore preferred under higher temperatures. A drawback of this container type is that the cells are stacked and not movable; when plants die this can cause spacing differences and variation in growth.

Container color is another important attribute affecting seedling root growth. It influences substrate temperature inside the container and temperatures > 30 ºC will reduce root growth significantly (Johnson and Ingram 1984). Root growth may stop at temperatures higher than 39 ºC (Mathers 2003). Black containers absorb more sunlight and can immediately increase the inside substrate temperature, but lighter colors can keep the substrate cooler (Luna et al. 2009). Research studies regarding container opacity reported various results. Markham et al. (2011) found that taller shoots were developed in red maple seedlings when grown in clear containers compared to black and green containers. In contrast, Blanchard and Runkle (2007) reported that container opacity did not have significant impacts on vegetative parts of two orchid cultivars, White Moon and Sharon Bay.

Assessment of Nursery Containers on Seedling Quality

Worldwide, container seedlings have been commonly used in forest restoration and regeneration programs. Selection of container type for seedling propagation is a significant part of forest nurseries' operations. Appropriate container types for forest tree seedling propagation should be affordable and biologically reliable (Landis et al. 1990). Readily available access to container types and space required by each container in the nursery are two fundamental features that one must ponder as economic bullet points. The most important biological attributes that should be contemplated during container type selection are seedling size and environmental condition of outplanting sites.

Container attributes (volume, design, root control methods) directly affect seedlings morphological and physiological characteristics, subsequently influencing outplanting performance. Seedlings planted out in harsh and hostile environments without irrigation and additional sustenance must have morphological and physiological characteristics in place to tolerate these conditions.

Root system deformity is one of the problems that must be avoided during seedlings production in containers. Recently, a variety of techniques (aerial, chemical, and mechanical root pruning) have been used to enhance root system fibrosity and architecture, and inhibit root spiraling (Kinghorn 1978; Riedacker 1978). Seedlings grown in smooth-walled containers such as polybags are likely to produce spiraled and deformed root systems and thus have issues after planting. Modern nursery systems use root spiraling control techniques to promote healthy root system architecture.

Chemically treated containers (coating on inner surface of container) have been used in modern containers to prevent root spiraling and develop more fibrous root systems. For example, copper-coated containers have been commonly used to modify seedling root systems and inhibit spiraling (McDonald et al. 1984; Ruehle 1985). Copper treatments stop lateral root elongation across the container perimeter and promote root configuration with production of higher order lateral roots (Watt and Smith 1999). Additionally, seedlings grown in copper-treated containers have shown significant improvements in morphological attributes (increased height, diameter and stem volume, number of lateral roots, root growth potential, total biomass, and quality index) compared to seedlings grown with no copper treatment (Tsakaldimi and Ganatsas 2006; Sayer et al. 2009). However, in another study, Dahoon Holly (*Ilex cassine* L.) seedlings grown in copper-coated containers had a reduction in root to shoot ratio and fine root dry weight compared to seedlings grown in non-treated containers (Gilman and Beeson 1995). As a result, we can argue that the seedling root system response to chemical pruning is also species specific.

Side-slits are commonly used in modern containers to reduce issues with root spiraling. The mechanism of this type of root control is when lateral roots grow and hit side-slits; they are pruned and suberized with aerial interception (Whitcomb 1981). Therefore, cessation of lateral root elongation stimulates seedlings to produce more branched root systems with an increased number of higher order lateral roots (Davis and Whitcomb 1975). Privett and Hummel (1992) reported that rooted cuttings of ‘Coral Beauty’ cotoneaster (*Cotoneaster dammeri* Schneid. ‘Coral Beaute’) and Leyland cypress [X *Cupresso-cyparis leylandii* (Jacks. And Dallim.) Dallim.] grown in side-slit container had greater height and few spiraled roots compared to the non-porous smooth-walled containers.

Mechanical barrier (ribs, ridges) placed on the inside of the container surface have been used to alter root system configuration (Kinghorn, 1978). The mechanism of this technique is that it stops lateral root circling across the container perimeter and forces roots to grow down toward the bottom drainage holes and pruned with the air interface (Kinghorn, 1978; Lindstrom, 1981). Therefore, mechanical pruning is an integration of both aerial and physical barriers. Brichell and Whitcomb, (1977) reported that vertical ribs across the container perimeter played a significant role in river birch (*Betual nigra*) seedlings root spiraling control. Based on these root training methods, many container production companies named their brands based on spiraling control techniques (Landis et al. 2010b). For instance, “Rootrainer^{TM}” (Beaver Plastic Ltd, Acheson), and “RootMaker$^{®}$” (Lacebark Inc, Stillwater, Oklahoma), Alberta), are two types of these containers that have air-slits for root spiraling prevention. Copperblock^{TM} (Beaver Plastic Ltd), is another type that have interior chemical-treated surfaces for root spiraling prevention.

Study Objectives and Hypothesis

The ultimate objective of this study is to develop technology for repurposing plastic beverage bottles to grow quality native plants, trees, and shrubs to benefit agroforestry, reforestation, restoration, and conservation programs worldwide. Specific objectives for this study were accomplished in two separate experiments, which were both conducted at the John T. Harrington Forestry Research Center in Mora, New Mexico. Each experiment included both a nursery and field (outplanting) phase. The first experiment (Container Comparison) compared root and shoot development for Afghan pine (*Pinus eldarica* Medw.) and Arizona walnut (*Juglans major* [Toor.] Heller) seedlings grown in four container types (Coca-Cola$^{®}$ beverage bottle, modern container Deepot^{TM}D27, polyethylene polybag and Sam’s Club$^{®}$ water bottle). The hypothesis for the Container Comparison experiment is that plastic bottle containers will produce seedlings with morphological attributes and root systems similar to that of the standard container type (Deepot^{TM} D27), while polybags will produce seedlings with deformed root systems and lower quality morphological attributes compared to the three other container types. The second experiment (Bottle Modification) examined root spiral control and opacity influences on Afghan pine seedling morphological attributes. In this second study, Coca-

Cola® beverage bottles were modified with three opacity levels (black, clear, green) and three spiral prevention techniques (side-slits, internal ridges and control with no alterations). The hypothesis for the Bottle Modification experiment is that container opacity and spiral control modifications will have a significant impact on seedling morphology and root growth dynamics. In both experiments, we expected differences observed in seedling quality during the nursery phase to translate to differences in outplanting establishment success during the field phase.

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CHAPTER 2. EVALUATION OF PLASTIC BOTTLES AS AN ALTERNATIVE CONTAINER TYPE FOR PROPAGATION OF FOREST TREE SEEDLINGS

Abstract

Modern nursery containers used to propagate forest tree seedlings have internal-surface barriers (ribs or ridges) or side-slits to prevent root spiraling. These containers are expensive in developing countries and so polybags (plastic bags) are more common, despite their tendency to produce seedlings with spiraled and deformed root systems that have less potential to establish and perform in harsh outplanting sites. Discarded plastic bottles may be a feasible alternative for seedling propagation in restoration programs of developing countries. We examined potential to repurpose plastic beverage bottles to grow quality native trees to benefit agroforestry, reforestation, restoration, and conservation programs. Specific objectives were accomplished in two separate experiments: 1) Container Comparison – to evaluate Arizona walnut (*Juglans major*) and Afghan pine (*Pinus eldarica*) seedling root and shoot development in two plastic bottle types compared to modern nursery containers and polybags, and 2) Bottle Modification – to examine the effects of root spiraling prevention techniques (side-slits, internal-ridges, and control) and container opacity (green, black, and clear) on Afghan pine seedling morphological attributes. We evaluated one season of nursery growth and first-year seedling field performance for both experiments. In the Container Comparison experiment, seedlings of both species had less spiraled roots in bottle containers compared to polybags. Arizona walnut had more fibrous root systems in polybags, while Afghan pine root system fibrosity was greater in bottle containers than in the two other types. First-year field height and diameter of both species were not affected by container type. In the Bottle Modification experiment, less spiraled roots occurred in containers with air-slits and interior-ridges compared to the control. The effects of container opacity on seedling morphology were inconsistent. Root spiral prevention and opacity had no significant influence on Afghan pine one-year field height and diameter, excepting opacity for height growth whereby, seedlings grown in green containers had taller shoots compared to black, but clear was similar among them. Plastic bottle containers may provide an effective alternative for production of high quality seedlings.

Introduction

Deforestation is a global issue that has been exacerbated by the fast growth of world population. Drivers of forest degradation and deforestation are regional and change over time (Rudel et al. 2009). Natural disasters, timber exploitation, illegal logging (Geist and Lambin 2002), agricultural land expansion (Gibbs et al. 2010) fuelwood collection and charcoal production (Anderson 1986; DeFries et al. 2007), grazing and ranching (Chakravarty et al 2012), squatter settlement (Kituyi et al 2001), lack of land ownership, and unsustainable land use (Lanly 2003; FAO 2011) are some of the current drivers of deforestation and forest degradation throughout the world.

Deforestation is prominent in poor and developing countries. Resource limitation is a major driver of deforestation because many people subside by exploiting forest resources (FAO 2010). In addition, there is a lack of effective systems to adequately reforest after forestlands have been disturbed, and governments of these countries are often unable to provide adequate services or effectively implement policies regarding natural resources conservation (FAO 2010). Thus, many restoration programs in developing countries are unsuccessful due to limited resources, expertise, and lack of quality planting materials (Nixon et al. 2000; Gregorio et al. 2008; Radoglou and Raftoyannis 2001; Gregorio et al. 2005; Roshetko et al. 2008; Harrington et al. 2012).

High quality nursery seedlings that are suitable for the environmental conditions of the outplanting site (Lavender et al. 1980) are vital for successful forest establishment (Schultz and Thompson 1997; Rose and Haase 1995; Landis 2003). Extensive research results suggest that nursery operations play a significant role in seedling quality and outplanting performance (Liu et al. 2000; Gao et al. 2007; Li et al. 2012; Takousting et al. 2012). However, the assurance of seedling quality for fitness of purpose is generally not taken into consideration in forest restoration programs in these countries (Lapis et al. 2001). Seedling height has often been used as a sole quality indicator rather than evaluation of seedling root system quality (Grossnickle 1992; Degrande et al. 2013). Planted seedlings often have poor root architecture and forked or deformed stems and are less likely to succeed when outplanted because they have a lower ability to overcome harsh environmental conditions (Sutton 1979; Sharma 1987). This reduces the effectiveness of forest restoration and regeneration operations.

Another problem that prevents nursery owners from investing in production of high quality stocktypes is limited and insecure market access (Mercado et al. 2009). Lack of demand causes seedlings to be grown too long in the nurseries (Mangaoang and Harrison 2003), which reduces the economic efficiency of nursery operations and leads to poor field performance after outplanting. Private nurseries in these countries compete by reducing their prices (Takoutsing 2014). However, these nurseries also tend to produce lower quality seedlings by using small containers, not allowing time for seedling hardening to environmental conditions, and using low-quality growing medium (Mercado et al. 2009). Quality control is also limiting in such forest nurseries and many producers and customers do not know how to properly check seedling quality (Degrande et al. 2013). Low quality seedlings attract customers because of low prices, which decrease the productivity of restoration efforts and prevent investment in high quality standard nursery operations and materials. Consequently, research on nursery practices and materials that may be readily transferable to enhance seedling quality is an important field of study for countries with limited resources.

Lack of appropriate container types in developing regions forces nursery operators to establish bare-root nurseries or use polybags as readily available containers in production nurseries (Jaenicke 1999; Harrington et al. 2012). Use of polybags filled with native topsoil and then placed on bare-ground is a common practice in nurseries in many developing countries (Mexal 1997; Harrington et al. 2012). However, seedlings grown in heavy topsoil in polybags with smooth inner-surfaces are prone to root-spiraling or J-rooting that causes poor outplanting performance (Bell 1978; Sharma 1987; Mexal et al. 1994; Mexal 1997; Gregorio et al. 2008). Root deformities, such as spiraled or J-shaped root systems, may reduce survival, stress resistance, water and nutrient uptake, vigor and mechanical stability after outplanting (Budy and Miller. 1984; Burdett et al. 1986; Lindstrom. 1990; Halter et al. 1993; Cedamon et al. 2004; Gregorio et al. 2005; Muriuki et al. 2007; Harrison et al. 2008; Bayala et al. 2009). A recent study (Cedamon et al. 2005) reported highly deformed root systems of two different species grown in polybags compared to those grown in a standard container type (hiko trays). Root egress from bags and growth into the soil below the polybag is another issue (Stein 1978) that may cause uneven seedling growth and root system damage during lifting. Furthermore, unlike other container types, polybags are not reusable and are typically used only once (Jaenicke 1999); discarded polybags can have a negative impact on the environment (Sanghi 2008; Adane and Muleta 2011).

Bottling companies around the world produce bottles for water and soft drinks, typically in 0.5 and 1.5 liter (L) sizes. These bottles are used briefly, usually only for the duration of consumption from a single user after which the bottle is discarded. Where recycling and waste management are limited, these bottles end up in streets, water ways, and open areas. In 2009, approximately 120 billion plastic water bottles (excluding carbonated beverages such as sodas) were used worldwide (Gleick 2010). Despite concentrated efforts, plastic bottles are often considered waste; however, they could be a cheap and readily available resource to help combat deforestation in developing countries. Many of these impoverished countries are facing severe environmental problems that can be ameliorated by planting trees in restoration projects (Bewket 2005). Restoration projects are limited because tree production nurseries in these areas do not have access to modern nursery containers to grow high quality seedlings. Plastic water bottles may provide an inexpensive and re-usable alternative for growing containers that has the two-fold advantage of reducing waste and extending the life of these products. Likewise, repurposing these bottles would reduce the use of plastic in agriculture. The United States used 237 million kg of plastic for agriculture in 1992, of which 66% was for nursery containers (Amidon 1994). In 2002, approximately 762 million kg of plastic was used in the agriculture sector (Levitan and Barros, 2003). The texture, pattern, color and thickness of these plastic bottles vary greatly, and if used as containers these properties may influence growing conditions for individual seedlings. Therefore, before using plastic bottles operationally as an alternative container type, research is needed to examine seedling growth and development in these bottles.

The overall objective of this study was to develop technology for repurposing plastic beverage bottles to use them as nursery containers for growing quality native trees to benefit agroforestry, reforestation, restoration, and conservation programs. Specific objectives for this study were accomplished in two separate experiments: 1) Container Comparison Experiment – to compare seedling root and shoot development in two plastic bottle types compared to seedlings grown in modern nursery containers and polybags, and 2) Bottle Modification Experiment – to examine the effects of root spiraling control techniques and container opacity on seedling morphological attributes. In addition, we evaluated seedling survival and performance for seedlings from both experiments in the field for one growing season.

Material and Methods

Plant Material and Experimental Treatments

In Experiment 1 (Container Comparison), we examined Afghan pine (*Pinus eldarica* Medw.) and Arizona walnut (*Juglans major* [Toor.] Heller) seedlings. Experiment 2 (Bottle Modification) used only Afghan pine. In Experiment 1, four container types Coca-Cola® beverage bottle (Coke), modern container Deepot™ D27

(D27), Polyethylene polybags (polybag) and Sam's Club® water bottle (Sams) were used. Plastic bottle containers were 0.5-L bottles from Coca-Cola® and Sam's Club® bottling companies, representing different qualities of plastic based on its durability. The plastic quality of the Coke bottles was thicker and more rigid than the Sams bottles. The depth and diameter of both bottle containers were 13 cm and 7 cm, respectively. Tops were removed from each bottle and six evenly spaced holes were placed in the bottom for drainage (Figure A2). Additionally, three vertical slits 12 cm in length spaced evenly around the bottle perimeter were created to control root spiraling. The volume of altered bottles, D27 and polybag was similar, approximately 500 ml. The primary difference between the Polybag and other container types was the lack of any root spiraling control mechanism. Deepot™ D27 containers were chosen to represent a standard industrial container type to compare with bottles and polybags in terms of seedling morphological parameters. No alterations were made to the D27 and this container type had internal ribs for root spiraling control and bottom holes for drainage. The depth and diameter of D27 were 17.8 cm and 6.4 cm, respectively.

Experiment 1 was established as a randomized complete block design with four container treatments and five blocks. Each block (replicate) contained 16 seedlings per treatment combination (64 seedlings per species per block, for a total of 640 seedlings). In Experiment 2 (Bottle Modification), we examined effects of three levels of bottle opacity (clear, green and completely opaque) (Figure A3) and three levels of root spiraling prevention method (side-slits, internal ridges, and control with no alterations). Coca-Cola® and Sprite bottles were used as container types. The top portion of each bottle was removed and six evenly spaced holes were placed in the bottom for drainage.

Three different colored bottles with various light penetration levels were tested in this study. The black color consisted of typical Coca-Cola® bottles coated with black paint, while green and clear were the original bottles of Sprite and Coca-Cola® beverages, respectively. All three colors were considered as opacity treatments. Two root spiraling control techniques (side-slits and internal ridges) were compared against one unaltered (control) treatment with no root spiraling prevention mechanism. For the side-slit treatment, three vertical side-slits, 12 cm in length, were evenly spaced around the circumference of the bottle. Likewise, three internal ridges, 12 cm in length and created using silicon adhesive, were evenly spaced around the inside perimeter of the bottle. This study was established as a completely randomized design with a 3 × 3 factorial structure (bottle opacity × root spiraling prevention). There were nine treatment combinations with sixty seedlings within each treatment combination for a total of 540 seedlings.

For both experiments and species, seed were sown in the first week of April 2013 at the John T Harrington Forestry Research Center (35º 58’ N, 105º 20’ W; 2207 m ASL) Mora, New Mexico, USA. Seedlings were reared for one growing season (2013) in a traditional greenhouse nursery with heating and cooling systems. Pad and fan evaporative coolers were installed in the greenhouse side-walls to convert hot air into a cool breeze. Artificial lighting was used to supplement natural light to ensure a minimum of a 12-hour photoperiod. The growing medium used in containers was a mixture of peat, vermiculite and perlite at a volume ratio of 2:1:1. Containers were kept moist until seed germination, after which irrigation was based on a gravimetric method (Bilderback et al. 2007; Newby 2013). A subsample of containers were selected by treatment for gravimetric weights and used to determine the irrigation schedule when those weights fell below 80% field capacity. The pH of irrigation water was controlled by mixing hydro phosphoric acid (H_3PO_4) and maintained at a pH of 6.0 to 6.5.

Water-soluble fertilizer was applied across the treatments in three different growing stages (i.e., starter, grower, and finisher) based on current operational nursery programs. Fertilizer rate was 25-150 mg/L started with minimum and reached to the peak and ended back with minimum concentrations. It was applied in every other irrigation for 30-45 minutes. Fertilization started in the beginning of June after seed germination and ceased in early-November in preparation for hardening off and storage. The “starter” fertilizer had a nutrient ratio of 10:30:20 (NPK). After two weeks in mid-June, the fertilizer type was shifted to the “grower” (NPK) of 21:5:20 to promote rapid root growth after germination (Edwards and Huber 1982). The “finisher” fertilizer was applied in mid-September after 12 weeks of using the “grower” and had a nutrient ratio of 4:25:35 (NPK). This fertilizer was applied for five weeks from late-September to early-November. This formulation had a higher concentration of phosphorus and potassium to harden seedlings so as to protect them from winter cold injury and a lower nitrogen concentration was chosen to slow down growth.

To initiate the hardening process in September, lighting and temperatures were reduced in the greenhouse using both shade cloth and an increase in the cooling conditions. At the end of the first growing season (December 2013) seedlings were moved to cold storage in a walk-in cooler (1°C) to maintain dormancy and prevent winter cold injury. Seedlings were weighed in storage using the gravimetric weight method to access dry down. No irrigation was required based a 65% field capacity irrigation point.

Seedlings for both experiments were outplanted at the beginning of their second growing season in June 2014 at the John T. Harrington Forestry Research Center. The soil type of outplanting site was silty loam well drained. This field was a bare-ground and was managed for Alfalfa experiment trial about 5 years ago. The outplanting site was disked before planting. Using a field sprinkler irrigation system, seedlings were watered twice a week for a two-hour period for seven weeks post outplanting. Weeds were removed during the early growing season through mechanical means. The outplanting component of Container Comparison experiment followed the same experimental design as in the nursery component, while the Bottle Modification experiment was a randomized complete block design with 3 × 3 (spiral prevention × opacity) factorial structure replicated with three blocks.

Measurements

Measurements were similar for both experiments. Seedling height and RCD were measured at the time seedlings were destructively sampled to examine root morphology and architecture. Destructive measurements occurred at two growth stages (August and November) using 4 seedlings per treatment combination per block (replicate) for the Container Comparison experiment and 15 seedlings per treatment combination for the Bottle Modification experiment at each of the harvest periods.

Seedlings that were destructively sampled at either period were evaluated for root morphology and architecture. Destructive measurements included shoot and root volumes, shoot and root dry mass, number of total lateral roots, number of spiraled roots, number of spiral controlled roots, number of bent roots and taproot length. “Spiraled roots” were defined as roots that once contacted the container wall begin to grow nearly horizontal to the ground with no interruption resulting in a spiraling effect around the container. Roots that began to spiral yet after 90º were controlled by root spiraling prevention barriers were designated “spiral controlled roots”. Roots that grew directly toward the container wall and after hitting the wall changed direction toward the bottom of containers were identified as “bent roots”. Additionally, the fresh mass of algae growth on the inner container walls of the emptied containers from the bottle modification study was assessed for the first destructive sampling period.

Destructive sampling procedures began with lifting seedlings from containers; roots were washed carefully to remove growing medium. Root and shoot volumes were measured using the water displacement method (Burdett 1979). Shoots were then separated from the root system at the root collar and placed into individual paper bags for determination of dry weight, while roots were used for further measurements.

Root architecture of each individual seedling was assessed systematically. Total root length was measured from the root collar to the end of the taproot. If multiple taproots existed, the longest was used for measurements. Subsequently, roots were separated into three segments: the top 5cm, middle 5cm and the bottom segment. The number of first order lateral roots was counted for each segment by removal from the taproot. In addition, the number of spiraled lateral roots, spiral controlled roots, and bent roots were counted within each segment. The separated lateral and tap roots for each segment were placed into individually labeled paper bags for drying. All plant material (shoots and separated roots) was placed into a drying oven at 70 °C for 48 hours. Once dried, the plant material from a single bag was weighed to the nearest 0.10 g.

Container substrate temperature was tested using Thermochron iButton Data Logger (Gasvoda et al. 2002) for the Bottle Modification experiment comparing the treatment combinations of three opacity levels and slit versus non-slit bottles (6 total). The design for this small trial was complete randomized design with 2×3 factorial structure (slits, no-slits × black, clear, green). There were 4 replications per treatment combination for a total of 24 bottles sampled for soil temperature.

Root fibrosity, root to shoot ratio, sturdiness quotient and quality index were also calculated after data collection. Root fibrosity was calculated based on percent of root dry mass indicated by the number of lateral roots (Tanaka et al. 1976). The formula for root fibrosity is shown as follow:

$$\text{Root fibrosity} = \left(\frac{\text{Lateral roots dry weight (g)}}{\text{Total root dry weight (g)}}\right) \times \text{Number of lateral roots}$$

For root to shoot ratio, root dry mass was divided by shoot dry mass, and the sturdiness quotient of seedlings was calculated as shoot height (cm) divided by root collar diameter (mm) (Thompson 1985). Quality index was calculated based on following formula (Bayala et al. 2009):

$$\text{Quality index} = \frac{\text{Seedling dry weight (g)}}{\frac{\text{Height (cm)}}{\text{Root collar diameter (mm)}} + \frac{\text{Shoot dry weight (g)}}{\text{Root dry weight (g)}}}$$

In the outplanting phase, seedlings were measured for height and ground line diameter at the time of planting (June 2014) and at the end of the growing season (November 2014) for both experiments. Survival was also recorded at the end of one growing season after outplanting. Relative growth was also calculated for field height and diameter growth based on the change in absolute height or diameter between specific time periods relative to the initial height or diameter of the seedling.

Statistical Analysis

All data were analyzed using the mixed model procedure (PROC MIXED) in SAS (SAS Institute Inc., Cary, NC, USA) with $\alpha = 0.05$. For the first experiment, effects of container types on seedling morphology were analyzed using analysis of variance (ANOVA) independently for each species for both the greenhouse and field components. When significant effects were detected within main effects, Tukey's honestly significant difference (HSD) test was performed to detect significant differences between means at $P < 0.05$. Residuals of all response variables were checked for normality and constant variance based on ANOVA assumptions. Analysis for the second experiment was similar to the first in that ANOVA was used to examine the effects of root spiraling prevention methods and opacity treatments on seedling morphological parameters.

Results

Container Comparison Experiment

Overall, container type had a significant influence on morphological responses for Arizona walnut seedlings in the nursery phase (Table 2.1). At the first sampling period in August, shoot height, shoot dry biomass, and root dry biomass were all significantly greater in D27 containers compared to Coke bottles and polybags, while Sams bottles did not differ among treatments (Table 2.2). Taproot length was significantly greater for Arizona walnut seedlings grown in D27 compared to Coke bottles and Sams bottles, but not different from the polybags (Table 2.2). Arizona walnut seedlings produced more fibrous root systems in polybags and D27 compared to Coke bottles, while Sams bottles were not different from all other containers (Table 2.2).

By the final measurement period (November) for Arizona walnut, all of the significant differences among treatments were found in root morphological responses (Table 2.1). The number of spiraled roots was significantly greater for seedlings grown in polybags compared to Coke and Sams bottles, but did not statistically differ from D27. However, absolute values showed that the number of spiraled roots in the D27 container type was almost half of that found in the polybag (Table 2.3). Taproot length continued to be significantly greater in D27 compared to all other container types (Table 2.3).

Container type also had a significant influence on Afghan pine shoot and root responses across both sampling periods (Table 2.1). Shoot height was significantly greater at the initial sampling period for seedlings grown in the Sams bottles compared to polybags, while Coke bottles and D27 did not differ among any treatments (Table 2.4). Root fibrosity was greater for seedlings grown in both Coke and Sams bottles compared to D27 and polybags. Similarly, the number of lateral roots was greater in Coke bottles compared to D27 and polybag containers (Table 2.4).

At the final sampling period (November 2013), no differences were detected in shoot response variables among treatments with the exception of shoot height (Table 2.5), which was significantly greater in Sams bottles compared to the D27; Coke bottles and polybags did not differ from any other treatment. There were more significant root responses to container type treatments at the final sampling period for Afghan pine (Table 2.5). As observed with Arizona walnut, the number of spiraled roots was significantly greater for seedlings grown in polybags compared to all other container types. Additional significant root responses included smaller taproot length for D27 compared to Sams bottles, less root fibrosity for D27 compared to Coke and Sams bottles, and fewer number of lateral roots in D27 compared to all other container types (Table 2.5).

Container impacts on final field height and diameter were non-significant for both Arizona walnut and Afghan pine at the end of first growing season (November 2014). First year field survival was not different among containers and was 100% for both Arizona walnut and Afghan pine. Only Arizona walnut relative height growth was significantly affected by container type ($P = 0.006$). Seedlings grown in Coke bottles had significantly greater relative height growth (11%) compared to the D27 (6%), Polybag (7%) and Sams (7%) bottle containers.

Bottle Modification Experiment

There were no significant interactions between root spiraling prevention techniques and opacity treatments for all morphological parameters of Afghan pine seedlings in either August or November. The only observed significant interaction was for algae growth on inner container walls ($P = 0.01$) in August. The side-slit, clear container resulted in significantly greater algae fresh weight compared to all other containers by opacity combinations with the exception of ridges, slits and control (Figure 2.1). Black color containers regardless of root spiraling prevention treatment produced significantly lower algae fresh weight compared to clear and green colors (Figure 2.1). The interaction between container opacity and slit treatments was not significant for container media temperature. Growing media temperatures did not vary based on bottle opacity, while bottles with side-slits were significantly (*P* <0.0001) cooler (23.8ºC ±0.08) than bottles with no slits (24.3ºC ±0.08) regardless of opacity.

Neither root spiraling prevention nor opacity treatments had any significant effects on seedlings shoot height, with the exception of opacity in August (Tables 2.6, 2.7 and 2.8). Seedlings grown in black containers produced significantly shorter shoots compared to clear and green containers (Table 2.9). Root collar diameter was only influenced at the final measurement period (November) in which containers with side-slits resulted in significantly greater RCD compared to internal ridges and the control (Table 2.8).

Our study results showed that taproot length was significantly affected by container opacity in both August and November; however, root spiraling prevention treatments had no effect on taproot length (Table 2.6). In August, seedlings grown in black containers produced significantly longer taproots compared to green containers, but taproot length of seedlings grown in clear containers did not differ from other treatments. Likewise, in November, seedlings grown in black containers produced significantly longer taproots than those in green or clear containers (Table 2.10).

Root spiraling prevention treatments had little impact on seedling performance at the initial sampling period (August). Bottles with internal ridges resulted in significantly greater root volumes compared to the side-slit treatment (Table 2.7). Root volume for the control treatment did not statistically differ from the other two root spiraling prevention treatments. By the final sampling period (November), the side-slit treatment resulted in significant increases in seedling RCD and shoot volume compared to both other treatments, as well as increase root volume, but only compared to the control treatment (Table 2.8). Root spiraling was significantly greater in the control treatment compared to the other treatments (Table 2.8).

Opacity treatments influence seedling performance at both measurement periods. In August, the black container resulted in significantly greater seedling height and an increase in the number of lateral roots compared to all other treatments (Table 2.9). The black container also resulted in significantly greater taproot length and more spiraled roots compared to the green container (Table 2.9). Shoot volume was significantly less for seedlings grown in black containers compared to all other treatments (Table 2.9).

At the final sampling period, the black container had significantly greater taproot length and more lateral roots compared to all other treatments (Table 2.10). Fibrosity was also greater for the black container but only compared to the green treatment (Table 2.10). Interestingly, the clear container resulted in significantly greater shoot volume and shoot biomass compared to the black container (Table 2.10).

There were no interactions between spiraling prevention and opacity treatments for one-year field height and diameter growth. Spiraling prevention and opacity treatments had no significant impact on one-year field height excepting opacity on final field diameter (P = 0.002). Final field diameter was smaller for black (8.5 mm) compared to green (9.2 mm) and clear (9.0 mm) containers. Field survival was not affected by root spiraling prevention or opacity treatments, and was 100% for all treatment combinations. Relative field height and diameter growth were not significantly affected by spiraling prevention or opacity treatments.

Discussion

In the Container Comparison experiment, there were few differences in plant growth responses at the final nursery sampling period among container types for both Arizona walnut and Afghan pine. One important difference found among container types was a pattern of increased root spiraling associated with the polybag container compared to all other treatments (except Arizona walnut in D27 containers) at the final nursery sampling period. This effect is the result of roots growing along the smooth, hard wall plastic of the polybag (Dumroese and Wenny 1997; Aldrete 2002; Landis et al. 2010). All of the other containers contained either vertical slits (bottles) or ribs (D27) to help prevent root spiraling. Outside of root spiraling, few significant differences were observed among treatments (root fibrosity, the number of lateral roots, taproot length, and height). In the case of Afghan pine, the plastic bottles actually had greater root fibrosity and number of lateral roots compared to the D27. This suggests that the side-slits may have been more effective than ribs; when lateral roots touch the side-slits, their growth ceases and this stimulates the taproot to produce more lateral roots (Davis and Whitcomb 1975). Another possible explanation is that media temperatures in the bottles may have been more favorable for plant growth; in the Bottle Modification study, containers with air slits regardless of color had reduced medium temperature compared to no-slit containers. Overall, these results suggest that plastic bottles (Coke and Sams) were similar to the industry standard container (D27) as well as the polybag. However, the deleterious root spiraling associated with the polybag suggests that this container type was less desirable for producing quality seedlings. Differences in taproot length between Arizona walnut and Afghan pine in D27 containers was likely associated with species-specific differences in taproot development as similarly reported by Al-zalzaleh (2013) for *Acacia saligna* and *Eucalyptus viminalis*. Arizona walnut seedlings produced a straight and hard taproot that was only stopped by air pruning; hence it was longer in the relatively deeper D27 containers compared to bottle containers. Afghan pine had a more flexible taproot that was less affected by container depth.

In the Bottle Modification experiment, both opacity and spiral prevention resulted in significant shoot and root morphology responses for Afghan pine (Table 2.6). By the final sampling period, seedlings grown in black (vs. green) containers had longer taproots, a greater number of lateral roots, and increased fibrosity (Table 2.10). This suggests that the higher light absorption of the black containers increased substrate temperatures and thus promoted root growth (Ingram 1981). Daily temperature records in our study showed that black containers had greater mid-day temperatures compared to the other two colors (Figure A1). Black containers promoted root development, and clear containers resulted in a significant increase of shoot volume and shoot biomass compared to the black containers (Tables 2.9 and 2.10). Similarly, Markham et al. (2011) found that red maple seedlings produced taller shoots when grown in clear containers compared to black and green containers. In contrast, Blanchard and Runkle (2007) reported that container opacity did not significantly impact biomass development of two orchid cultivars, White Moon and Sharon Bay.

Black containers also had less algae on the inner wall compared to both clear and green containers (Figure 2.3). Similar results were reported by Blanchard and Runkle (2007), who observed less algae growth inside the surfaces of containers with higher opacity. For both studies, plant performance was not affected by algal growth but this effect may impact the longevity and durability of the plastic bottle container.

In the Bottle Modification experiment, we found that the control or containers with no spiral prevention method resulted in significant increases in root spiraling. This concurs with previous findings that seedlings grown in solid-wall containers with no means of root spiraling prevention produced a greater number of spiraled and deformed roots (Marshall and Gilman 1998; Ortega et al. 2006). Regardless of opacity, modifying the plastic bottle using a side-slit resulted in significant gains in RCD, shoot volume, and shoot biomass. This is in contrast to results of Ortega et al. (2006) who reported lower shoot dry mass due to air-pruning in side-slit compare to solid-wall containers. The observed increase in shoot response to side-slits may be the result of better root medium conditions that promoted better gas exchange, as indicated by Al-zalzaleh (2013) who similarly found improved shoot responses in air-slit containers compared to solid-wall containers for *Acacia saligna* and *Eucalyptus viminalis.* Donahue et al. (1983) also reported that seedling growth is improved with better water movement, and good aeration. Restricted aeration in container medium will reduce photosynthesis, translocation and growth (Sutherland and Day 1988). Our results for Afghan pine shoot height concurred with Irmak et al. (2005) who reported longer shoots in clear compared to black containers; they also found that substrate temperatures for clear containers was always optimum and more favorable for root growth compared to the black color which exceeded from 40 ºC. In our Bottle Modification study, the black color container produced more lateral and spiraled roots. One possible reason for this is the longer taproot length in black containers compared to other colors. Because the longer taproot has more area for lateral roots and as the number of lateral roots is increased the number of spiraled roots might increases correspondingly.

In the outplanting phase, container type did not have any significant impact on Arizona walnut or Afghan pine seedling shoot height and diameter growth. The one-year period may have been insufficient to observe responses associated with treatment variation in nursery seedling root development. Lack of effects may have also been associated with post-planting irrigation, which was used to reduce potential mortality following the relatively late planting date. In the Bottle modification study root spiraling prevention treatment had no effects on Afghan pine height growth, which conflicts with reports of shorter shoot height in side-slit containers compared to solid-wall containers (Ortega et al. 2006). Correspondingly, Rune (2003) reported similar above-ground responses for seedlings grown in solid-wall and side-slit containers six years after outplanting. Container opacity effects were significant for final field diameter with green containers producing greater diameter compared to black though relative growth analyses showed no significant treatment effects. In both studies, field irrigation may have reduced potential to detect significant differences in early outplanting performance.

Conclusion

Use of plastic bottles as an alternative container type in production nurseries may offer a cost-effective opportunity for incorporation into reforestation and restoration programs, especially in developing countries that lack access to modern container types. Our results showed that bottle containers produced seedlings with better root architecture compared to polybags and similar to the modern container type. Use of side-slits in these bottle containers is a feasible means of preventing root spiraling and improving seedling root system quality. Production of seedlings with quality root systems will improve outplanting survival and performance on heavily degraded sites. Container opacity did not have important impacts on seedling above- and below-ground morphology. In warm temperature nursery conditions, seedlings may benefit from lighter color containers because of lower sunlight absorptive capacity and maintenance of optimum substrate temperature. Both Afghan pine and Arizona walnut seedlings produced longer shoots in lighter containers compared to the black color. Use of these bottles as nursery containers will also reduce consumption of plastic in the agricultural sector and provide a good alternative for waste management. Future research should examine alternative media types from locally available resources and the performance of a variety of native species in these bottle container types

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Table 2.1. Morphological parameters analysis of variance test (ANOVA) results for Arizona walnut and Afghan pine seedlings grown in four different container types; Coca-Cola® bottle, DeePot™ D27, Polyethylene polybag, and Sam's Club® bottle. Seedlings were destructively sampled in two time periods, August and November 2013. Significant effects are in bold at (α = 0.05). FOLRs stands for first order lateral roots.

Parameters	Arizona walnut				Afghan pine			
	August		November		August		November	
	F value	P value	F value	P value	F value	P value	F value	P value
Shoot height (cm)	3.59	**0.018**	2.55	0.062	3.52	**0.020**	3.20	**0.032**
Root collar diameter (mm)	1.42	0.245	1.27	0.291	1.17	0.326	2.43	0.072
Taproot length (cm)	13.19	**<.0001**	70.69	**<.0001**	7.81	**0.0001**	5.11	**0.003**
Shoot volume (cm3)	1.66	0.183	0.61	0.612	2.21	0.095	2.55	0.062
Root volume (cm3)	3.48	**0.020**	2.13	0.103	0.55	0.648	1.24	0.301
Shoot dry mass (g)	5.45	**0.002**	0.96	0.416	1.67	0.182	2.54	0.064
Root dry mass (g)	4.43	**0.006**	1.72	0.170	0.17	0.916	0.88	0.456
Lateral roots dry mass (g)	8.79	**<.0001**	1.74	0.167	0.83	0.482	0.64	0.590
Taproot dry mass (g)	3.84	**0.013**	1.69	0.177	1.01	0.393	2.02	0.119
Total dry mass (g)	5.71	**0.002**	1.64	0.189	0.63	0.507	1.94	0.130
Root fibrosity	4.50	**0.006**	3.01	**0.036**	10.37	**<.0001**	8.49	**<.0001**
Total FOLRs (#)	0.75	0.524	3.36	**0.018**	7.63	**0.0002**	8.64	**<.0001**
Spiraled roots (#)	1.93	0.133	6.47	**0.0006**	2.83	**0.044**	47.59	**<.0001**
Spiral controlled roots (#)	7.25	**0.0003**	7.59	**0.0002**	1.16	0.331	17.19	**<.0001**
Bent roots (#)	0.25	0.860	2.22	0.093	1.74	0.168	0.79	0.501
FOLRs in top segment (#)	0.90	0.443	1.49	0.224	3.48	**0.02**	2.64	0.056
FOLRs in middle segment (#)	1.65	0.186	1.24	0.301	12.48	**<.0001**	3.32	**0.025**
FOLRs in bottom segment (#)	8.98	**<.0001**	7.19	**0.0003**	4.67	**0.005**	5.31	**0.003**
Root: shoot ratio	0.45	0.721	0.40	0.756	1.42	0.243	0.63	0.597
Sturdiness quotient	1.87	0.142	7.76	**0.0001**	2.51	0.656	0.10	0.960
Dickson's quality index	2.02	0.120	2.39	0.076	0.15	0.929	1.09	0.360

Table 2.2. Arizona walnut seedling morphological parameters (Mean ±SE) in August sampling period. Seedlings were grown in the nursery in four different container types: Coca-Cola® bottle (Coke), Deepot™ D27 (D27), Polyethylene polybag (polybag) and Sam's Club® bottle (Sams). Reading across the rows means not followed by same lower-case letters are significantly different (*α*= 0.05) according to Tukey's HSD test. FOLRs stands for first order lateral roots.

Parameters	Arizona walnut							
	Coke		D27		Polybag		Sams	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Shoot height (cm)	23.1b	±2.1	29.1a	±2.1	22.6b	±2.1	23.7ab	±2.2
Root collar diameter (mm)	5.7a	±0.3	6.3a	±0.3	5.7a	±0.3	6.0a	±0.3
Taproot length (cm)	11.9bc	±0.5	14.9a	±0.5	13.4ab	±0.5	11.2c	±0.5
Shoot volume (cm3)	25.1a	±2.1	30.0a	±2.1	25.4a	±2.1	28.0a	±2.1
Root volume (cm3)	17.3b	±2.0	23.4a	±2.0	19.1ab	±2.0	19.7ab	±2.0
Shoot dry mass (g)	4.9b	±0.6	7.0a	±0.6	4.9b	±0.6	5.8ab	±0.6
Root dry mass (g)	4.1b	±0.6	5.9a	±0.6	4.0b	±0.6	4.5ab	±0.6
Lateral roots dry mass (g)	0.3c	±0.1	0.8a	±0.1	0.6ab	±0.1	0.4bc	±0.1
Taproot dry mass (g)	3.8ab	±0.5	5.1a	±0.5	3.4b	±0.5	4.1ab	±0.5
Total dry mass (g)	9.0b	±1.1	12.9a	±1.1	8.9b	±1.1	10.3ab	±1.2
Root fibrosity	3.3b	±0.7	6.4a	±0.7	6.5a	±0.7	4.6ab	±0.7
Total FOLRs (#)	37.7a	±4.0	44.9a	±4.0	44.1a	±4.0	43.6a	±4.0
Spiraled roots (#)	0.8a	±0.5	0.2a	±0.5	1.9a	±0.5	0.9a	±0.5
Spiral controlled roots (#)	0.4ab	±0.3	1.5a	±0.3	0.0b	±0.3	0.7ab	±0.3
Bent roots (#)	2.7a	±0.7	3.1a	±0.7	2.4a	±0.7	2.6a	±0.7
FOLRs in top segment (#)	19.1a	±2.0	17.3a	±2.0	17.3a	±2.0	20.0a	±2.0
FOLRs in middle segment (#)	12.9a	±1.6	14.8a	±1.6	14.1a	±1.6	17.7a	±1.6
FOLRs in bottom segment (#)	5.8b	±1.5	12.8a	±1.5	12.7a	±1.5	4.9b	±1.5
Root: shoot ratio	0.9a	±0.06	0.8a	±0.06	0.8a	±0.06	0.8a	±0.07
Sturdiness quotient	4.1a	±0.2	4.6a	±0.2	4.0a	±0.2	3.9a	±0.2
Dickson's quality index	1.8a	±0.2	2.3a	±0.2	1.7a	±0.2	2.0a	±0.2

Table 2.3. Arizona walnut seedling morphological parameters (Mean ±SE) in November sampling period. Seedlings were grown in the nursery in four different container types: Coca-Cola® bottle (Coke), Deepot™ D27 (D27), Polyethylene polybag (polybag) and Sam's Club® bottle (Sams). Reading across the rows means not followed by same lower-case letters are significantly different (*α*= 0.05) according to Tukey's HSD test. FOLRs stands for first order lateral roots. FOLRs stands for first order lateral roots.

Parameters	Arizona walnut							
	Coke		D27		Polybag		Sams	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Shoot height (cm)	25.4a	±2.1	32.5a	±2.1	27.5a	±2.1	26.4a	±2.1
Root collar diameter (mm)	11.5a	±0.7	10.9a	±0.7	10.3a	±0.7	10.1a	±0.7
Taproot length (cm)	13.0c	±0.3	16.0a	±0.3	15.0b	±0.3	12.3c	±0.3
Shoot volume (cm3)	10.4a	±1.4	12.0a	±1.4	10.4a	±1.4	9.4a	±1.4
Root volume (cm3)	57.1a	±5.7	66.4a	±5.7	67.3a	±5.7	51.3a	±5.7
Shoot dry mass (g)	5.4a	±0.7	5.5a	±0.7	5.0a	±0.7	4.2a	±0.7
Root dry mass (g)	29.2a	±3.0	32.0a	±3.0	32.1a	±3.0	24.1a	±3.0
Lateral roots dry mass (g)	2.6a	±0.7	4.3a	±0.7	4.2a	±0.7	2.9a	±0.7
Taproot dry mass (g)	26.6a	±2.5	27.6a	±2.5	27.9a	±2.5	21.2a	±2.5
Total dry mass (g)	34.6a	±3.4	37.5a	±3.4	37.1a	±3.4	28.2a	±3.4
Root fibrosity	2.3b	±0.4	3.5ab	±0.4	4.1a	±0.4	3.0ab	±0.4
Total FOLRs (#)	29.7ab	±2.4	28.6ab	±2.4	35.0a	±2.4	26.6b	±2.4
Spiraled roots (#)	0.8b	±0.6	1.9ab	±0.6	3.8a	±0.6	0.9b	±0.6
Spiral controlled roots (#)	2.2ab	±0.5	3.0a	±0.5	0.4c	±0.5	1.1bc	±0.5
Bent roots (#)	2.5a	±0.7	3.7a	±0.7	4.7a	±0.7	2.8a	±0.7
FOLRs in top segment (#)	11.6a	±1.2	10.4a	±1.2	11.3a	±1.2	8.4a	±1.2
FOLRs in middle segment (#)	11.3a	±1.0	9.8a	±1.0	11.7a	±1.0	12.1a	±1.0
FOLRs in bottom segment (#)	6.8b	±1.1	8.5ab	±1.1	12.1a	±1.1	6.2b	±1.1
Root: shoot ratio	6.6a	±0.6	6.2a	±0.6	6.8a	±0.6	6.3a	±0.6
Sturdiness quotient	2.2b	±0.1	3.0a	±0.1	2.7a	±0.1	2.7a	±0.1
Dickson's quality index	15.1a	±1.4	12.4a	±1.4	13.3a	±1.4	10.5a	±1.4

Table 2.4. Afghan pine seedling morphological parameters (Mean ±SE) in August sampling period. Seedlings were grown in the nursery in four different container types: Coca-Cola® bottle (Coke), Deepot^{TM} D27 (D27), Polyethylene polybag (polybag), and Sam's Club® bottle (Sams). Reading across the rows means not followed by same lower-case letters are significantly different (α= 0.05) according to Tukey's HSD test. FOLRs stands for first order lateral roots.

Parameters	Afghan pine							
	Coke		D27		Polybag		Sams	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Shoot height (cm)	15.8ab	±0.7	15.2ab	±0.7	15.0b	±0.7	16.4a	±0.7
Root collar diameter (mm)	2.4a	±0.1	2.3a	±0.1	2.2a	±0.1	2.3a	±0.1
Taproot length (cm)	17.1ab	±0.7	17.2ab	±0.7	19.5a	±0.7	14.7b	±0.7
Shoot volume (cm3)	6.8a	±0.5	6.1a	±0.5	5.6a	±0.5	6.9a	±0.5
Root volume (cm3)	5.0a	±0.3	5.1a	±0.3	4.7a	±0.3	5.3a	±0.3
Shoot dry mass (g)	1.1a	±0.1	1.0a	±0.1	0.9a	±0.1	1.1a	±0.1
Root dry mass (g)	0.35a	±0.04	0.39a	±0.04	0.39a	±0.04	0.37a	±0.04
Lateral roots dry mass (g)	0.24a	±0.02	0.23a	±0.02	0.23a	±0.03	0.27a	±0.02
Taproot dry mass (g)	0.11a	±0.03	0.15a	±0.03	0.15a	±0.03	0.10a	±0.03
Total dry mass (g)	1.4a	±0.1	1.4a	±0.1	1.3a	±0.1	1.4a	±0.1
Root fibrosity	41.0a	±1.7	30.1b	±1.8	31.4b	±1.7	39.3a	±1.7
Total FOLRs (#)	60.4a	±2.6	47.3b	±2.7	49.6b	±2.6	54.5ab	±2.6
Spiraled roots (#)	0.4a	±0.1	0.0b	±0.1	0.2ab	±0.1	0.1ab	±0.1
Spiral controlled roots (#)	0.2a	±0.1	0.2a	±0.1	0.0a	±0.1	0.2a	±0.1
Bent roots (#)	1.7a	±0.5	2.9a	±0.5	1.7a	±0.5	1.6a	±0.5
FOLRs in top segment (#)	14.1a	±0.7	11.7b	±0.7	12.6ab	±0.7	13.6ab	± 0.7
FOLRs in middle segment (#)	17.8ab	±0.9	13.3c	±0.9	15.7bc	±0.9	21.0a	±0.9
FOLRs in bottom segment (#)	28.5a	±2.0	22.3ab	±2.1	21.3b	±2.0	20.0b	±2.0
Root: shoot ratio	0.35a	±0.04	0.41a	±0.04	0.43a	±0.04	0.35a	±0.04
Sturdiness quotient	6.8a	±0.3	6.6a	±0.3	7.0a	±0.3	7.3a	±0.3
Dickson's quality index	0.1a	±0.01	0.2a	±0.01	0.1a	±0.01	0.1a	±0.01

Table 2.5. Afghan pine seedling morphological parameters (Mean ±SE) in November sampling period. Seedlings were grown in the nursery in four different container types: Coca-Cola® bottle (Coke), Deepot™ D27 (D27), Polyethylene polybag (polybag), and Sam's Club® bottle (Sams). Reading across the rows means not followed by same lower-case letters are significantly different (α= 0.05) according to Tukey's HSD test. FOLRs stands for first order lateral roots. FOLRs stands for first order lateral roots.

Parameters	Afghan pine							
	Coke		D27		Polybag		Sams	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Shoot height (cm)	33.6ab	±1.9	29.9b	±1.9	32.0ab	±1.9	34.7a	±1.9
Root collar diameter (mm)	5.9a	±0.2	5.1a	±0.2	5.6a	±0.2	6.0a	±0.2
Taproot length (cm)	22.3ab	±2.1	16.3b	±2.1	22.2ab	±2.1	26.5a	±2.1
Shoot volume (cm3)	38.9a	±3.0	31.7a	±3.0	33.2a	±3.0	42.0a	±3.0
Root volume (cm3)	26.6a	±2.9	20.3a	±2.9	23.3a	±2.9	25.1a	±2.9
Shoot dry mass (g)	8.2a	±0.6	6.7a	±0.6	7.0a	±0.6	8.9a	±0.6
Root dry mass (g)	3.6a	±0.4	2.9a	±0.4	3.1a	±0.4	3.5a	±0.4
Lateral roots dry mass (g)	2.7a	±0.3	2.2a	±0.3	2.3a	±0.3	2.6a	±0.3
Taproot dry mass (g)	0.8a	±0.07	0.7a	±0.07	0.8a	±0.07	0.9a	±0.07
Total dry mass (g)	11.8a	±1.0	9.6a	±1.0	10.1a	±1.0	12.4a	±1.0
Root fibrosity	48.0a	±3.0	34.4b	±3.0	44.3ab	±3.0	53.4a	±3.0
Total FOLRs (#)	66.0a	±4.7	46.7b	±4.7	61.8a	±4.7	75.0a	±4.7
Spiraled roots (#)	0.0b	±0.3	0.3b	±0.3	3.6a	±0.3	0.1b	±0.3
Spiral controlled roots (#)	2.0b	±0.3	3.4a	±0.3	0.1c	±0.3	2.1b	±0.3
Bent roots (#)	6.9a	±0.7	6.0a	±0.7	5.7a	±0.7	5.9a	±0.7
FOLRs in top segment (#)	15.1a	±1.0	11.9a	±1.0	15.2a	±1.0	15.3a	±1.0
FOLRs in middle segment (#)	18.2ab	±1.0	15.4b	±1.0	19.0a	±1.0	19.1a	±1.0
FOLRs in bottom segment (#)	32.8ab	±4.0	19.5b	±4.0	27.7ab	±4.0	40.7a	±4.0
Root: shoot ratio	0.43a	±0.02	0.43a	±0.02	0.44a	±0.02	0.39a	±0.02
Sturdiness quotient	5.9a	±0.3	5.8a	±0.3	5.7a	±0.3	5.9a	±0.3
Dickson's quality index	1.5a	±0.2	1.2a	±0.2	1.3a	±0.2	1.5a	±0.2

Table 2.6. Analysis of variance (ANOVA) results for morphological parameters of Afghan pine seedling grown in beverage bottles with three root spiraling prevention (df=2) and three opacity (df=2) treatments. Reading under p-value column, significant effects are in bold at (*α*= 0.05). FOLRs stands for first order lateral roots.

Parameters	Root spiral prevention effects				Opacity effects			
	August		November		August		November	
	F value	P value	F value	P value	F value	P value	F value	P value
Shoot height (cm)	0.86	0.424	0.28	0.760	9.77	**0.0001**	1.98	0.142
Root collar diameter (mm)	1.73	0.181	8.27	**0.0004**	1.44	0.240	1.95	0.147
Taproot length (cm)	0.12	0.887	0.38	0.684	6.57	**0.002**	15.42	**<.0001**
Shoot volume (cm3)	2.31	0.103	5.90	**0.004**	6.15	**0.003**	3.03	0.0515
Root volume (cm3)	5.92	**0.004**	0.14	0.872	0.78	0.459	0.29	0.752
Shoot dry mass (g)	2.71	0.07	3.94	**0.013**	4.13	**0.018**	3.61	**0.030**
Root dry mass (g)	2.47	0.089	0.21	0.812	4.51	**0.022**	0.61	0.5459
Lateral roots dry mass (g)	1.68	0.190	0.16	0.854	1.66	0.193	0.96	0.386
Taproot dry mass (g)	3.20	**0.044**	1.06	0.350	7.06	**0.001**	4.42	**0.014**
Total dry mass (g)	2.89	0.059	2.49	0.087	1.42	0.244	2.79	0.065
Root fibrosity	1.43	0.243	0.85	0.430	1.41	0.248	4.99	**0.008**
Total FOLRs (#)	0.39	0.679	1.21	0.300	4.72	**0.011**	7.53	**0.001**
Spiraled roots (#)	0.63	0.534	6.27	**0.003**	4.35	**0.015**	1.42	0.245
Spiral controlled roots (#)	0.46	0.631	16.87	**<.0001**	0.04	0.957	3.75	**0.026**
Bent roots (#)	0.02	0.984	1.10	0.335	1.98	0.142	0.11	0.899
FOLRs in top segment (#)	0.16	0.853	0.25	0.782	1.02	0.363	1.36	0.261
FOLRs in middle segment (#)	0.36	0.701	0.77	0.464	1.26	0.288	0.11	0.892
FOLRs in bottom segment (#)	0.67	0.516	0.94	0.392	11.79	**<.0001**	9.52	**0.0001**
Root: shoot ratio	1.37	0.258	1.35	0.264	16.38	**<.0001**	1.42	0.245
Sturdiness quotient	1.37	0.257	1.25	0.289	6.56	**0.0019**	0.29	0.748
Dickson's quality index	3.20	0.050	1.43	0.242	1.00	0.369	0.87	0.423

Table 2.7. Effects of root spiraling prevention treatment on morphological parameters of Afghan pine seedling (means ± SE) in August sampling period. Reading across the rows, means not followed by the same lower-case letters are significantly different (*α* = 0.05) according to Tukey's HSD test. FOLRs stands for first order lateral roots.

Parameters	Root Spiral prevention treatment					
	Control		Ridges		Slits	
	Mean	SE	Mean	SE	Mean	SE
Shoot height (cm)	15.0a	±0.3	15.5a	±0.3	15.1a	±0.3
Root collar diameter (mm)	2.5a	±0.05	2.6a	±0.05	2.5a	±0.05
Taproot length (cm)	15.7a	±0.5	16.1a	±0.5	16.0a	±0.5
Shoot volume (cm3)	9.5a	±0.4	10.6a	±0.4	9.4a	± 0.4
Root volume (cm3)	6.7ab	±0.2	7.4a	±0.2	6.3b	± 0.2
Shoot dry mass (g)	1.5a	±0.1	1.7a	±0.1	1.5a	±0.1
Root dry mass (g)	0.5a	±0.02	0.6a	±0.02	0.5a	±0.02
Lateral roots dry mass (g)	0.3a	±0.02	0.4a	±0.02	0.3a	±0.02
Taproot dry mass (g)	0.19ab	±0.01	0.21a	±0.01	0.17b	±0.01
Total dry mass (g)	2.03a	±0.1	2.26a	±0.1	1.99a	±0.1
Root fibrosity	34.9a	±1.1	36.8a	±1.1	37.6a	±1.1
Total FOLRs (#)	56.4a	±1.9	58.7a	±1.9	57.1a	±1.9
Spiraled roots (#)	0.7a	±0.1	0.6a	±0.1	0.4a	±0.1
Spiral controlled roots (#)	0.1a	±0.05	0.1a	±0.05	0.1a	±0.05
Bent roots (#)	5.0a	±0.4	5.1a	±0.4	5.0a	±0.4
FOLRs in top segment (#)	14.2a	±0.4	14.3a	±0.4	14.0a	±0.4
FOLRs in middle segment (#)	18.1a	±0.7	17.9a	±0.7	18.7a	±0.7
FOLRs in bottom segment (#)	24.1a	±1.5	26.4a	±1.5	24.5a	±1.5
Root: shoot ratio	0.3a	±0.02	0.4a	±0.02	0.4a	±0.02
Sturdiness quotient	0.6a	±0.1	6.0a	±0.1	6.2a	±0.1
Dickson's quality index	0.22a	±0.01	0.25a	±0.01	0.22a	±0.01

Table 2.8. Effects of root spiraling prevention treatment on morphological parameters of Afghan pine seedling (means ± SE) in November sampling period. Reading across the rows, means not followed by the same lower-case letters are significantly different (*α*= 0.05) according to Tukey's HSD test. FOLRs stands for first order lateral roots.

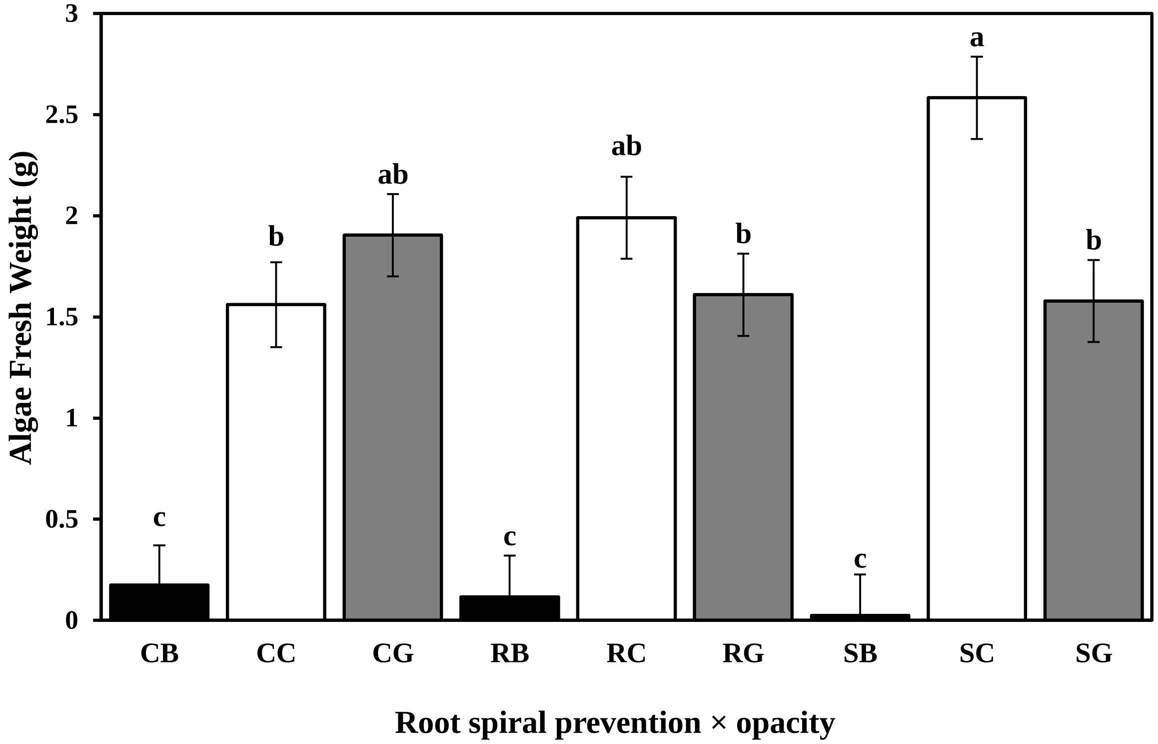
Parameters	Root spiraling prevention treatment					
	Control		Ridges		Slits	
	Mean	SE	Mean	SE	Mean	SE
Shoot height (cm)	40.2a	±0.8	40.3a	±0.9	41.0a	±0.9
Root collar diameter (mm)	7.0b	±0.1	7.0b	±0.1	7.5a	±0.1
Taproot length (cm)	24.5a	±1.7	22.7a	±1.7	22.7a	±1.7
Shoot volume (cm3)	63.0b	±2.5	65.4b	±2.5	74.4a	±2.5
Root volume (cm3)	40.6a	±2.2	39.7a	±2.2	41.4a	±2.2
Shoot dry mass (g)	14.5b	±0.5	15.0ab	±0.5	16.5a	±0.5
Root dry mass (g)	5.5a	±0.25	5.8a	±0.25	5.7a	±0.25
Lateral roots dry mass (g)	4.3a	±0.2	4.5a	±0.2	4.4a	±0.2
Taproot dry mass (g)	1.29a	±0.05	1.39a	±0.05	1.29a	±0.05
Total dry mass (g)	20.1a	±0.7	20.8a	±0.7	22.2a	±0.7
Root fibrosity	56.5a	±2.8	53.6a	±2.8	51.3a	±2.8
Total FOLRs (#)	75.0a	±3.8	70.6a	±3.9	66.7a	±3.8
Spiraled roots (#)	0.2a	±0.05	0.001b	±0.06	0.003b	±0.06
Spiral controlled roots (#)	0.002b	±0.1	1.0a	±0.1	1.1a	±0.1
Bent roots (#)	6.9a	±0.5	7.9a	±0.5	7.2a	±0.5
FOLRs in top segment (#)	17.8a	±0.9	18.4a	±0.9	17.4a	±0.9
FOLRs in middle segment (#)	18.4a	±0.7	17.7a	±0.7	17.1a	±0.7
FOLRs in bottom segment (#)	38.9a	±3.5	34.5a	±3.6	32.1a	±3.5
Root: shoot ratio	0.4a	±0.06	0.5a	±0.07	0.3a	±0.07
Sturdiness quotient	5.8a	±0.1	5.8a	±0.1	5.5a	±0.1
Dickson's quality index	2.44a	±0.1	2.52a	±0.1	2.72a	±0.1

Table 2.9. Effects of container opacity treatment on Afghan pine seedling morphological parameters (means ± SE) in August sampling period. Reading across the rows, means not followed by the same lower-case letters are significantly different ($\alpha = 0.05$) according to Tukey's HSD test. FOLRs stands for first order lateral roots.

Parameters	Opacity treatment					
	Black		Clear		Green	
	Mean	SE	Mean	SE	Mean	SE
Shoot height (cm)	14.2b	±0.3	15.8a	±0.3	15.7a	±0.3
Root collar diameter (mm)	2.5a	±0.1	2.6a	±0.1	2.5a	±0.1
Taproot length (cm)	17.3a	±0.5	15.8ab	±0.5	14.7b	±0.5
Shoot volume (cm3)	8.7b	±0.4	10.2a	±0.4	10.7a	±0.4
Root volume (cm3)	6.8a	±0.2	6.6a	±0.2	7.0a	±0.2
Shoot dry mass (g)	1.4b	±0.1	1.6ab	±0.1	1.7a	±0.1
Root dry mass (g)	0.58a	±0.02	0.49b	±0.02	0.52ab	±0.02
Lateral roots dry mass (g)	0.4a	±0.02	0.3a	±0.02	0.3a	±0.02
Taproot dry mass (g)	0.22a	±0.01	0.18b	±0.01	0.17b	±0.01
Total dry mass (g)	1.99a	±0.1	2.10a	±0.1	2.19a	±0.1
Root fibrosity	37.8a	±1.1	35.2a	±1.2	36.3a	±1.1
Total FOLRs (#)	62.1a	±1.9	55.3b	±1.9	54.8b	±1.9
Spiraled roots (#)	0.9a	±0.1	0.5ab	±0.1	0.3b	±0.1
Spiral controlled roots (#)	0.1a	±0.05	0.09a	±0.05	0.1a	±0.05
Bent roots (#)	5.6a	±0.4	5.0a	±0.4	4.4a	±0.4
FOLRs in top segment (#)	13.8a	±0.4	14.1a	±0.5	14.6a	±0.4
FOLRs in middle segment (#)	17.4a	±0.7	18.5a	±0.7	18.8a	±0.7
FOLRs in bottom segment (#)	31.0a	±1.5	22.7b	±1.5	21.4b	±1.5
Root: shoot ratio	0.4a	±0.02	0.3b	±0.02	0.3b	±0.02
Sturdiness quotient	5.8b	±0.1	6.2a	±0.1	6.2a	±0.1
Dickson's quality index	0.24a	±0.01	0.22a	±0.01	0.23a	±0.01

Table 2.10. Effects of container opacity treatment on Afghan pine seedling morphological parameters (means ± SE) in November sampling period. Reading across the rows, means not followed by the same lower-case letters are significantly different ($\alpha = 0.05$) according to Tukey's HSD test. FOLRs stands for first order lateral roots.

Parameters	Opacity treatment					
	Black		Clear		Green	
	Mean	SE	Mean	SE	Mean	SE
Shoot height (cm)	39.2a	±0.8	41.0a	±0.9	41.4a	±0.9
Root collar diameter (mm)	7.0a	±0.1	7.3a	±0.1	7.2a	±0.1
Taproot length (cm)	30.3a	±1.7	22.4b	±1.7	17.2b	±1.7
Shoot volume (cm3)	63.8b	±2.5	72.3a	±2.5	66.7ab	±2.5
Root volume (cm3)	39.4a	±2.2	41.8a	±2.2	40.5a	±2.2
Shoot dry mass (g)	14.3b	±0.5	16.3a	±0.5	15.5ab	±0.5
Root dry mass (g)	5.6a	±0.25	5.9a	±0.25	5.5a	±0.25
Lateral roots dry mass (g)	4.2a	±0.2	4.6a	±0.2	4.4a	±0.2
Taproot dry mass (g)	1.42a	±0.05	1.35ab	±0.05	1.2b	±0.06
Total dry mass (g)	19.9a	±0.7	22.2a	±0.7	21.1a	±0.7
Root fibrosity	60.1a	±2.8	53.8ab	±2.8	47.6b	±2.8
Total FOLRs (#)	82.0a	±3.8	68.9b	±3.8	61.4b	±3.9
Spiraled roots (#)	0.04a	±0.05	0.04a	±0.06	0.2a	±0.06
Spiral controlled roots (#)	0.6ab	±0.1	0.5b	±0.1	1.1a	±0.1
Bent roots (#)	7.4a	±0.5	7.1a	±0.5	7.3a	±0.5
FOLRs in top segment (#)	17.8a	±0.9	16.9a	±0.9	19.0a	±0.9
FOLRs in middle segment (#)	17.9a	±0.7	17.5a	±0.7	17.8a	±0.7
FOLRs in bottom segment (#)	46.4a	±3.5	34.6b	±3.5	24.6b	±3.6
Root: shoot ratio	0.5a	±0.1	0.4a	±0.7	0.4a	±0.1
Sturdiness quotient	5.6a	±0.1	5.7a	±0.1	5.8a	±0.1
Dickson's quality index	2.48a	±0.1	2.69a	±0.1	2.5a	±0.1



Figue 2.1. Effects of root spiraling prevention method by opacity treatment combinations on algae growth on inner container walls. The data were collected during the August 2013 destructive sampling period. The interaction between root spiraling prevention method and opacity treatments was statistically significant (P = 0.01). Abbreviations stand for: CB (control with black color), CC (control with clear color), CG (control with green color), RB (internal ridges with black color), RC (internal ridges with clear color), RG (internal ridges with green color), SB (side-slits with black color), SC (side-slits with clear color), and SG (side-slits with green color). Means (±SE) not accompanied by the same lowercase letters are significantly different (α=0.05) according to Tukey's HSD test.

APPENDIX

APPENDIX

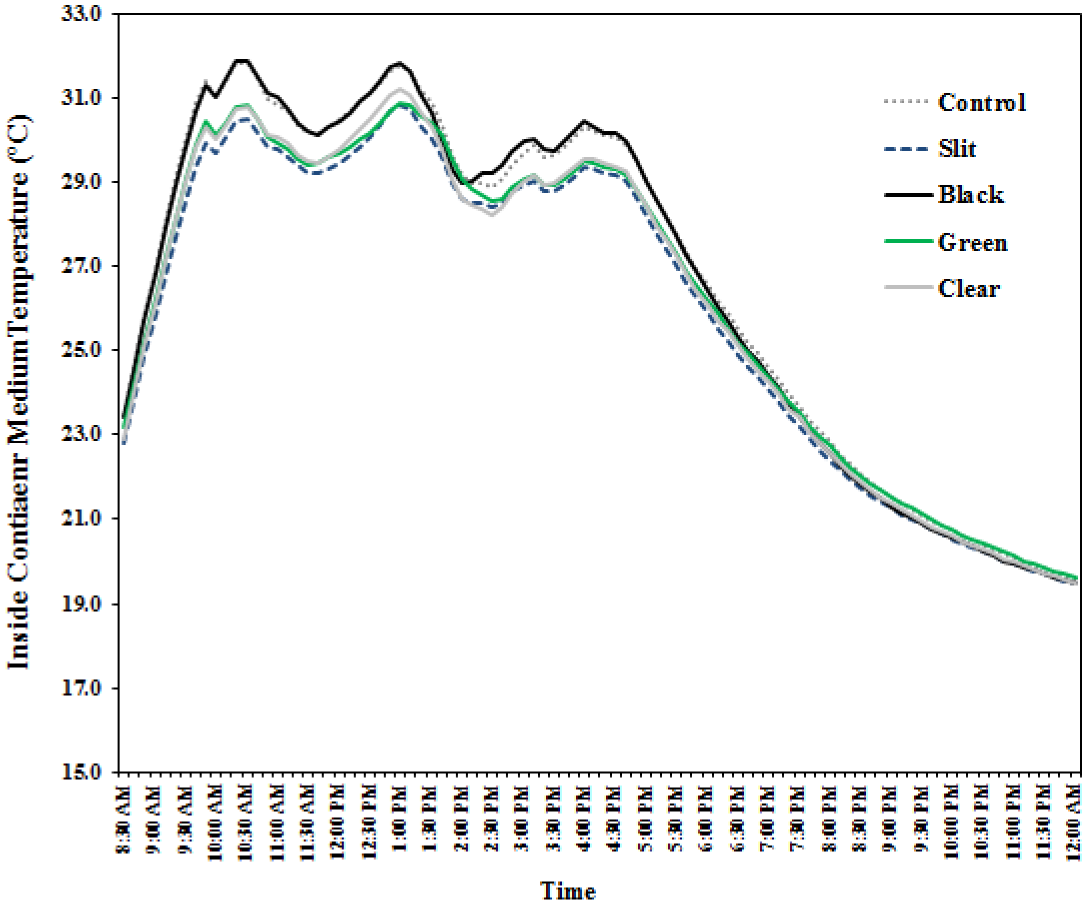


Figure A1. Daily temperature change inside container substrate with different opacities and with and without air-slits.

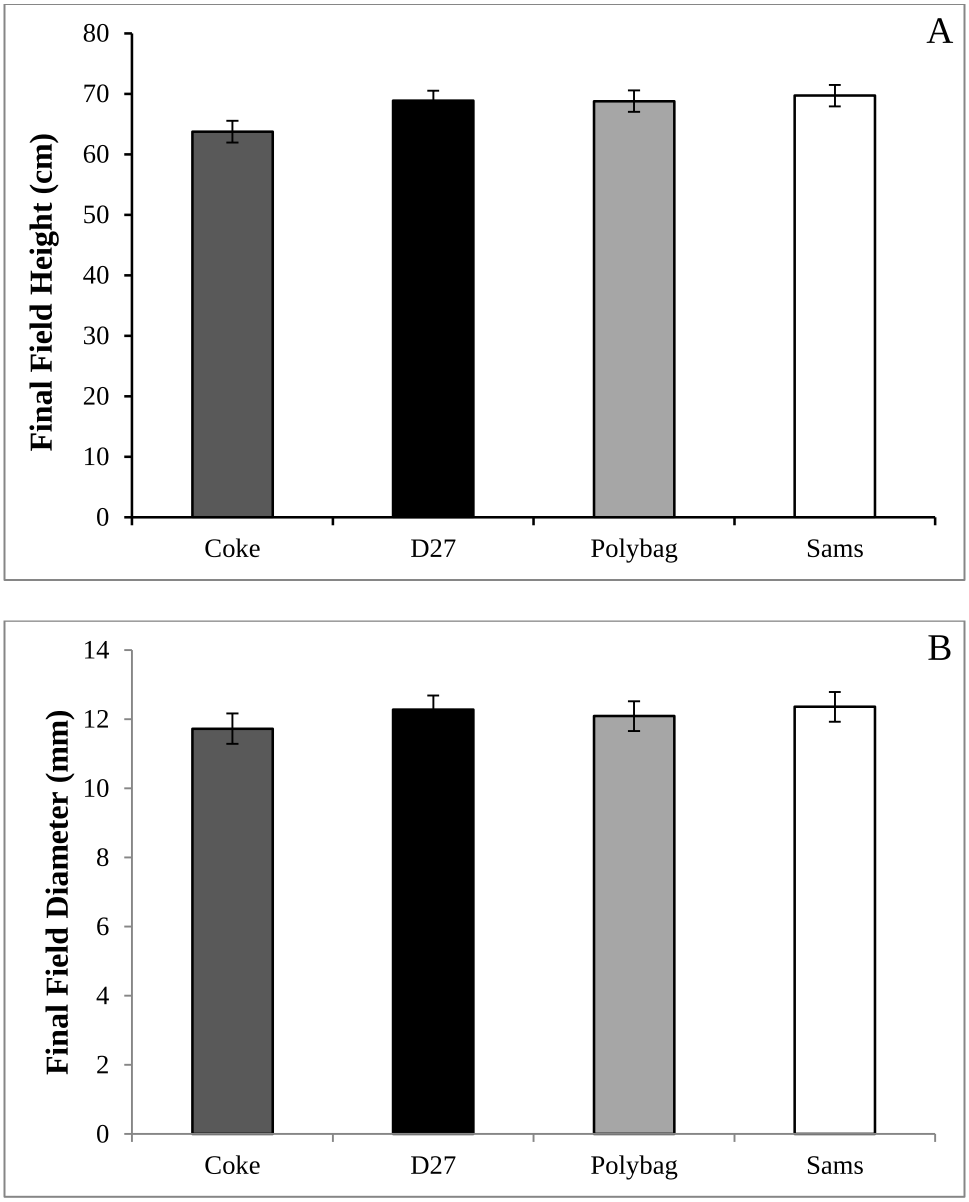


Figure A2. Arizona walnut seedling final field height and diameter (Means ± SE) for seedlings grown in the nursery for one growing season in four container types: Coca-Cola® bottles (Coke), Deepot™ D27 containers (D27), polyethylene polybags (polybag), and Sam's Club® bottles (Sams). Seedlings were outplanted in June 2014, and final height (A) and diameter (B) were measured in the November 2014. Non-significant differences between means (α =0.05) according to Tukey's HSD test, are shown in bars without letters.

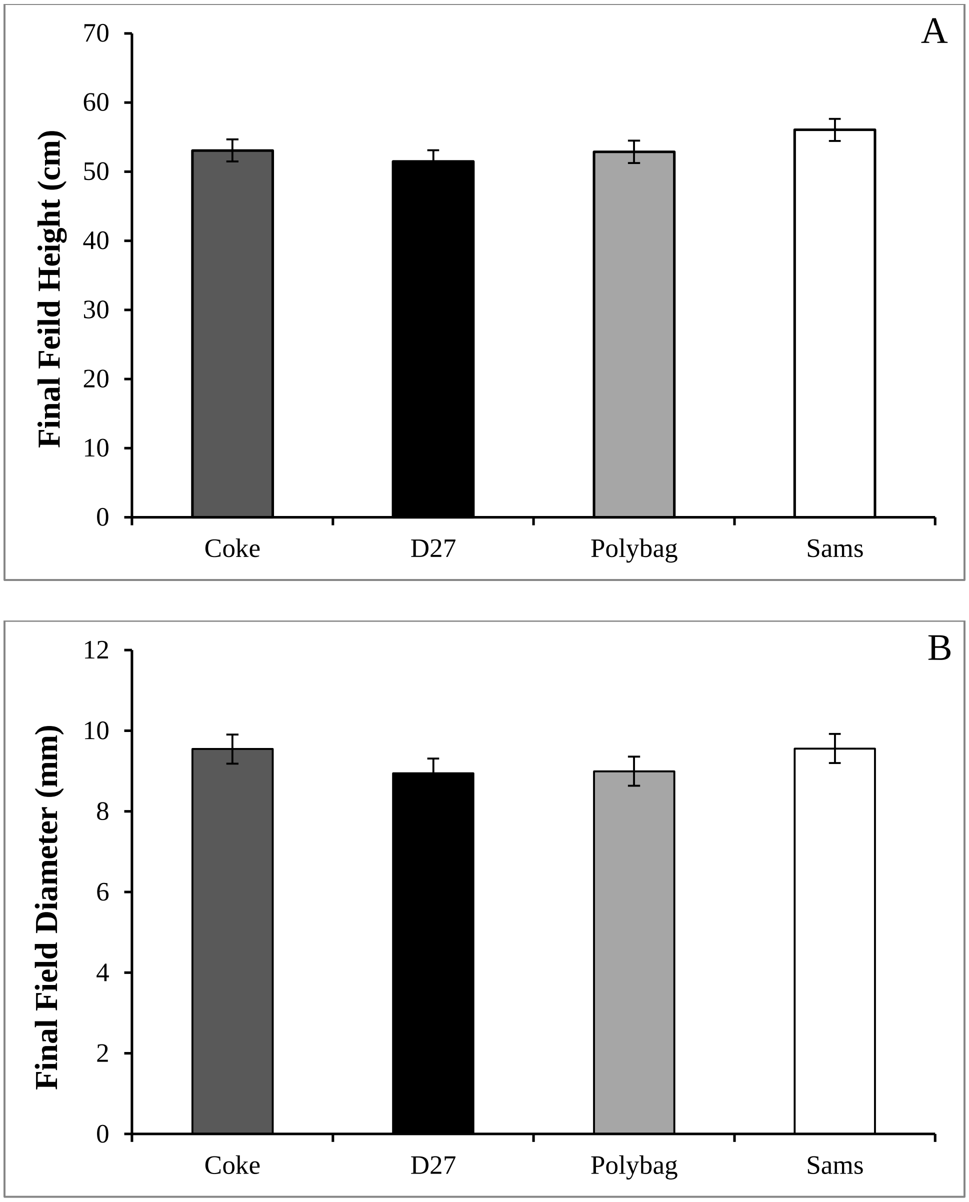


Figure A3. Afghan pine seedling final field height and diameter (Means ± SE) for seedlings grown in the nursery for one growing season in four container types: Coca-Cola® bottles (Coke), Deepot™ D27 containers (Deepot), polyethylene polybags (polybag), and Sam's Club® bottles (Sam's). Seedlings were outplanted in June 2014, and final height (A) and diameter (B) were measured in the November 2014. Non-significant differences between means (α =0.05) according to Tukey's HSD test, are shown in bars without letters.

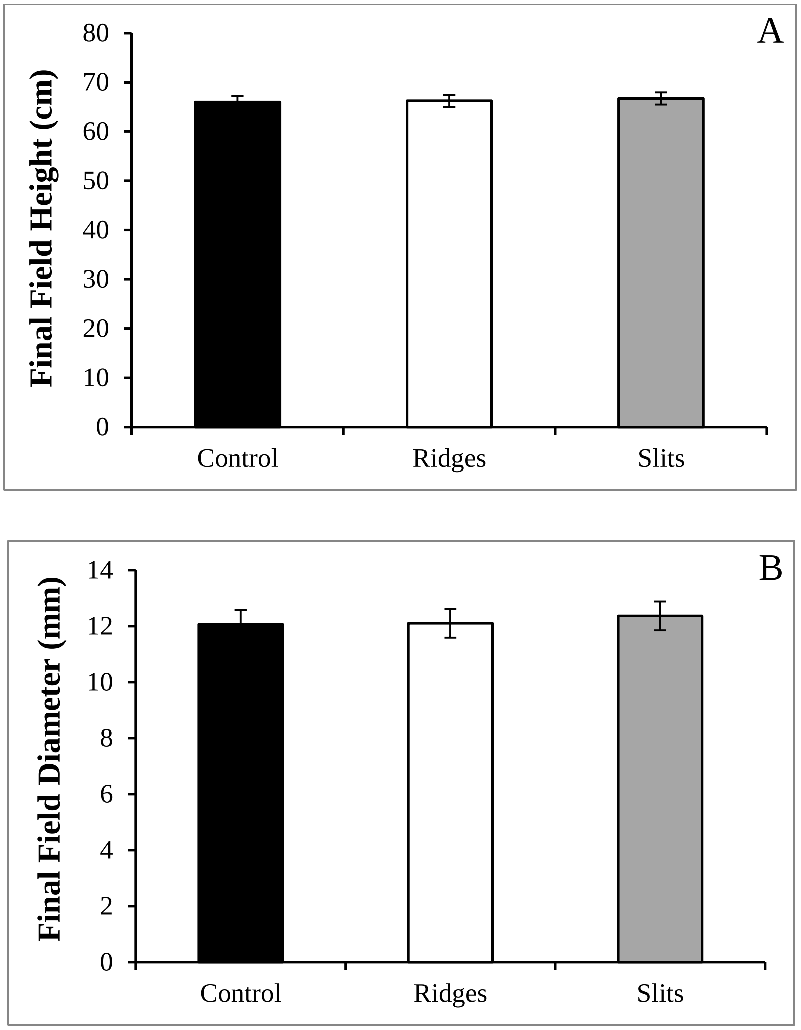


Figure A4. Afghan pine seedling final field height and diameter (Means ± SE) under root spiraling prevention treatments. Seedlings were grown in the nursery for one growing season in plastic bottles with three root spiraling prevention and three opacity treatments. Seedlings were outplanted in June 2014, and final height (A) and diameter (B) were measured at the end of the growing season in November 2014. Non-significant differences between means (α =0.05) according to Tukey’s HSD test, are shown in bars without letters.

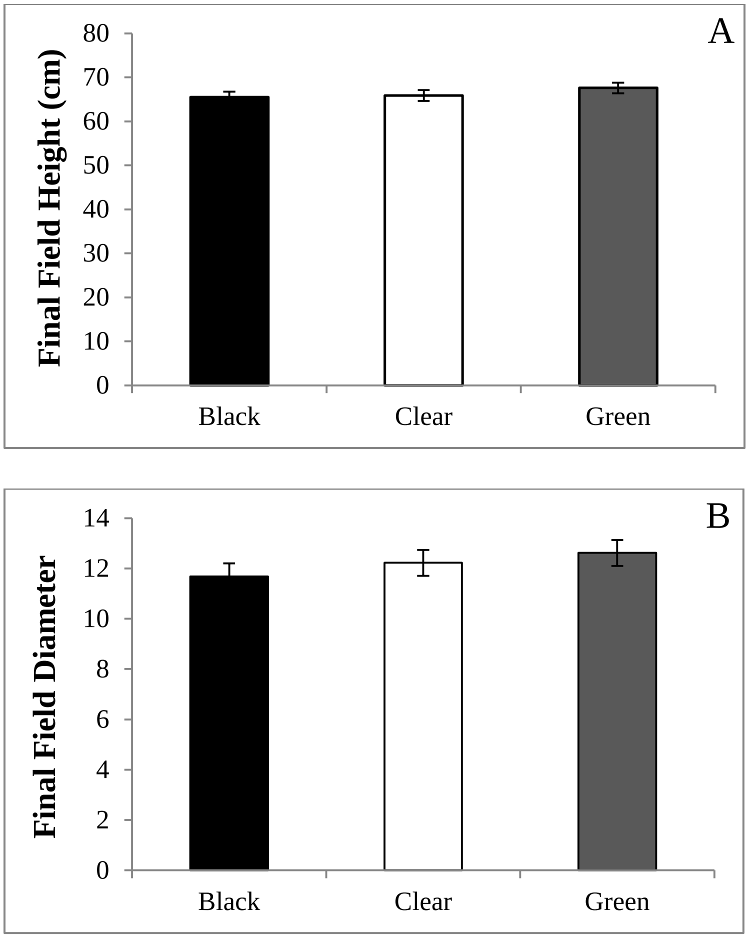


Figure A5. Afghan pine seedling final field height and diameter (Means ± SE) under opacity treatment. Seedlings were grown in the nursery for one growing season in plastic beverage bottles with three root spiraling prevention and three opacity treatments. Seedlings were outplanted in June 2014, and final height (A) and diameter (B) were measured at the end of the growing season in November 2014. Non-significant differences between means (α =0.05) according to Tukey's HSD test, are shown in bars without letters.

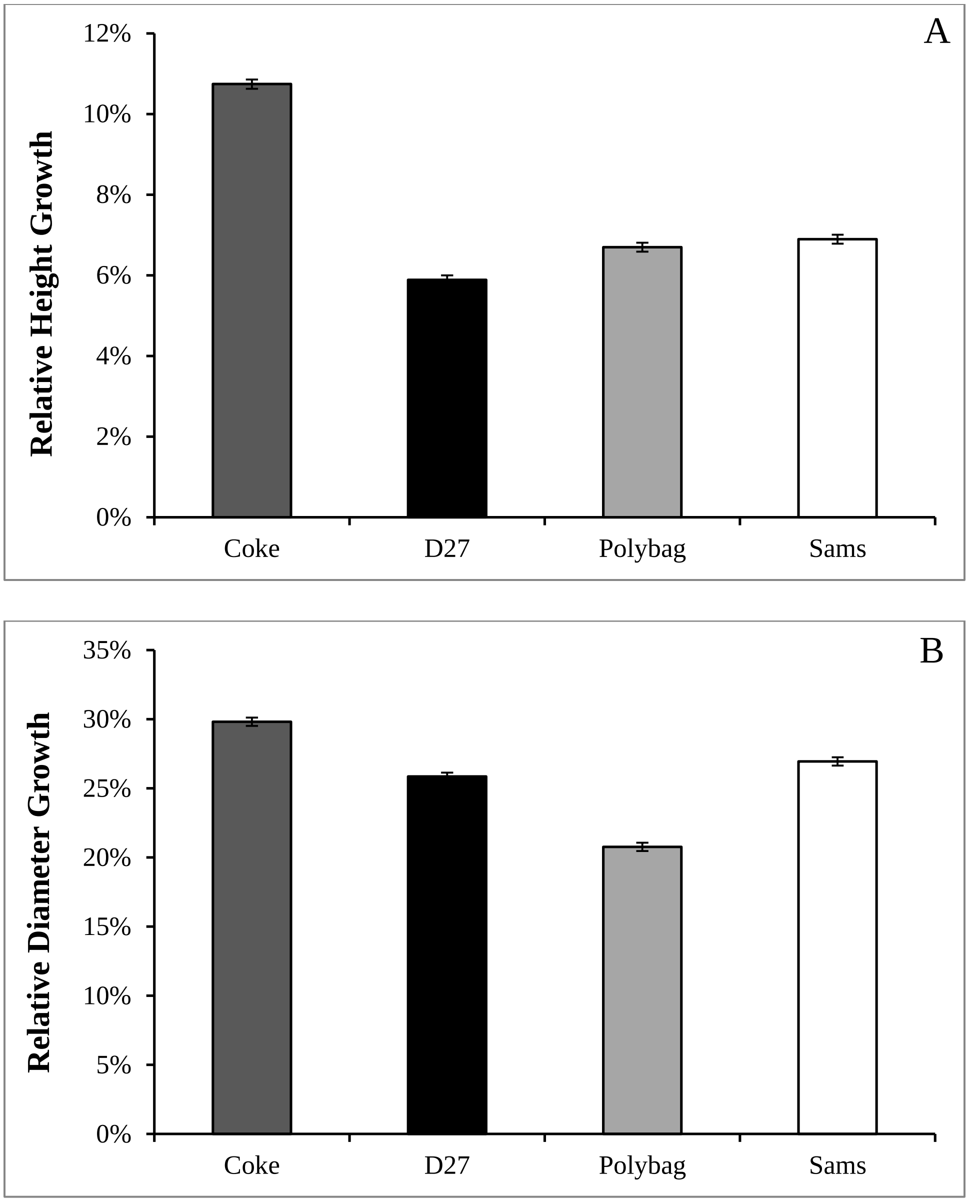


Figure A6. Arizona walnut seedling field relative height and diameter growth (Means ± SE) for seedlings grown in the nursery for one growing season in four container types: Coca-Cola® bottles (Coke), Deepot™ D27 containers (D27), polyethylene polybags (polybag), and Sam's Club® bottles (Sams). Seedlings were outplanted in June 2014, and final height (A) and diameter (B) were measured in the November 2014. Non-significant differences between means (α =0.05) according to Tukey's HSD test, are shown in bars without letters. Relative growth was calculated based on the change in absolute height or diameter between specific time periods relative to the initial height or diameter of the seedling.

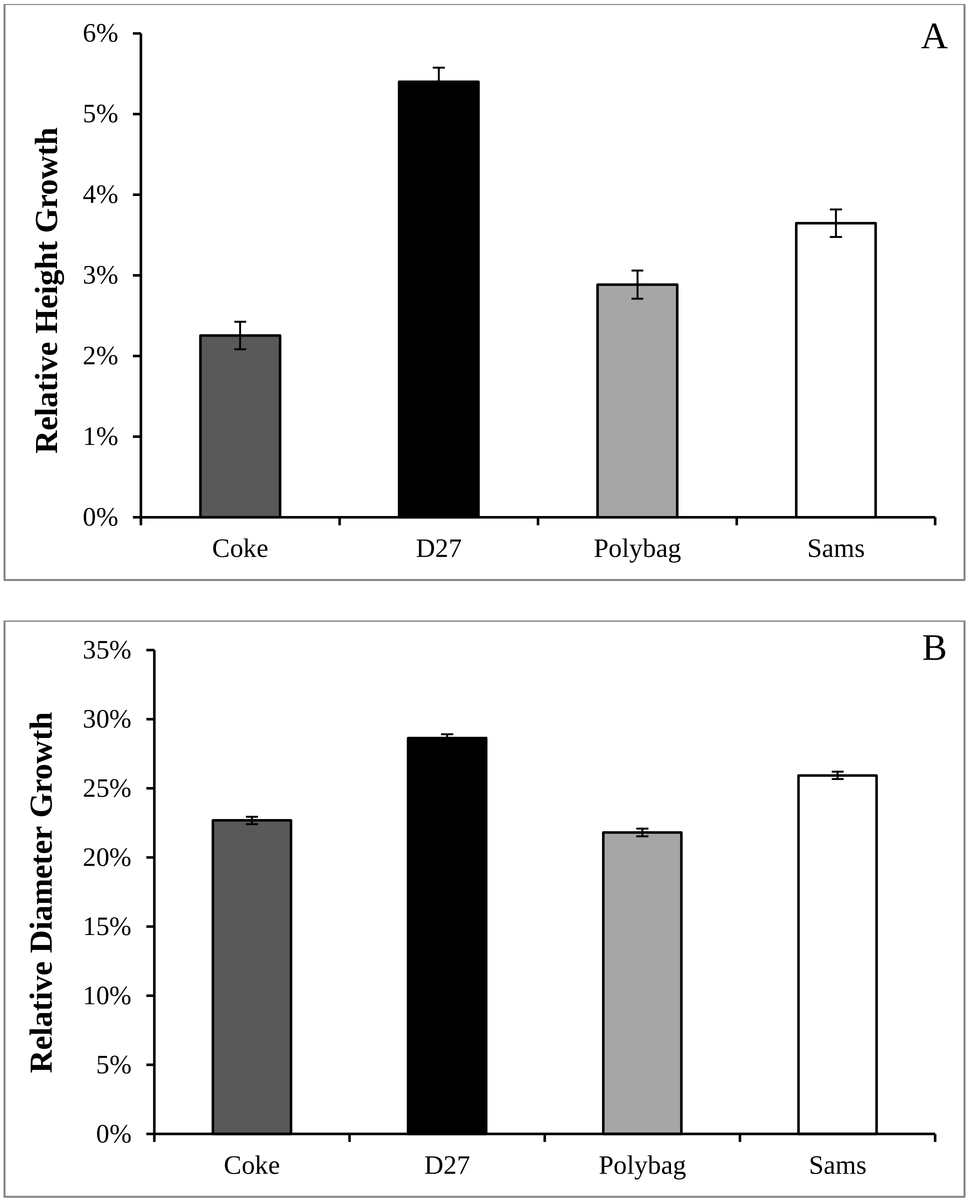


Figure A7. Afghan pine seedling field relative height and diameter growth (Means ± SE) for seedlings grown in the nursery for one growing season in four container types: Coca-Cola$^{\circledR}$ bottles (Coke), Deepot^{TM} D27 containers (D27), polyethylene polybags (polybag), and Sam's Club$^{\circledR}$ bottles (Sams). Seedlings were outplanted in June 2014, and final height (A) and diameter (B) were measured in the November 2014. Non-significant differences between means (α =0.05) according to Tukey's HSD test, are shown in bars without letters. Relative growth was calculated based on the change in absolute height or diameter between specific time periods relative to the initial height or diameter of the seedling.

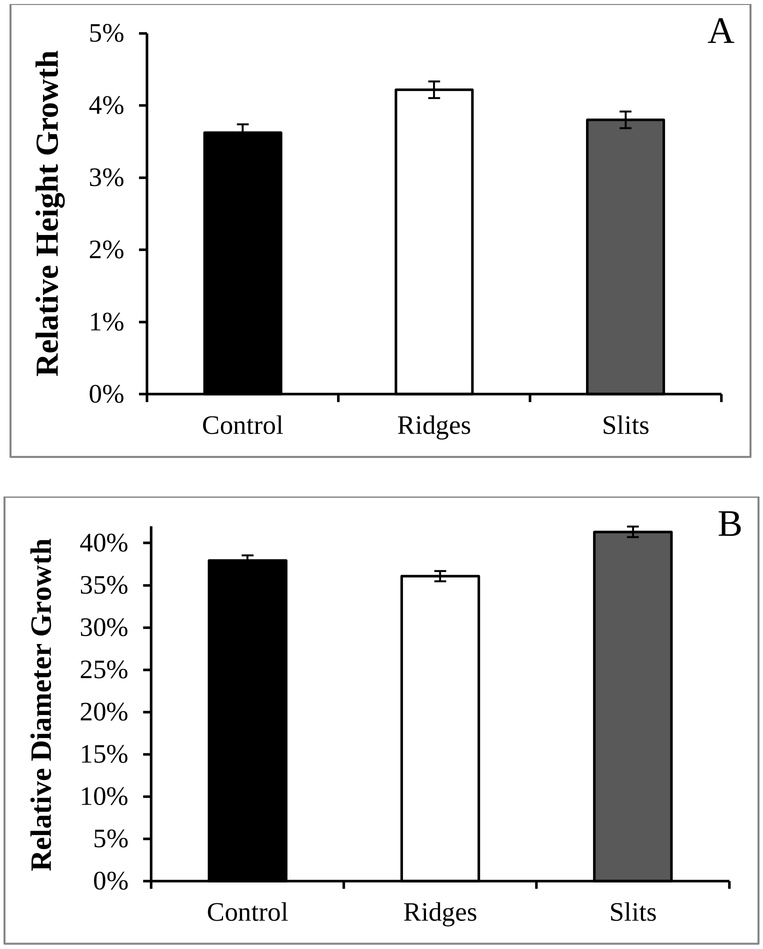


Figure A8. Afghan pine seedling field relative height and diameter growth (Means ± SE) under root spiraling prevention treatments. Seedlings were grown in the nursery for one growing season in plastic bottles with three root spiraling prevention and three opacity treatments. Seedlings were outplanted in June 2014, and final height (A) and diameter (B) were measured at the end of the growing season in November 2014. Non-significant differences between means (α =0.05) according to Tukey's HSD test, are shown in bars without letters. Relative growth was calculated based on the change in absolute height or diameter between specific time periods relative to the initial height or diameter of the seedling.

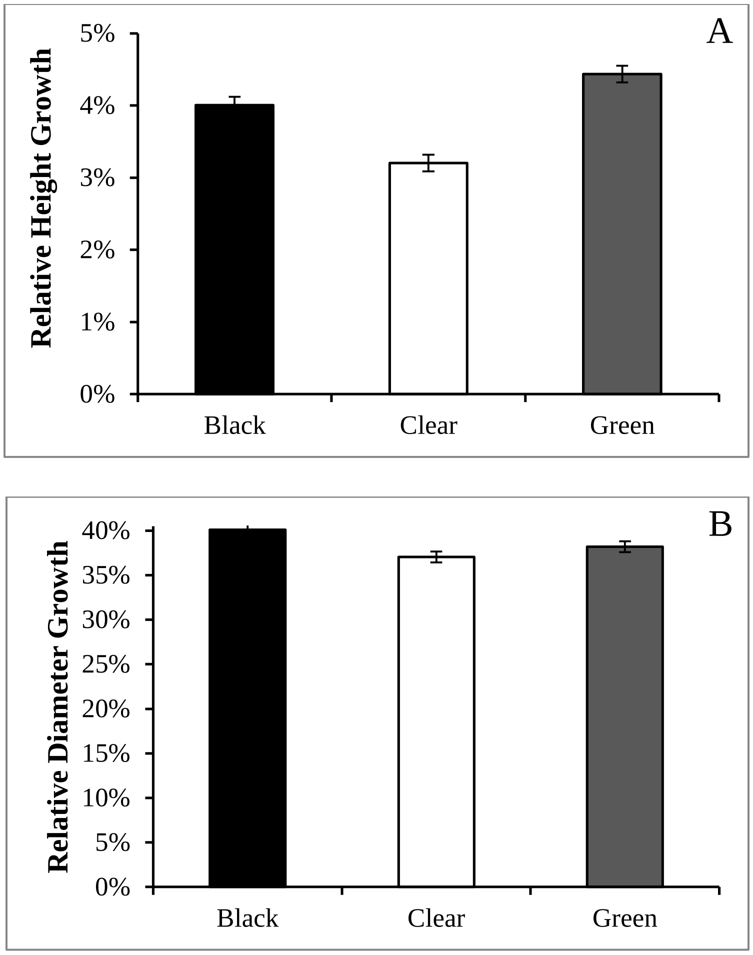


Figure A9. Afghan pine seedling field relative height and diameter growth (Means ± SE) under opacity treatment. Seedlings were grown in the nursery for one growing season in plastic beverage bottles with three root spiraling prevention and three opacity treatments. Seedlings were outplanted in June 2014, and final height (A) and diameter (B) were measured at the end of the growing season in November 2014. Non-significant differences between means (α =0.05) according to Tukey's HSD test, are shown in bars without letters. Relative growth was calculated based on the change in absolute height or diameter between specific time periods relative to the initial height or diameter of the seedling.

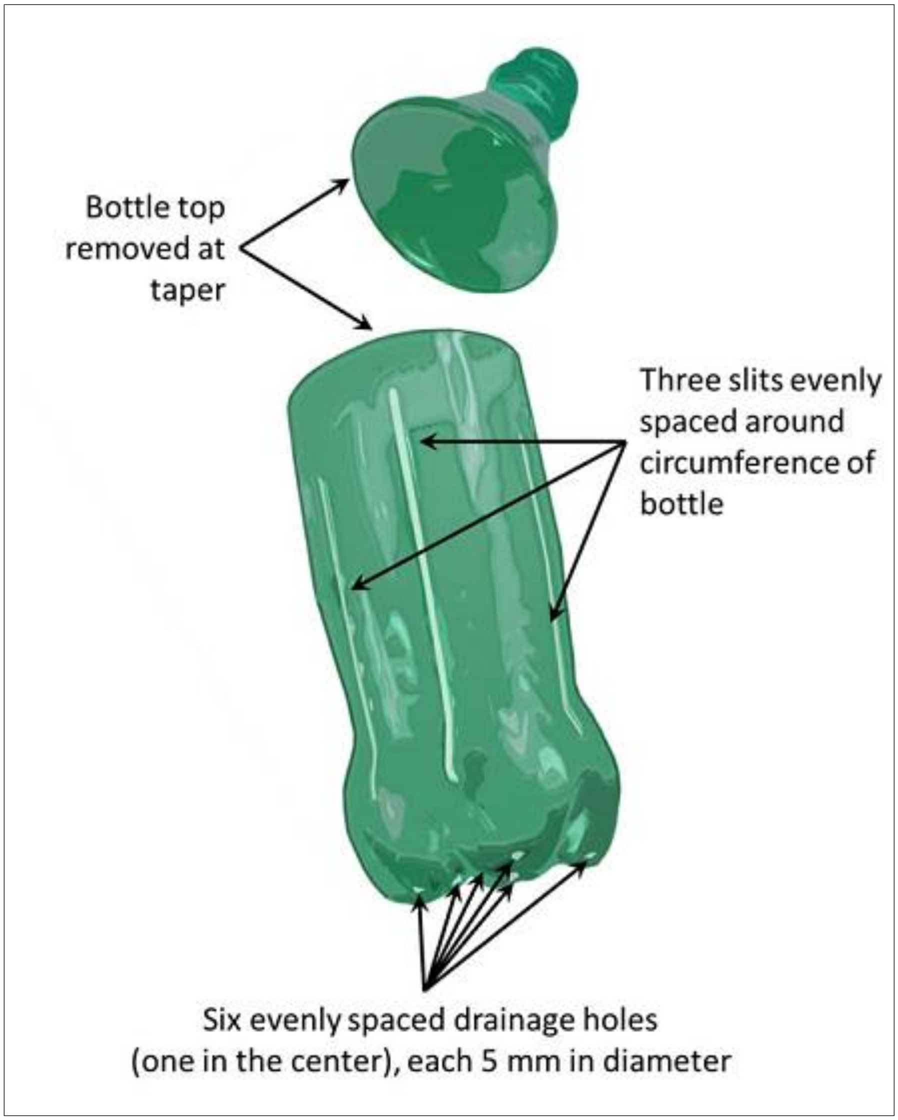


Figure A10. Example of modified plastic bottle used as container type. Bottom holes were placed for drainage and side-slits were created for root spiraling prevention.

Figure A11. Comparison of three opacities (clear, green, black; left to right) and three spiral control techniques (control, internal ridges, side-slits; left to right) in Bottle Modification Experiment.

Figure A12. Nursery trial layout for Bottle Modification Experiment.

Figure A13. Nursery trial layout for Container Comparison Experiment.

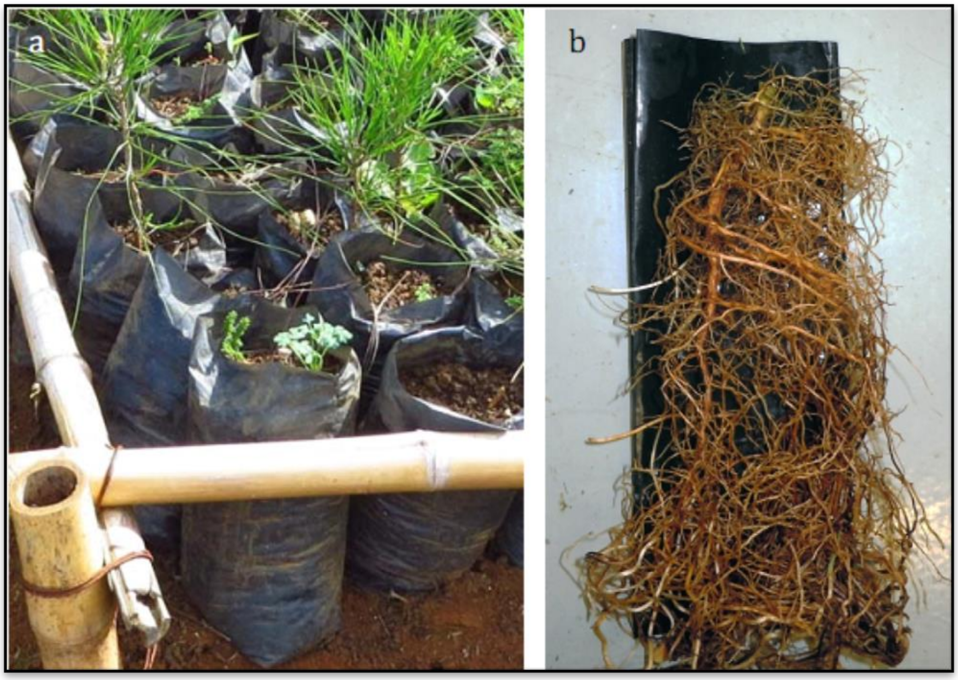


Figure A14. a) Polybag containers; b) Root spiraling typical of seedlings grown in polybags.

Figure A15. Four container types used in container comparison study. A) Sam's Club® bottle B) Coca-Cola® bottle C) Polybag D) DeePots™ D27.

Figure A16. Root spiraling of Arizona walnut seedling grown in polybag container.

Figure A17. Afghan pine root system without any spiraled roots grown in bottle container.

Figure A18. Field study sites at the John T Harrington Forestry Research Center, Mora, NM, USA.