CONTAINER CONFIGURATION INFLUENCES WESTERN LARCH AND BIG SAGEBRUSH SEEDLING DEVELOPMENT

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AUTHORIZATION TO SUBMIT THESIS

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Abstract

Big sagebrush (*Artemisia tridentata* Nutt.*),* a woody shrub, and western larch (*Larix occidentalis* Nutt.*),* a deciduous conifer, are among many western North American species that have suffered a decline in presence and natural regeneration across their native ranges. These species are economically, ecologically, and intrinsically valuable, therefore many current restoration and management efforts are working to mitigate their decline. Outplanting container-grown seedlings can expedite the regeneration process and create future propagule sources on sites where other regeneration strategies may be more difficult. Research was conducted with the intention of improving survival and growth of both big sagebrush and western larch seedlings through optimizing container nursery culture. Two general objectives were to 1) evaluate the influence of container volume and growing density on seedlings grown at the nursery, and 2) examine the post-transplant effect of stocktype on seedling growth through simulations of optimal and suboptimal field conditions. Results indicate that big sagebrush grown in a high-density arrangement was beneficial to shoot growth at the nursery and following transplant. Also, container volume did not contribute to differences in sagebrush growth during simulated field conditions. Western larch root:shoot were not different after nursery culture, despite differences in container volume; this suggests the benefit of a tailored nutrient regime. Additionally, growing larch seedlings in the low-density arrangement increased root production at the nursery and following transplant. For both species, nursery and post-transplant growth was influenced less by container volume and more by density arrangement. Survival in the field simulations was 100% for both species, indicating that seedlings were of high quality and warranting field testing of these stocktypes and production methods under a wide range of conditions. Further, robust growth across the tested container sizes implies the potential for cost savings nurseries through reduced materials consumption without the loss of seedling quality; a well grown seedling, of phenotypic traits tailored to the specific field and economic constraints of the outplanting project, should be able to outperform a seedling that does not have those same attributes. This thesis contributes to a larger body of research aimed at improving target seedling production for these species and their congeners.

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Chapter 1

Container volume and growing density influence big sagebrush (*Artemisia tridentata* Nutt.) seedling development

Abstract

What remains of big sagebrush (*Artemisia tridentata* Nutt.) habitat is declining, and restoration of this species through direct seeding has been limited in success. Though more costly, greater success will come from efforts using transplanted nursery stock to facilitate the establishment of sagebrush communities. This study had two objectives: The first was to determine the relative contribution of container volume (as influenced solely by depth) and density to the morphology of big sagebrush seedlings after one year of nursery culture. The second was to determine the relative contribution of the same container variables to seedling establishment when subject to simulated field conditions the following year. Four distinct stocktypes were developed using a modified Jiffy® 50150 container to attain desired volumes (111, 143, 175 and, 207 ml) at a fixed width; each was paired with a volume-dependent nutrient regime. Seedlings grown at high density and in smaller containers during nursery culture ultimately had larger shoots and had a higher root:shoot than seedlings grown at low density and in larger containers. Second year experimentation evaluated rhizosphere temperatures (20°C or 10°C) and watering regimes (High 4.4 L, medium 2.2 L, and low 0.44 L, seasonal rate sdlg^{-1}) in simulated field experiments. Seedling morphology was not influenced by container volume during simulations. Homogeneous post-transplant growth, despite difference in initial seedling size, was linked to tailored nutrient application during nursery culture. Big sagebrush seedlings grown at high density had increased shoot growth after transplant, without differences in root development. This study demonstrates the possibility of reducing productions costs through manipulating material consumption without the loss of seedling quality – an important consideration for growers and restoration specialists. Additionally, high survival and homogeneous performance across stocktypes subject to suboptimal soil moisture and temperature conditions warrants research trials for testing an expanded outplanting window.

Introduction

Big sagebrush (*Artemisia tridentata* Nutt.) is a woody shrub with widespread distribution in western North America; it is most abundant in the Great Basin and the Columbia Plateau (Ward 1953; Freeman et al. 1991). The sagebrush ecosystem is one of the most imperiled in the United States (Noss et al. 1995), with more than 350 sagebrush-associated plants and animals identified as species of conservation concern due to habitat decline (Suring et al. 2005a, b; Wisdom et al. 2005). Estimates have indicated that sagebrush currently occupies only 56% of its original range, and what remains is highly fragmented (Knick et al. 1997). The remaining habitat is declining through conversion to conifer woodlands and cropland, degradation due to the spread of exotic grasses, and the subsequent increase in fire frequency (Davies et al. 2011).

The maintenance and restoration of sagebrush habitat can be costly and difficult due to the large scale involved (often vast parcels of land) and the absence of natural regeneration following intervention. Active restoration of sagebrush is often necessary because large burns and other stochastic or anthropogenic events can reduce or eliminate propagule production (Ziegenhagen and Miller 2009). While the absence of propagules is one problem, additional issues with sagebrush seed can further diminish the chance of natural regeneration. Wijayratne and Pyke (2009) saw a 50% reduction in the viability of the native seed bank within two years following fire. Big sagebrush seeds are primarily wind dispersed and can only travel relatively short distances (Young and Evans 1989); this requires considerable amounts of time to move across large burned or disturbed areas (Davies et al. 2011). To mitigate these issues, transplanted nursery or wild stock (e.g. Monsen et al 2004) are used to establish small sagebrush communities that serve as propagule sources on larger restoration areas (Reever-Morghan and Sheley 2005; Ziegenhagen and Miller 2009).

The primary advantage of nursery plant production is the ability to manipulate environmental and physical conditions to produce high-quality seedlings that are more likely to establish and grow on an outplanting site. Inherent in this manipulation, is the ability to change a seedling’s phenotype, an important measurable trait that can be used to assess seedling quality and health (Haase 2008). Because phenotypes are a product of genotypes and propagation environments (Pinto 2005), it follows that changes in environmental conditions and container variables (i.e. diameter, depth, volume, and seedling density) can have significant impact. Numerous researchers have examined which variables have the greatest influence on seedling phenotype and subsequent performance (Dominguez-Lerena 2005; Pinto et al. 2011 a, b) and found the response to be species-dependent. Practitioners have also noted a correlation between container cell density and rooting volume, also concluding that optimal container shapes are species-specific (Endean and Carlson 1975; Carlson and Endean 1976). Therefore, examining the separate and combined effects of these two factors is important for optimizing greenhouse production space (Aphalo and Rikala 2002), while maximizing seedling quality.

Seedling phenotypes are intimately tied to the referential term "stocktype," which describes how a seedling is produced and ultimately how it looks. The "target plant concept" (TPC, Rose et al. 1990; Landis 2003; Pinto et al. 2011a) posits the importance of stocktype selection in overcoming site limiting factors to a seedling's establishment and subsequent development (Pinto et al. 2011b). Seedling quality is thus determined at the outplanting site, and results from practices implemented by a grower throughout nursery culture. Specific practices that weigh highly on the quality of the resulting seedlings are stocktype selection and the corresponding fertilizer regime. Thus, numerous studies have investigated which stocktypes exhibit the highest survival and growth under drought (Amidon et al. 1982; Pinto et al. 2011b; Rose et al. 1997), vegetative competition (Hunt 2002), cost effectiveness (Miller and Schaefer 1985), and other potentially limiting factors found after outplanting (Arnott 1975; Simpson 1991; Bowden 1993; South et al. 2005; Pinto et al. 2011a). These types of investigations, are attempting to link specific container parameters and the resulting seedling morphology with physiological performance and ultimately outplanting success.

Past studies have evaluated the effect of container size (potential rooting volume) and tray density on a variety of hardwood and conifer species. The results indicate that generally, seedlings with larger morphological features—height and root-collar diameter, for example—outperform smaller seedlings following outplanting. Larger container volumes were found to provide more water and nutrient availability, along with more space for root development, resulting in better seedling growth (McConnoghay and Bazzar 1991; Hsu et al. 1996) and survival (Ward et al. 1981; Matthes-Sears and Larson 1999) after planting in field trials (Dominguez-Lerena et al. 2005). Many of these studies put an emphasis on investigating root development in varying container sizes and linking those results to outplanting success (Grossnickle 2005).

Root system quality, largely driven by nursery culture, is critical for determining seedling establishment on a site immediately following outplanting (Davis and Jacobs 2005). The original root system size determines the seedling's ability to take up water so it can initiate the establishment process (Carlson and Miller 1990). Among many contributing factors, relatively low temperatures and low moisture in the rhizosphere can provide the most significant limitations to root growth and hinder the establishment process (Grossnickle 2005). Further, the threshold at which seedlings respond to varying edaphic conditions following transplanting is species dependent and is influenced by both stocktype selection and nursery culture (Aphalo and Rikala 2002; Grossnickle 2005; Dominguez-Lerena et al. 2005; Pinto et al. 2011a). Consequently, investigations into the potential of seedling root systems both during nursery culture and following transplant, are critical to stocktype enhancement.

There are a wide variety of containers available on the market for producing seedlings. Therefore, past research often used a variety of containers within individual studies, which often meant the presence of confounding variables when evaluating the resulting seedlings. Previous stocktype investigations have evaluated parameters of plug volume, depth, diameter, and tray density, often using an assortment of container types and sizes (i.e. Simpson 1991; Lamhamedi et al. 1997; Pinto et al. 2011a; Pinto et al. 2012). Most containers available to nurseries, and those used experimentally, have a degree of taper from the top to the bottom of individual cells, which can influence the movement of water vertically and laterally, alter root position, and in general provide unaccounted for variables, which may translate into potential sources of confounding in those previous studies. This study aimed to minimize such potential sources of confounding when investigating volume and growing density as independent and linked variables in container configuration.

The materials selection and design of this study allowed for an evaluation of seedling response to changes in container volume without accounting for changes in width. Width contributes exponentially to container volume and previous studies have found that, given equal container volumes, containers with larger diameters result in better seedling growth (Hocking and Mitchell 1974; Dominguez-Lerena et al. 2006). Thus width was not examined in this study. Further, the Jiffy® forestry pellet (Jiffy Products of America, Inc., Norwalk, OH) was selected for this study because it would provide a homogeneous propagation medium across large sample sizes. This industrially produced container type comes assembled with growing media composed of sorted and sterilized peat moss and thus provides a highly uniform rooting media and containment system. The Jiffy® pellet is uniform in width, thus eliminating the influence of taper. Each expanded container provides a cylindrical tube that can be easily adjusted to desired volumes without compromising the integrity of the mesh containment system or altering media density.

This study had two objectives. The first was to determine the relative contribution of container volume (as influenced solely by depth) and density to the morphology of big sagebrush seedlings after one year of nursery culture. Evaluating these parameters, paired with a tailored culturing regime, was an initial step towards understanding this species-specific relationship of how container attributes contribute to morphological variables. The second objective was to determine the relative contribution of the same container variables to seedling establishment when subject to simulated field conditions the following year. This was accomplished with two experiments. The first experiment simulated varying degrees of soil water availability throughout a four-month growing period. The second experiment simulated either low or high rhizosphere temperature for six weeks following transplant. Root volume, a focal point, along with a suite of morphological measurements, was used to assess seedling quality and relative performance following transplant. The evaluation of stocktype performance in these simulations contributes to a greater body of work with this species that will ultimately benefit target seedling production for a variety of restoration sites.

Materials and Methods

Big sagebrush seeds were provided by the Boise District of the Bureau of Land Management and collected in the Great Basin region of southern Idaho. Seedlings were grown at the University of Idaho Pitkin Forest Nursery (UIPFN) in Moscow, Idaho (46° 43'N, 117° 00'W) using Jiffy® forestry pellet containers (Jiffy Products of America, Inc., Norwalk, OH).

The Jiffy® forestry pellets were hydrated to the fully expanded dimensions of 13 × 5 cm (height × diameter). Containers were then adjusted to plug depths of 13, 11, 9 or 7 cm. Four experimental volumes (270 containers per volume) resulted: 207, 175, 143 and 111 ml, respectively. Each 2 cm decrease in plug depth provided a 15% decrease in total plug volume. Container size adjustments were done by hand using a metric ruler and a pair of standard, large scissors. Care was taken to provide a uniform sowing surface across adjusted container sizes. After height adjustments, the containers were placed in Jiffy® air trays (6 × 6 configuration) at two density arrangements – high and low. Full trays comprised the high-density treatment with 36 pellets per tray (224 seedlings m^{-2}), while the low-density treatment was achieved by removing every other pellet from a full tray (18 pellets per tray; 112 seedlings m^{-2}).

The experiment followed a randomized complete block design (RCBD) with a factorial treatment structure (4 volume treatments × 2 density treatments × 5 replications) arranged across 40 trays. Randomization took place at the block level, as each tray representing a volume and density treatment combination was considered to be a block and randomized weekly. The individual seedlings within each block were the experimental units and were maintained in their initial arrangement.

Nursery Culture

Seeds were stratified in the dark for 5 months at 1 to 7 °C before being sown into the adjusted Jiffy® containers on 1 June 2010. Five to ten seeds were sown per pellet to ensure all pellets contained one seedling for the experiment. The sown containers were covered with Deluxe Seed Guard™ germination fabric (DeWitt Company Inc., Sikeston, Missouri) and irrigated 3 times a day using a mist nozzle on a standard garden hose. Using this form of irrigation prevented movement of the small seeds (<1 mm) from the surface of the propagation medium. Germination was considered complete after 5 days, at which time the first thinning (to 3 individuals per pellet) took place. After 25 days, 2 to 4 true leaves were evident on all seedlings and a final thinning (to 1 individual per cavity) was completed. Following thinning, seedlings were cultivated using an adaptation of a propagation protocol by Luna et al. (2008). Irrigation timing was based on gravimetric container weights for each treatment, 70% during establishment and 60% during the rapid growth and hardening phases. Irrigation was timed so that all 40 trays received the same frequency and amount of water. However, an independent rate of fertilizer was applied to each container volume treatment amounting to 4 separate rates per fertigation event.

As a result of containers being modified, the mesh did not enclose the growing medium surrounding the seeds at the top of the pellet. So initially, seedlings were fertilized and irrigated via subirrigation to prevent the unnecessary loss of the growing medium. Later, root development was sufficient for stabilizing the medium so that fertilizer could be applied with the overhead traveling boom system. Use of the traveling boom for water and fertilizer application persisted for the duration of this crop's nursery culture. Fertilizer (The Scotts Company, Marysville, Ohio) was applied using solutions of Peters® Professional Conifer Starter™, Grower™, or Finisher™ along with calcium nitrate and S.T.E.M. (Soluble Trace Element Mix) were applied to seedlings depending on the respective growing phase. A target N was established for the 207 ml containers (56 mg N $^{-1}$) and proportionally reduced for the other volumes (Table 1.1). On 2 February 2011, seedlings were lifted, placed into plastic bags, and cold stored (0 to 5 °C) for 5 months.

Sampling

The morphological plant growth characteristics of height (HT), root-collar diameter (RCD), root volume (RV), root dry mass (RDM), and shoot dry mass (SDM) were measured on all seedlings post-harvest on 31 January 2011. Seedling HT was measured from the cotyledon scar at the root-collar to the terminal end of the longest branch. If multiple stems were present then total growth was the cumulative length of all shoots.

RCD measurements were obtained at the root-collar. On a subset of 5 seedlings derived from each treatment replicate (total n=200), destructive sampling was conducted to determine RV, RDM, and SDM. First, root systems were carefully washed free of all peat media. Second, RV was determined by water displacement (Burdett 1979; Harrington et al. 1994). Next, seedlings were severed at the root-collar and roots and shoots were bagged separately and dried at 60°C for 72 hours. Following drying, RDM and SDM were measured and subsequently used to assess seedling root-to-shoot ratio (R:S).

Statistical Analysis

Analyses of variance using SAS (SAS Institute, Inc., Version 9.2, Cary, NC), PROC MIXED for a RCBD was carried out for each response variable (HT, RCD, RV, SDM, RDM, and R:S) after nursery culture and destructive harvest. The model included the main effects of volume and density as well as their interaction. The data met all assumptions for normality and thus treatment comparisons were evaluated using the least significant difference of means; differences were deemed significant at $\alpha=0.05$.

Simulated Field Trials

Watering Regime

Seedlings representing all treatment combinations were obtained from the first experiment and transplanted into 5.05 L TP 430 “Long Pots” (Stuewe and Sons, Inc., Tangent, OR). Seedlings were transplanted directly into pots, containing a 2:1 of sand and vermiculite growing media, after removal from cold storage. Watering regime treatments were carried out on each stocktype, representing all density and depth configurations. For this the experiment employed 3 different moisture treatments: High (100% field capacity [FC]), medium (50% of FC) and low (10% of FC) (Table 1.2). Each treatment was randomly assigned to 1 seedling and all treatments were applied 4 times for the duration of the experiment. Water was applied at transplant, and then tapered throughout the growing season to simulate a decrease in soil water availability that a seedling might encounter in the field (Table 1.2). A total of 120 big sagebrush seedlings were transplanted into this experiment and grown for 4 months before harvest.

The experiment was established as a RCBD with a factorial treatment structure (4 volume treatments × 2 density treatments × 3 FC treatments) represented in 120 tree pots. The randomization took place at the block level (residual from nursery culture) and groups of 3 seedlings were considered to be a block and randomized bi-weekly.

Rhizosphere Temperature

Immediately following removal from cold storage, seedling root systems were extracted from their peat-pellet containers by removing the mesh cover and washing the roots free of peat medium. Before transplant, seedling status was evaluated through HT, RCD and RV measurements. Less than 30 minutes elapsed between seedling removal from cold storage and placement into regulated rhizosphere conditions.

Extracted seedlings representing all treatment combinations were transplanted into 2.83 L TP 414 "Tall Ones" Stuewe and Sons, Inc. (Tangent, OR) filled with a media mixture of 2:1 sand:vermiculite (v:v; 1.86:0.93 l). Two rhizosphere temperature treatments were implemented using a hydroponic cooling system to create a temperature regulated water bath for submerged pots. To prevent leakage from the water bath into the media, containers were placed in a plastic liner and inserted into a second pot to produce a watertight seal. For the water bath, Coleman® 100 quart standard coolers were modified by first removing the original lids and then by retrofitting the coolers with 1" polyisocyanurate foam insulation designed to hold 16 pots per cooling unit. A thermoelectric cooling assembly (TE Technologies, Inc. 2010 Traverse City, MI) in each cooler unit maintained set treatment temperatures as a closed system (Figure 1, Appendix A). Thermoelectric cooling units were programmed to hold either 8°C or 18°C (± 1°C) water temperatures. Water bath temperatures maintained media temperatures of 10°C or 20°C. Temperatures were monitored in real-time using calibrated digital aquarium thermometers with probes that were inserted both into the water bath and the container media up to a 10 cm depth.

A total of 10 cooling units were assembled for this experiment. Pairs of cooling units constituted complete blocks with each unit assigned either 10°C or 20°C media temperatures. The experiment had 5 blocks and thus 5 repetitions of each temperature, for a total of 80 randomized big sagebrush seedlings. Each cooling unit contained 8 individuals (Figure 1, Appendix A). The experiment was established in a RCB split-plot design (4 volume treatments × 2 density treatments × 2 temperature treatments) with volume and density as whole-plot factors and temperature as the split-plot factor. Once randomized and inserted into a cooling unit, seedlings remained in that configuration for the duration of the experiment.

Sampling

Seedlings were destructively harvested following each respective experiment. The morphological measures of HT, RCD, RV, RDM, and SDM were determined using the methods previously described.

Statistical Analysis

For both experiments, analyses of variance PROC MIXED for a RCBD were carried out (ANOVA; SAS Institute, Inc., Version 9.2, Cary, NC) on each response variable (HT, RCD, RV, SDM, RDM, FC, TEMP) following destructive harvests. The data met all assumptions for normality and thus treatment comparisons were evaluated using the least significant difference of means; differences were deemed significant at $\alpha=0.05$.

Results

Nursery Culture

For the morphological response variables measured, interactions between container volume × growing density were absent (Table 1.3). However, when the main effects of each treatment were considered independently, seedling morphology was influenced by container volume and/or growing density (Table 1.4). Important to consider are the large sample sizes allowing for significant differences between treatments despite absence of significance as indicated by main effect values. For seedlings grown at high density, shoot HT was taller than those grown at low density ($p < 0.0001$). The seedlings cultivated in the 111 ml containers had significantly larger RCD than those cultivated in the 207 ml and 175 ml containers, respectively ($p = 0.0090$ and $p = 0.0022$). Seedlings grown in the 143 ml containers also had significantly larger RCD than those grown in the 207 ml containers (p = 0.0209). Destructive sampling failed to reveal any significant differences among seedling RV or RDM, irrespective of container volume or growing density. Seedlings grown in the 111 ml containers had significantly more SDM, however, than those grown in the 207 ml containers (p = 0.0420). Lastly, seedlings grown in the smallest container had a significantly lower mean R:S than those in the largest container (p = 0.0071).

Simulated Field Trials

Watering Regime

Analysis indicated 2- or 3-way interactions between volume, density, and watering regime were absent from this experiment (Table 1.5). However, individual treatment effects elicited different responses (Table 1.6). Seedlings grown at high density during nursery culture produced significantly more shoot biomass than those reared at half density (p = 0.0338). Seedlings grown in the 207 ml containers produced significantly more root biomass than seedlings grown in the 111 ml containers (p = 0.0462).

Rhizosphere Temperature

Analysis indicated 2- or 3-way interactions among volume, density, and watering regime were absent in this experiment (Table 1.7). Examining individual treatment effects did elicit differential responses only in two measures, RCD and SDM (Table 1.8). Seedling RCD growth in the 10°C media temperature was significantly greater than RCD growth at 20°C (p = 0.0151). With regard to seedlings grown in the 111 ml containers, RV was significantly smaller in response to 10°C (1.7 ± 0.74 cm^3) soil temperatures than to 20°C (3.7 ± 0.67 cm^3) soil temperatures (p = 0.0472). At both temperatures, seedlings grown in the 207 ml container had significantly larger RV if they were cultivated at high density (4.3 ± 0.79 cm^3) than at low density (1.6 ± 0.7 cm^3) (p = 0.0162). Also, seedlings grown at high density exhibited significantly more SDM than those grown at low density (p = 0.0138) (Table 1.8).

Discussion

Nursery Culture

Seedlings grown at high density had taller shoot HT than those grown at low density. Canopy density is known to alter whole seedling growth through shading (Aphalo and Ballare 1995). Specifically, the shoot phenotype and rate of growth are affected by correlated increases or decreases in photosynthetically active irradiance (PAR) and light quality (Aphalo and Rikala 2002). In this study, it is likely that the high density growing arrangement translated to increased shading, lower PAR, and likely the competitive response of the seedlings, which was greater HT growth. Seedlings' RCD, RV, RDM, and SDM were unaffected by growing density; indicating that growth was not occurring at the expense of root development, and that an increase in density altered seedling HT without affecting the distribution of biomass in this plant during nursery culture.

Container size contributed to a specific morphological response from this species. An increase in container size resulted in a reduced RCD in big sagebrush seedlings, whereas seedling RV remained static. That result contradicts the findings of previous work with containerized angiosperm seedlings in which larger HT, RCD, and RV were a result of cultivation in larger containers (Jacobs et al. 2005). Additionally, other past work has found that big sagebrush seedlings grown in large containers, with ample water and nutrients, responded with greater shoot growth and less root development resulting in lower R:S (Herriman 2009). Possibly, when water or nutrients are not limiting factors, sagebrush seedlings invest more in shoot development, despite the container size available to them.

Further, the similarity of RV across container volumes should be examined. A homogeneous RV, despite available container volume, supports the possibility that sagebrush root development responded to the tailored nutrient regimes assigned to each container. A seedling, which produces excessive shoot material during nursery culture, is often considered unbalanced due to undesirable below-optimum R:S. Also, a low R:S can be detrimental to seedling establishment by contributing to moisture stress following planting (Bernier et al. 1995; Burdett 1990), because the amount of transpiring shoot tissue outweighs the absorptive root tissue. An abundance of past work, summarized by

Landis et al. (1990) supports the notion that an imbalance in R:S growth in this and many other woody species can be mitigated by extending the nursery culture period or through altered fertilizer regimes. In this study specific fertilizer regimes, tailored to container size, were examined.

The findings of this study indicate that adapting fertilizer regimes to match container parameters will impact R:S ratio. Incrementally (when compared to the next container size up or down), stocktypes were not significantly different, and relatively equal in proportion. However, the comparison of R:S from seedlings produced by the 111 ml versus the 207 ml indicated significant difference. Proper root-to-shoot balance is an important morphological attribute, as it is a proxy measure of seedling water loss and water uptake capability at the time of planting (Ritchie 1984; Thompson 1985; Burdett 1990; Grossnickle 2000). Results indicated an increase in the ratio with increasing container volume. With balanced R:S as the predictor for seedling tolerance to planting stress (Grossnickle 2004), one would expect that seedling performance would be similar by incremental size comparison of stocktype, but diverge between the smallest and largest stocktypes following transplant; with the largest stocktype outperforming the smallest.

One of the novel aspects of this study is that accounting for changes in container width was unnecessary as container volume increased. Width contributes exponentially to container volume, and previous studies have found that, given equal container volumes, containers with larger diameters resulted in better growth (Hocking and Mitchell 1974; Dominguez-Lerena et al. 2006). Thus, for this study evaluation examined changes in container volume via depth alterations. Volume could be viewed as a proxy for investigating the results of altered container depth or vice versa. In the present investigation, container volume played a role in seedling development. Seedlings in the shallowest (111 ml) containers had root systems with similar RV to those produced in deepest (207 ml) containers. However, plants in the shallowest container produced the most SDM. This unexpected response of seedling growth to container volume supports the TPC in that optimal container shape is species-specific and can affect field performance (Endean and Carlson 1975; Carlson and Endean 1976). Container depth has often been considered to be a more critical variable in influencing seedling phenotype because it is directly related to moisture holding capacity, humidity, and the ventilation of the root system (Landis et al. 1990). Future work investigating the specific effects of container width versus depth without the confounding corresponding to taper is thus warranted.

Certain container characteristics are often unaccounted for in stocktype studies and could influence seedling development. For example, roots proliferate differently for seedlings grown in air-pruning and non-air-pruning containers (Chapman and Colombo 2006). Some container designs, particularly hard-walled containers, can lead to root spiraling, restricted taproots and other disadvantageous root forms that can result in instability and less desirable growth habits following transplant (Rune 2003a,b). Whereas, air-pruning containers are designed to promote lateral root development and to facilitate a horizontal distribution of the root system that is more desirable after transplant (Burdett et al.1987). Chapman and Colombo (2006) found that air-pruning plugs required mechanical separation due to extensive inter-rooting, which can result in significant reductions of root mass. In this study, inter-rooting was absent, and likely a result of the tray design and greenhouse bench design which allowed for sufficient airflow between containers and promoted a dry container exterior between irrigation events, which in turn prevented root egress from container perimeters. Root architecture was excluded from this study; however, the assessment of root development (RV, RDM) following transplant allowed for a qualitative evaluation of residual effects of altered container parameters and density arrangements in an air-pruning system. The effects of using this container type are discussed in the following sections.

Lastly, volume and density considerations are relevant to the economics of container seedling production and the field performance following outplanting (Kinghorn 1974; Bowden 1993). Higher container density and reduced container volume are methods of decreasing costs at the nursery. Following this nursery culture period, results indicated that a well-balanced big sagebrush seedling could be produced at higher densities and in the shallower containers (lower volume). This implies the potential for cost savings without a reduction in quality; however, this implication still remains to be tested *in situ*, as true seedling quality is dependent on performance (e.g. growth, establishment potential) under true field conditions (Rose et al. 1990).

Simulated Field Trials

Watering Regime

In regards to methodology, consider the contrasting amount of water, in addition to the increasing interval between irrigation dates. Watering regimes were designed this way to simulate a broad spectrum of soil moisture across treatments. Seedlings subject to the high watering regime received 10 times the amount of water throughout the growing season as those subject to the low watering regime (4,440ml vs. 440ml, respectively). To better illustrate the difference note that the low treatment provided the equivalent of just under a pint (US) of water applied to container media and the high provided over a gallon. Nevertheless, following the duration of the experiment, measures of seedling phenotype - with the exception of HT - were indistinguishable despite their assigned watering regime treatment. In terms of biological significance, big sagebrush seedling development was neither facilitated nor hindered by the moisture available to them in the rhizosphere.

Also, there were few residual effects of treatments from nursery culture (i.e. container volume, density). The mean RDM and mean RV were different between the 207 ml and 111 ml containers; indicating that a 45% difference in container volume resulted in diverging root characteristics following transplant, despite watering regime. The high density arrangement facilitated greater HT growth during nursery culture and that in turn appeared to provide increased photosynthetic surface area evidenced by the greater SDM in those seedlings three months after transplant.

Similar to findings by Pinto et al. (2011b), seedlings in this study were grown under a tailored, container volume-specific nutrient regime in order to further examine the influence of container volume on field performance. Therefore, it was expected that differences in root and shoot phenotype corresponding to container volume after first-year nursery culture would persist under low water availability conditions. When restricted by container size, seedlings are known to have reduced water and mineral uptake capacity, which can impose a physical limit on the growth of roots and negatively impact overall plant development (Tschaplinski and Blake 1985; Will and Teskey 1997). The results support the contrary in this study; seedling growth under the tested watering regimes did not appear to be limited by container volume. It is possible, however, that tailoring nutrient application by stocktype may have created a disadvantage for the smallest stocktype – evidenced by distinguishable morphological traits of seedlings between the 111 ml and 207 ml stocktypes.

The low watering regime, which provided the least water to seedlings over three months, still appeared to be above a physiological threshold for root development of these containerized sagebrush seedlings. Seedling water status was not evaluated. Instead, seedling growth, specifically by measure of RV, was considered a proxy for determining water stress versus water surplus conditions. Planting stress does not occur when recently transplanted seedlings have ample soil water and can thus meet the atmospheric demand for water (Grossnickle 2004). Under these optimal conditions, new root growth is unneeded because root system size is sufficient at transporting water to the shoot system in order to meet transpirational demands (Simpson and Ritchie 1997). Big sagebrush seedling RV growth was virtually equal across water treatments, and likely a reflection of the species' growth habit and the evolved physiological potential to withstand low moisture conditions (Noss et al. 1995). Furthermore, water deficit is known to enhance root growth and can stimulate leaf abscission (Taiz and Zeiger 2006) , which is analogous to a recession of seedling growth. Thus, a homogeneity between big sagebrush seedlings' root and shoot dry mass (as well as HT and RCD) further suggests a uniform response to varying degrees of soil moisture.

Rhizosphere Temperature

The high density growing arrangement, a treatment applied during nursery culture, resulted in more shoot biomass growth at both 10°C and 20°C rhizosphere temperatures during this second year experiment. This result was predicted based on the already robust shoot resulting from the high density treatment, as well as R:S with a value closer to 1. A review of related studies on seedling root growth by Grossnickle (2005) indicates that a higher R:S can be a predictor of seedling success after transplant in a variety of angiosperm tree seedlings. Based on findings similar to the water regime experiment, it appears that density arrangement may be more deterministic than container volume for big sagebrush seedling development post-transplant.

Examining the effect of temperature on seedling growth independently indicates no difference in HT, RV, SDM and RDM in response to either 10°C or 20°C rhizosphere temperatures. These results correspond to a study by Alvarez-Uria and Korner (2007), in which rhizosphere temperatures from 6 to 23°C did not have a significant effect on shoot growth of several sub-alpine conifer and broadleaf species during a 10-week experimental period. Despite being quite different from those previously studied species, big sagebrush is still subject to physiological limitations in the extremes of its growing environments; thus, understanding what those thresholds are through controlled studies is important. The lower tested temperature, much like the low water regime treatment in the previously discussed experiment, did not clearly demarcate the lower threshold for tolerance in this species.

Seedling growth was relatively unaffected by rhizosphere temperature; however, RCD did show some enlargement; possibly a result of the species evolved growth habit. Environments like those found in the Columbia, Great, and Wyoming Basins are characterized as having cold winters and arid to semiarid climates, resulting in plants that possess distinguishing morpho-physiological features like those of *Artemisia spp.*. Most vegetation native to these environments have adapted to take advantage of the winter recharge in soil moisture in addition to moderate spring temperatures before the onset of extreme summer temperature fluctuations and arid edaphic conditions (Comstock and Ehleringer 1992). Thus, when provided with ample water at low rhizosphere temperature (as in this study), sagebrush seedling RCD growth can be interpreted as advantageous; likely an attempt to store carbohydrates for later use in root development when the environment becomes increasingly arid. Awareness of species-specific physiological mechanisms should be taken into account when designing future *in situ* testing of outplanting tolerance.

Conclusions

Conventionally, seedling growers have adjusted container volume as the primary method of manipulating seedling size. This practice may be effective for many tree species; however, some plants should be expected to respond differently. The results of this study indicated the seedling phenotype was more significantly affected by changes to growing density than changes in container volume. Specifically, growing seedlings at high density was beneficial to shoot growth and did not hinder root development. Also, the study findings indicated that producing high quality big sagebrush seedlings was possible in stocktypes ranging in volume from 111 ml to 207 ml. Seedling phenotype and subsequent field performance were relatively unaffected by changes to container volume; specifically by changes to plug depth. Seedling growth, as determined by assessment of root volume and biomass, was distinguishable only between the smallest and largest container sizes tested; this difference between stocktypes would not be considered biologically significant, however.

Changes from low to high density in this study allowed doubling of the number of seedlings produced per m^2. Also, each container volume treatment, in descending order, reflected a 15% decrease in materials (peat and fertilizer) used throughout the growing season. The 111 ml container reflected a 45% decrease in materials from the 207 ml container and produced seedlings of relatively similar phenotype. The range of materials input per seedling produced for this study, presents the possibility for growers of containerized sagebrush to decrease production costs through manipulating materials consumption, without the loss of quality. While the performance of big sagebrush during simulated field trials was focused on evaluating seedling quality, this study provides a better understanding of the influence of nursery cultural practices on seedling phenotype. Additionally, the study aimed to gather empirical data on the relationship of cultural practice to post-transplant performance, which can ultimately help define target seedling parameters for future restoration projects with this arid-land shrub.

Prior to transplant it was hypothesized that subjected to optimal conditions (i.e., ample water and warm rhizosphere temperature), initial differences in seedling phenotype after nursery culture would be transient and disappear quickly; and that suboptimal conditions (i.e., low moisture and cold rhizosphere temperature) would result in the persistence of initial stocktype differences. Contrarily, the response of big sagebrush was a relatively homogeneous expression of seedling root and shoot growth, despite watering regime and despite rhizosphere temperature. Contributing to these results were stockypes produced with tailored nutrient regimes and the physiological potential evolved by this species. But generally, such a response will be attributed to the production of a high quality, container grown seedling.

Also, previously unmentioned, the survival rate of seedlings following transplant was 100%. When evaluating the second objective of this study, which was evaluating stocktype performance post-transplant, a high survival rate across suboptimal conditions is an interesting and useful result. Within the tested ranges of rhizosphere temperature and moisture, seedling physiological thresholds were not lapsed. These findings warrant true field tests with stocktypes similar to those developed in this study being tested in true outplanting scenarios, with temperature and moisture as known site limitations.

In the great basin, early spring seeding of sagebrush has been common practice. Snow-melt recharges soil moisture and light precipitation is frequent, along with a warming trend in daytime air temperatures (West 1979). Planting container or bareroot sagebrush seedlings is less common because of the associated costs. It is known that from the few outplanting trials conducted, first-year survival rates ranged from 50-80% and varied by stocktype (Welch et al. 1992; Lysne 2005; Herriman 2009). While past work tells us that sagebrush seedlings grown in larger containers may have higher survival rates (Herriman 2009), the present findings suggest that container size and density are both important considerations (and the later more so). Cultural practices such as growing density and tailored nutrient regimes can supplement seedling quality across a variety of container sizes.

Finally, adapting the timing of outplanting to maximize the potential of a particular stocktype is part of the process when developing a target seedling. To date, no studies with sagebrush have attempted to correlate the establishment of particular stocktypes with optimal planting dates. Performance of the stocktype developed for this thesis research yielded results suggesting future trials should examine success during later fall or earlier spring outplanting dates. Specifically, sagebrush had increased shoot growth and unhindered root growth and lower temperatures and in drier conditions. Other recent research on containerized big sagebrush seedlings (Herriman and Davis 2012), found that cold hardiness peaks in the late fall and early winter (November to January). Thus warranting tests of a fall outplanting window using containerized big sagebrush seedlings.

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Table 1.1 Total nitrogen (N) application by container volume during big sagebrush nursery culture.

Container Volume (ml)	Total N (mg) Seedling^{-1}
207	56
175	48
143	39
111	31

*estimate based on 5 applications of starter, 9 applications of grower, and 4 applications of finisher per container treatment (432 seedlings)

Table 1.2 Assigned field capacity treatments for big sagebrush seedlings for the duration of the simulated field experiment. Respective amount of water and total water applied to n=40 (per low, medium, or high treatment) seedlings over a four month growing period. Also included is the irrigation schedule for the watering regime treatments; each seedling was watered once on the listed calendar dates.

Treatment	Low	Medium	High
% Field Capacity (FC)	10%	50%	100%
H_2O per Irrigation Event (mL)	110	550	1100
Total H^2O Application (mL)	440	2200	4400

Irrigation Frequency	Irrigation Interval
17-Jun (Transplant)	Initial
1-Jul	2 weeks
22-Jul	3 weeks
19-Aug	4 weeks
14-Oct (Harvest)	8 weeks

Table 1.3 Big sagebrush seedling morphological growth response to container volume, growing density, and their interaction.

Nursery Culture	P-values		
	Volume	**Density**	**Volume×Density**
Root Volume	0.4488	0.4721	0.5356
Root-collar Diameter	0.0049*	0.3233	0.5803
Height	0.3845	<.0001*	0.5286
Shoot Dry Mass	0.2374	0.7622	0.4610
Root Dry Mass	0.7377	0.7199	0.7328
Root:Shoot	0.0501	0.2599	0.4779

*indicates significance (α=0.05)

Table 1.4 Morphological growth characteristics of big sagebrush seedlings following nursery culture. Statistical means for measured values and the associated standard error; height, root-collar diameter (RCD), root volume (RV), root dry mass (RDM), shoot dry mass (SDM) and Root:Shoot (R:S) were measured.

Height (cm)		**RCD (mm)**		**RV (cm^3)**		**RDM (g)**		**SDM (g)**		**R:S**	
18.08 (0.59)	a	3.26 (0.09)	a	2.78 (0.24)	a	0.52 (0.04)	a	1.05 (0.07)	a	0.54 (0.03)	a
17.24 (0.60)	a	3.17 (0.09)	ab	2.76 (0.24)	a	0.51 (0.04)	a	0.94 (0.07)	ab	0.58 (0.03)	ab
16.75 (0.59)	a	2.94 (0.09)	bc	3.24 (0.24)	a	0.57 (0.04)	a	0.96 (0.07)	ab	0.61 (0.03)	ab
16.86 (0.61)	a	2.89 (0.09)	c	2.92 (0.24)	a	0.54 (0.04)	a	0.83 (0.07)	b	0.67 (0.03)	b
Height (cm)		**RCD (mm)**		**RV (cm^3)**		**RDM (g)**		**SDM (g)**		**R:S**	
18.78 (0.40)	a	3.11 (0.11)	a	2.84 (0.17)	a	0.53 (0.03)	a	0.96 (0.05)	a	0.58 (0.03)	a
15.69 (0.44)	b	3.02 (0.11)	a	3.02 (0.17)	a	0.54 (0.03)	a	0.93 (0.05)	a	0.62 (0.02)	a

*different letters indicate significant differences (α=0.05); n=270 for container volume treatments, n=360 for tray density treatments.

Table 1.5 Big sagebrush seedling morphological growth response to container volume, growing density, and watering regime treatments and their interactions.

Watering Regime	P-values						
	Volume	**Density**	**Water**	**Volume×Density**	**Density×Water**	**Volume×Water**	**Water×Volume×Density**
Root Volume	0.0504	0.4209	0.8511	0.8881	0.2575	0.4639	0.8121
Root-collar Diameter	0.7684	0.2055	0.1966	0.0747	0.6849	0.9782	0.8058
Height	0.7891	0.6834	0.0226*	0.8164	0.9572	0.5590	0.4562
Shoot Dry Mass	0.0338*	0.0972	0.0014*	0.3707	0.5058	0.8560	0.2367
Root Dry Mass	0.1556	0.4548	0.2853	0.6580	0.9705	0.1251	0.3438

*indicates significance (α=0.05)

Table 1.6 Morphological characteristics of big sagebrush seedlings following the watering regime experiment. Values are statistical means for measured values (time 1 – time zero) and the associated standard error; container volume, tray density and watering regime treatments (% field capacity, FC) were evaluated by measures of height, root-collar diameter (RCD), root volume (RV), root dry mass (RDM) and shoot dry mass (SDM).

Container Volume (ml)	Height (cm)		RCD (mm)		RV (cm^3)		RDM (g)		SDM (g)	
111	4.78 (0.51)	a	0.68 (0.15)	a	10.17 (0.88)	a	1.69 (0.15)	a	1.70 (0.11)	a
143	4.43 (0.51)	a	0.72 (0.15)	a	13.57 (0.88)	b	2.09 (0.15)	ab	1.79 (0.11)	a
175	4.73 (0.51)	a	0.72 (0.15)	a	11.11 (0.88)	ab	1.87 (0.15)	ab	1.75 (0.11)	a
207	5.17 (0.51)	a	0.88 (0.15)	a	12.02 (0.88)	ab	2.13 (0.15)	b	1.97 (0.11)	a
Tray Density (sdlgs m^{-2})	**Height (cm)**		**RCD (mm)**		**RV (cm^3)**		**RDM (g)**		**SDM (g)**	
High (224)	4.68 (0.36)	a	0.66 (0.10)	a	12.07 (0.62)	a	2.00 (0.11)	a	1.93 (0.08)	a
Low (112)	4.88 (0.36)	a	0.84 (0.10)	a	11.35 (0.62)	a	1.89 (0.11)	a	1.69 (0.08)	b
Watering Regime (% FC)	**Height (cm)**		**RCD (mm)**		**RV (cm^3)**		**RDM (g)**		**SDM (g)**	
10%	3.83 (0.44)	a	0.80 (0.11)	a	11.53 (0.71)	a	1.81 (0.13)	a	1.65 (0.09)	a
50%	5.56 (0.44)	b	0.61 (0.11)	a	11.58 (0.71)	a	2.10 (0.13)	a	1.89 (0.09)	a
100%	4.95 (0.44)	ab	0.84 (0.11)	a	12.03 (0.71)	a	1.93 (0.13)	a	1.87 (0.09)	a

*different letters indicate significant differences (α=0.05); n=40 for watering regime treatments.

Table 1.7 Big sagebrush seedling morphological growth response to container volume, growing density, and rhizosphere temperature treatments and their interactions.

Rhizosphere Temperature	P-values						
	Volume	Density	Temperature	Volume×Density	Density×Temp.	Volume×Temp.	Volume×Temp.×Density
Root Volume	0.9694	0.373	0.1679	0.1126	0.0597	0.4422	0.1346
Root-collar Diameter	0.5669	0.3467	0.0151*	0.979	0.3192	0.1676	0.4221
Height	0.8893	0.7877	0.9771	0.3561	0.8032	0.6374	0.6931
Shoot Dry Mass	0.7024	0.0138*	0.5361	0.1986	0.9256	0.4126	0.5582
Root Dry Mass	0.8289	0.2627	0.0636	0.2600	0.6836	0.4833	0.1332

*indicates significance (α=0.05)

Table 1.8 Morphological growth characteristics of big sagebrush seedlings following the rhizosphere temperature experiment. Values are statistical means for measured values (time 1 – time zero) and the associated standard error; height, root-collar diameter (RCD), root volume (RV), root dry mass (RDM) and shoot dry mass (SDM) were measured.

Container Volume (ml)	Height (cm)		RCD (mm)		RV (cm^3)		RDM (g)		SDM (g)	
111	2.01 (0.70)	a	0.25 (0.08)	a	2.68 (0.50)	a	0.99 (0.17)	a	1.29 (0.14)	a
143	2.52 (0.70)	a	0.37 (0.08)	a	2.75 (0.49)	a	1.15 (0.17)	a	1.32 (0.14)	a
175	1.73 (0.74)	a	0.21 (0.09)	a	2.55 (0.52)	a	1.09 (0.18)	a	1.10 (0.15)	a
207	2.10 (0.72)	a	0.30 (0.09)	a	2.91 (0.51)	a	1.21 (0.17	a	1.26 (0.14)	a
Tray Density (sdlgs m^{-2})	**Height (cm)**		**RCD (mm)**		**RV (cm^3)**		**RDM (g)**		**SDM (g)**	
High (224)	2.19 (0.52)	a	0.32 (0.06)	a	2.95 (0.37)	a	1.21 (0.13)	a	1.43 (0.10)	a
Low (112)	1.99 (0.48)	a	0.24 (0.06)	a	2.49 (0.34)	a	1.02 (0.12)	a	1.06 (0.10)	b
Rhizosphere Temperature	**Height (cm)**		**RCD (mm)**		**RV (cm^3)**		**RDM (g)**		**SDM (g)**	
10°C	2.08 (0.50)	a	0.39 (0.06)	a	2.36 (0.36)	a	0.95 (0.12)	a	1.19 (0.10	a
20°C	2.10 (0.50)	a	0.17 (0.06)	b	3.08 (0.36)	a	1.28 (0.12)	a	1.29 (0.10)	a

*different letters indicate significant differences (α=0.05); n=40 for rhizosphere temperature treatments.

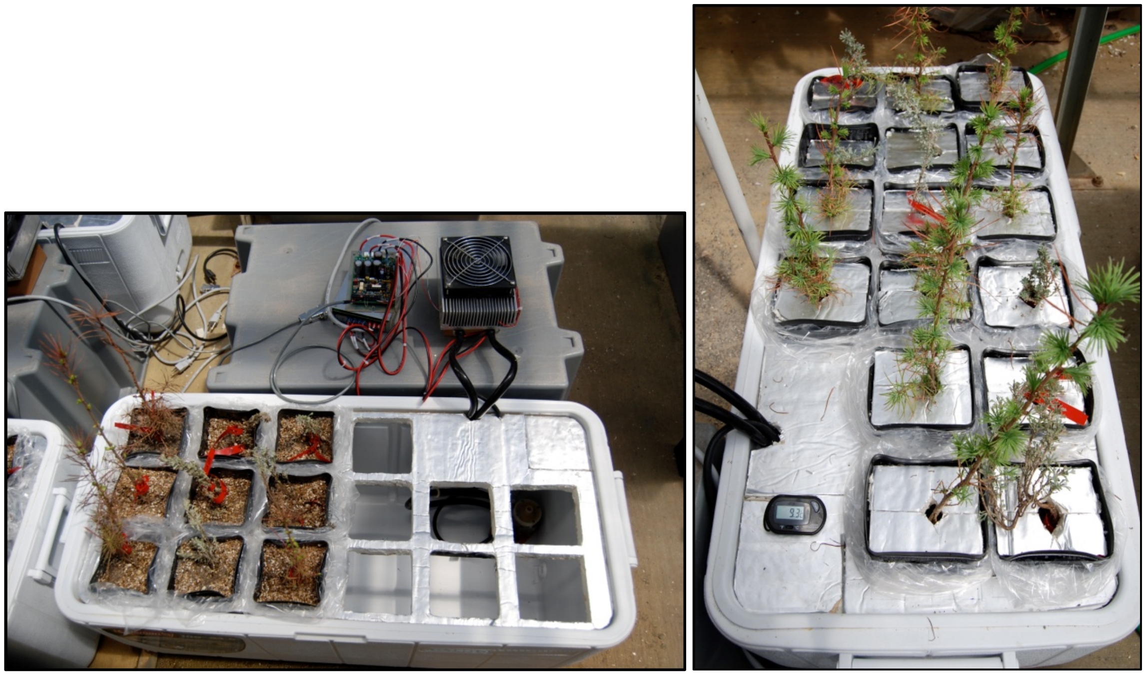

Figure 1.1 Coleman 100 quart coolers retrofitted with 1” polyisocyanurate foam insulation to include 16 “Tall Ones” tree pots. Using a thermoelectric cooling assembly (left) from TE Technologies, Inc. (©2010 Traverse City, MI), a circulating water bath kept soil media at assigned experimental temperatures of 10°C or 20°C, respectively. Western larch and big sagebrush seedlings were randomized and mixed into each cooling unit (right). Media and water temperatures were kept stable with thorough insulation and monitored via digital aquarium thermometers.

Chapter 2

Container volume and growing density influence western larch (*Larix occidentalis* Nutt.) seedling development

Abstract

Western larch (*Larix occidentalis* Nutt.) regeneration has become limited in its historic range due to land use changes and a shifting climate. Because western larch is both ecologically and commercially valuable, it is the focus of many habitat related and commercial reforestation efforts in the intermountain areas of western North America. This study had two objectives with intentions to contribute to target seedling production. The first was to determine the relative contribution of container volume (as influenced solely by depth) and density to the morphology of western larch seedlings after one year of nursery culture. The second was to determine the relative contribution of the same container variables to seedling establishment when subject to simulated field conditions the following year. Four distinct stocktypes were developed using the modified Jiffy® 50150 container to attain desired volumes (111, 143, 175 and, 207 ml) at a fixed width; each was paired with a volume-dependent nutrient regime. Seedling phenotype was more greatly affected by container density than by container volume. Despite changes to container volume, root:shoot was found to be similar, implying the benefits of a tailored nutrient regime during nursery culture. Simulated field trials revealed that a low density growing arrangement will improve post-transplant seedling growth, specifically root growth. Also, the 207 ml container facilitated greater growth in dry soil conditions compared to smaller containers. Lower (10°C) rhizosphere temperature hindered root growth, however, seedling survival was 100%, indicating a potential for testing earlier outplanting windows for this species. Lastly, this study presents the possibility for seedling growers to increase cost savings through manipulating materials consumption at a container nursery with reduced concern for loss of quality.

Introduction

The deciduous conifer western larch (*Larix occidentalis* Nutt.) is a valued timber species and the most productive of its North American congeners. Its current range includes the intermountain areas of Western North American, specifically the Cascade and Rocky Mountain regions of Canada and the USA (Parker 1993; Parish et al. 1996). Recently, bioclimatic models have been used to examine the current and future range of western larch. The findings suggest that within the next century the natural range of this species will be greatly constricted and that optimal habitats will exist outside of areas where natural regeneration can be expected. Because western larch is both ecologically and commercially valued in North America, it has been part of ongoing breeding programs with the goal of mitigating range loss (Rehfeldt and Jaquish 2010). With the rapid rate of climate change threatening present western larch habitat, seed source establishment and artificial reforestation for this species will likely be primary solutions to its maintenance on the landscape.

Nurseries provide many advantages that contribute to higher quality seedling production and better results following outplanting. Essentially those advantages come with the ability to manipulate environmental and physical conditions during plant propogation and growth. With those controls, there is the ability to change a seedling's phenotype; an important measurable trait that can be used to assess seedling quality and health (Haase 2008). Because phenotypes are a product of genotypes and propagation environments (Pinto 2005), it follows that changes in environmental conditions and container variables (i.e. diameter, depth, volume, and seedling density) can have significant impact. Numerous researchers have examined which variables have the greatest influence on seedling phenotype and subsequent performance (Dominguez-Lerena 2005; Pinto et al. 2011 a, b) and found the response to be species-dependent. Practitioners have also noted a correlation between container cell density and rooting volume, also concluding that optimal container shapes are species-specific (Endean and Carlson 1975; Carlson and Endean 1976). Therefore, examining the separate and combined effects of these two factors is important for optimizing greenhouse production space (Aphalo and Rikala 2002), while maximizing seedling quality.

The referential term “stocktype” describes the methods by which a seedling was produced, and should give some indication of size. The “target plant concept” (TPC, Rose et al. 1990; Landis 2003; Pinto et al. 2011a) posits the importance of stocktype selection in overcoming site limiting factors to a seedling’s establishment and subsequent development (Pinto et al. 2011b). Ultimately, outplanting site conditions will influence seedling growth and until then, the quality of a particular stocktype cannot be determined. Specific practices that weigh highly on the quality of the resulting seedlings are stocktype selection, and the corresponding fertilizer regime. Thus, numerous studies have investigated which stocktypes exhibit the highest survival and growth under drought (Amidon et al. 1982; Pinto et al. 2011b; Rose et al. 1997), vegetative competition (Hunt 2002), cost effectiveness (Miller and Schaefer 1985), and other potentially limiting factors found after outplanting (Arnott 1975; Simpson 1991; Bowden 1993; South et al. 2005; Pinto et al. 2011a). The objectives in studies of this type are often to link specific container parameters and the resulting seedling morphology with physiological performance and ultimately outplanting success.

We know from past research that generally, seedlings with larger morphological features—height and root-collar diameter, for example—outperform smaller seedlings following outplanting. Larger container volumes were found to provide more water and nutrient availability, along with more space for root development, resulting in better seedling growth (McConnoghay and Bazzar 1991; Hsu et al. 1996) and survival (Ward et al. 1981; Matthes-Sears and Larson 1999) after planting in field trials (Dominguez-Lerena et al. 2005). Many of these studies put an emphasis on investigating root development in varying container sizes and linking those results to outplanting success (Grossnickle 2005).

The original size and root:shoot of a transplanted seedling determines the ability of that plant to take up water and initiate the establishment process (Carlson and Miller 1990). Root system quality, largely driven by nursery culture, will also be central to seedling establishment following transplant (Davis and Jacobs 2005). Among many contributing factors, relatively low temperatures and low moisture in the rhizosphere can provide the most significant limitations to root growth and hinder the establishment process (Grossnickle 2005). Further, the threshold at which seedlings respond to varying edaphic conditions following transplanting is species dependent and is influenced by both stocktype selection and nursery culture (Aphalo and Rikala 2002; Grossnickle 2005; Dominguez-Lerena et al. 2005; Pinto et al. 2011a). Consequently, enhancements in seedling growth and survival can be made through investigations linking stocktype to root system quality.

An abundant variety of container types and configurations are available to growers for producing seedlings. Many of the most popular varieties have been used in research projects as well. However, often containers with different parameters or unique features were tested against each other in the same study, which meant the presence of confounding variables when evaluating the resulting seedlings. Previous stocktype investigations have evaluated parameters of plug volume, depth, diameter, and tray density, often using an assortment of container types and sizes (i.e. Simpson 1991; Lamhamedi et al. 1997; Pinto et al. 2011a; Pinto et al. 2012). Most containers available to nurseries, and those used experimentally, have a degree of taper from the top to the bottom of individual cells, which can influence the movement of water vertically and laterally, alter root position, and in general provide unaccounted for variables, which may translate into potential sources of confounding in those previous studies. This study aimed to minimize such potential sources of confounding when investigating volume and growing density as independent and linked variables in container configuration.

The materials selection and design of this study allowed for an evaluation of seedling response to changes in container volume without accounting for changes in width. Width contributes exponentially to container volume and previous studies have found that, given equal container volumes, containers with larger diameters result in better seedling growth (Hocking and Mitchell 1974; Dominguez-Lerena et al. 2006). Thus, width was not examined in this study. Further, the Jiffy® forestry pellet (Jiffy Products of America, Inc., Norwalk, OH) was selected for this study because it would provide a homogeneous propagation media across large sample sizes. This industrially produced container type comes assembled with growing media composed of sorted and sterilized peat moss and thus provides a highly uniform rooting media and containment system. The Jiffy® pellet is uniform in width, thus eliminating the influence of taper. Each expanded container provides a cylindrical tube that can be easily adjusted to desired volumes without compromising the integrity of the mesh containment system or altering media density.

Two objectives were met during this two year investigation. The first was to determine the relative contribution of container volume (as influenced solely by depth) and density to the morphology of western larch seedlings after one year of nursery culture. Evaluating these parameters, paired with a tailored culturing regime, was an initial step towards understanding this species-specific relationship of how container attributes contribute to morphological variables. The second objective was to determine the relative contribution of the same container variables to seedling establishment when subject to simulated field conditions the following year. This was accomplished with two experiments. The first experiment simulated varying degrees of soil water availability throughout a four-month growing period. The second experiment simulated either low or high rhizosphere temperature for six weeks following transplant. Root volume, a focal point, along with a suite of morphological measurements, was used to assess seedling quality and relative performance following transplant. The evaluation of stocktype performance in these simulations contributes to a greater body of work with this species that will ultimately benefit target seedling production for a variety of reforestation sites.

Materials and Methods

This study used western larch seeds, obtained from Silvaseed Co. (Roy, WA), that contained a mixture of seeds collected from intermountain sites throughout Idaho, Oregon, and Washington, USA. Seedlings were grown at the University of Idaho Pitkin Forest Nursery (UIPFN) in Moscow, Idaho (46° 43’N, 117° 00’W) using Jiffy® forestry pellet containers (Jiffy Products of America, Inc., Norwalk, OH).

The Jiffy® forestry pellets were hydrated to the fully expanded dimensions of 13 × 5 cm (height × diameter). Containers were then adjusted to plug depths of 13, 11, 9 or 7 cm.

Four experimental volumes (270 containers per volume) resulted: 207, 175, 143 and 111 ml, respectively. Each 2 cm decrease in plug depth provided a 15% decrease in total plug volume. Container size adjustments were done by hand using a metric ruler and a pair of standard, large scissors. Care was taken to provide a uniform sowing surface across adjusted container sizes. After height adjustments, the containers were placed in Jiffy® air trays (6 × 6 configuration) at two density arrangements – high and low. Full trays comprised the high-density treatment with 36 pellets per tray (224 seedlings m^{-2}), while the low-density treatment was achieved by removing every other pellet from a full tray (18 pellets per tray; 112 seedlings m^{-2}).

The experiment followed a randomized complete block design (RCBD) with a factorial treatment structure (4 volume treatments × 2 density treatments × 5 replications) arranged across 40 trays. Randomization took place at the block level, as each tray representing a volume and density treatment combination was considered to be a block and randomized weekly. The individual seedlings within each block were the experimental units and were maintained in their initial arrangement.

Nursery Culture

Seeds were stratified at temperatures averaging 0 to 5°C for 30 days and subsequently imbibed by submergence in oxygenated water for 48 hours prior to sowing (Tanaka 1984). Seeds were then sown into the adjusted containers on 1 June 2010 at 3 seeds per cavity to ensure all containers were full. The sown containers were covered with Deluxe Seed Guard™ germination fabric (DeWitt Company Inc., Sikeston, MO) and irrigated twice daily using an overhead traveling boom system. Irrigation water was adjusted to a pH of 6.0 with phosphoric acid (H_3PO_4) injection. Germination in every cavity occurred after 7 days and seedlings were maintained under the same irrigation regime for 22 days, at which point all of the seed coats had fallen off. The germination fabric was then removed and seedlings were subsequently thinned to 1 individual per container. No fertilizer was applied during this portion of plant propagation.

Following thinning, seedlings were cultivated by using an adaptation of the Dumroese (2009) protocol. Irrigation timing was determined gravimetrically and maintained equally across treatments at 85% during establishment, 75% during rapid growth, and 65% during hardening. All applied water and fertilizer solutions were acidified to a pH of 6.0 using phosphoric acid for the duration of this crop's nursery culture. Initially, seedlings were fertilized and irrigated via subirrigation to prevent the unnecessary loss of the growing medium held within each mesh container. After eight weeks, roots sufficiently stabilized the growing medium so that fertilizer could be applied with the overhead traveling boom system. Use of the traveling boom for water and fertilizer application persisted for the duration of this crop's nursery culture.

Nutrient solutions (The Scotts Company, Marysville, OH) of Peters® Professional Conifer Starter™, Grower™, or Finisher™ along with calcium nitrate and S.T.E.M. (Soluble Trace Element Mix) were applied to seedlings depending on the respective growing phase. A target N was established for the 207 ml containers (56 mg N^{-1}) and proportionally reduced for the other volumes (Table 2.1). On 1 February 2011, seedlings were lifted and placed into plastic bags for cold storage (-3°C to 0°C) for 5 months.

Sampling

The morphological plant growth characteristics of height (HT), root-collar diameter (RCD), root volume (RV), root dry mass (RDM), and shoot dry mass (SDM) were measured on all seedlings post-harvest on 31 January 2011. Seedling HT was measured from the cotyledon scar at the root-collar to the terminal end of the longest branch. If multiple stems were present then total growth was the cumulative length of all shoots. RCD measurements were obtained at the root-collar. On a subset of 5 seedlings derived from each treatment replicate (total n=200), destructive sampling was conducted to determine RV, RDM, and SDM. First, root systems were carefully washed free of all peat media. Second, RV was determined by water displacement (Burdett 1979; Harrington et al. 1994). Next, seedlings were severed at the root-collar and roots and shoots were bagged separately and dried at 60°C for 72 hours. Following drying, RDM and SDM were measured and subsequently used to assess seedling root-to-shoot ratio (R:S).

Statistical Analysis

Analyses of variance using SAS (SAS Institute, Inc., Version 9.2, Cary, NC), PROC MIXED for a RCBD was carried out for each response variable (HT, RCD, RV, SDM, RDM, and R:S) after nursery culture and destructive harvest. The model included the main effects of volume and density as well as their interaction. The data met all assumptions for normality and thus treatment comparisons were evaluated using the least significant difference of means; differences were deemed significant at α=0.05. Due to large sample sizes, there were circumstances where no statistical significance was determined among the main effects of treatments, yet significant differences existed among individual parameters. Because of the discrepancy these values are omitted from the results. However, deemed significant by the statistical software, they are displayed in data tables and considered in the discussion portion of this thesis.

Simulated Field Trials

Watering Regime

Seedlings representing all treatment combinations were obtained from the first experiment and transplanted into 5.05 L TP 430 “Long Pots” (Stuewe and Sons, Inc., Tangent, OR). Seedlings were transplanted directly into pots, containing a 2:1 of sand and vermiculite growing media, after removal from cold storage. Watering regime treatments were carried out on each stocktype, representing all density and depth configurations. For this the experiment employed 3 different moisture treatments: High (100% field capacity [FC]), medium (50% of FC) and low (10% of FC) (Table 2.2). Each treatment was randomly assigned to 1 seedling and all treatments were applied 4 times for the duration of the experiment. Water was applied at transplant, and then tapered throughout the growing season to simulate a decrease in soil water availability that a seedling might encounter in the field (Table 2.2). A total of 120 western larch seedlings were transplanted into this experiment and grown for 4 months before harvest.

The experiment was established as a RCBD with a factorial treatment structure (4 volume treatments × 2 density treatments × 3 FC treatments) represented in 120 tree pots. The randomization took place at the block level (residual from nursery culture) and groups of 3 seedlings were considered to be a block and randomized bi-weekly.

Rhizosphere Temperature

Immediately following removal from cold storage, seedling root systems were extracted from their peat-pellet containers by removing the mesh cover and washing the roots free of peat medium. Before transplant, seedling status was evaluated through HT, RCD and RV measurements. Less than 30 minutes elapsed between seedling removal from cold storage and placement into regulated rhizosphere conditions.

Extracted seedlings representing all treatment combinations were transplanted into 2.83 L TP 414 "Tall Ones" (Stuewe and Sons, Inc. Tangent, OR).The pots were filled with a media mixture of 2:1 sand:vermiculite (v:v; 1.86:0.93 l). Two rhizosphere temperature treatments were implemented using a hydroponic cooling system to create a temperature regulated water bath for submerged pots. To prevent leakage from the water bath into the media, containers were placed in a plastic liner and inserted into a second pot to produce a watertight seal. For the water bath, Coleman® 100 quart standard coolers were modified by first removing the original lids and then by retrofitting the coolers with 1" polyisocyanurate foam insulation designed to hold 16 pots per cooling unit. A thermoelectric cooling assembly (TE Technologies, Inc. 2010 Traverse City, MI) in each cooler unit maintained set treatment temperatures as a closed system (Figure 1, Appendix A). Thermoelectric cooling units were programmed to hold either 8°C or 18°C (± 1°C) water temperatures. Water bath temperatures maintained media temperatures of 10°C or 20°C. Temperatures were monitored in real-time using calibrated digital aquarium thermometers with probes that were inserted both into the water bath and the container media up to a 10 cm depth.

A total of 10 cooling units were assembled for this experiment. Pairs of cooling units constituted complete blocks with each unit assigned either 10°C or 20°C media temperatures. The experiment had 5 blocks and thus 5 repetitions of each temperature, for a total of 80 randomized western larch seedlings. Each cooling unit contained 8 individuals (Figure 1, Appendix A). The experiment was established in a RCB split-plot design (4 volume treatments × 2 density treatments × 2 temperature treatments) with volume and density as whole-plot factors and temperature as the split-plot factor. Once randomized and inserted into a cooling unit, seedlings remained in that configuration for the duration of the experiment.

Sampling

Seedlings were destructively harvested following each respective experiment. The morphological measures of HT, RCD, RV, RDM, and SDM were determined using the methods previously described.

Statistical Analysis

For both experiments, analyses of variance PROC MIXED for a RCBD were carried out (ANOVA; SAS Institute, Inc., Version 9.2, Cary, NC) on each response variable (HT, RCD, RV, SDM, RDM, FC, TEMP) following destructive harvests. The data met all assumptions for normality and thus treatment comparisons were evaluated using the least significant difference of means; differences were deemed significant at α=0.05.

Results

Nursery Culture

For the morphological response variables measured, interactions between container volume × growing density were insignificant (Table 2.3). Thus, each treatment main effect was considered independently when evaluating response variables. Morphological characteristics differed between treatments in response to varying container volume and growing density (Table 2.4). Specifically, HT was significantly greater for seedlings grown at high vs. low density (p = 0.0002). Conversely, RCD was significantly lower for seedlings cultivated at high vs. low density (p = 0.0125). With respect to HT, seedlings were similar among the tested container volumes (p = 0.5050). Seedlings cultivated in the 111 ml containers had significantly smaller RCD than those cultivated in the 207 ml containers (p = 0.0125).

Across container volume and density treatments, seedling RV was found to me similar (p > 0.05 and p = 0.0858, respectively). SDM of seedlings cultivated in the 207 ml containers were significantly higher than of those produced in the 111 ml containers (p =

0.0104). RDM was significantly greater for seedlings cultivated in the 207 ml than for those cultivated in the two smallest containers (111 ml and 143 ml) (p = 0.0009 and p = 0.0485, respectively). Seedlings cultivated at low density had significantly more RDM than those at high density (p = 0.0182). Seedling R:S (RDM:SDM) was similar across container volumes ($p > 0.05$). Seedlings grown at low density had significantly higher R:S than those at high density (p = 0.0014).

Simulated Field Trials

Watering Regime

Analysis indicated 2- or 3-way interactions between volume, density, and watering regime were absent from this experiment (Table 2.5). However, individual treatment effects elicited different responses (Table 2.6). Seedlings receiving the low water treatment produced significantly less RV growth than those receiving the medium water treatment (p = 0.0078). With respect to nursery culture, seedlings grown at low density had significantly more RV than those grown at high density, irrespective to watering regime (p = 0.01).

Rhizosphere Temperature

Analysis indicated no 3-way interaction among volume, density, and rhizosphere temperature treatments in this experiment, however, a 2-way interaction between volume and temperature was detected in SDM (Table 2.7). Seedling RV was significantly higher for seedlings transplanted into 20°C medium temperature, than for those transplanted into 10°C medium temperature (p = 0.0327)(Table 2.8). Seedlings grown at high density during nursery culture had significantly less RDM than those grown at low density after transplant into the 10°C medium temperature (p = 0.0028).

Discussion

Nursery Culture

It is suspected that the HT growth occurred at the expense of RCD and RDM because both these values were lower for high, versus low, density trays. This response to resource availability aligns with results reported for *Pseudotsuga menziesii* (Timmis and Tanaka 1976) and *Betula pendula* (Aphalo and Rikala 2002). When provided with ample rooting medium, water, and fertilizer, space-related resources appear to be the limiting factors in seedling development. Canopy density is known to alter whole seedling growth through shading (Aphalo and Ballare 1995). Specifically, shoot phenotype and rate of root growth are affected by altering photosynthetically active irradiance (PAR) and light quality (Aphalo and Rikala 2002). Characteristic to most woody tree species, particularly a conifer, the western larch seedlings in this study displayed exceptional HT growth in response to greater density in the given propagation environment.

Also important to consider is the belowground response to growing density and the materials of which container walls are constructed. One of those considerations, air-pruning, is not often quantified in its effect on seedling phenotype, specifically roots, in stocktype studies. However, Chapman and Colombo (2006) have shown significant differences in root proliferation among seedlings grown in air-pruning and non-air-pruning containers. Another consideration is that hard-walled containers can lead to root spiraling, restricted taproots, and other disadvantageous root forms that can result in instability and less desirable growth habits following transplant (Rune 2003a,b). Air-pruning containers are designed to promote lateral root development and to facilitate the horizontally distributed root systems that are more desirable after transplant (Burdett et al. 1987). Chapman and Colombo (2006) found that air-pruning plugs required mechanical separation due to extensive inter-rooting, which can result in significant reductions of root mass. In this study, inter-rooting did not occur and may have instead facilitated increased lignification and suberization in the roots of seedlings due to increase airflow.

Interestingly, measures of seedling RV remained static among the growing density treatments. Thus, the difference in RDM could be explained by greater root density or the anecdotally observed metacutization of roots that appeared to occur more markedly among seedlings derived from the low density arrangement. Early investigations into the function of metacutization in roots of tree seedlings provided mixed results; with studies suggesting it to be a response to water deprivation (Mager 1913) or a feature of "nongrowing (non-egressing)" roots (Wilcox 1968). In this study the increased airflow allowed by greater spacing between containers in the low density arrangement perpetuated an increased drying rate of media between irrigation events that may have lead to greater metacutization of roots in those containers. This was anecdotally observed during experimentation and thus warrants future investigation into the occurrence of metacutization in tree seedlings responding to container type and growing conditions.

With R:S balance as a predictor of seedling tolerance to planting stress (Grossnickle 2005), higher values among treatment groups could be linked with better performance in the simulated field trials. At low growing density the resulting greater RDM of seedlings constituted an increase in seedling R:S; this implies a potential post-transplant advantage over seedlings produced at high density. No significant difference in R:S was found among the four container volumes; that suggests a relatively similar phenotypic response despite available rooting medium. Proper R:S balance is an important morphological attribute because it is a measure of seedling water loss and water uptake capacity at the time of planting (Ritchie 1984; Thompson 1985; Burdett 1990; Grossnickle 2000). Previous studies evaluating optimal container selection (Pinto 2005; Pinto et al. 2011b) used a tailored culturing regime to better determine container effects on seedling success following transplant. Larch seedlings in this study were grown subject to a nutrient regime tailored to container size. Likely this contributed to the similar R:S values among the four tested stocktypes. That similarity also has implications on outplanting performance that are discussed in later sections.

The role of container volume was found to be only marginally significant in driving larch seedling development. Differences in seedling phenotype existed when there was a 30% or more change in container volume (Table 2.4), specifically among measures of RCD, SDM, and RDM. Container depth has often been considered to be the most critical variable influencing seedling phenotype because it is directly related to moisture holding capacity, humidity, and the ventilation of the root system (Landis et al. 1990). Also, when restricted by container size, seedlings are known to have reduced water and mineral uptake capacity, which can impose a physical limit on the growth of roots, thus negatively impacting overall plant development (Tschaplinski and Blake 1985; Will and

Teskey 1997). Because container width was static among all container seedlings in this study, the response of seedling phenotype to volume differences demonstrates sensitivity to changes in rooting depth.

Volume and density considerations are relevant to the economics of container seedling production and later field performance (Kinghorn 1974; Bowden 1993). Greater container density (more seedlings per area^2) and reduced container volume can reduce production costs. Operationally, measures of RCD are used by nursery managers to determine seedling quality, and can be used to project seedling potential following outplanting. Therefore, an increase in seedling height at the expense of RCD may not be a favorable tradeoff in terms of quality per area^2. However, increases in RDM, RCD, and R:S achieved through a low density growing arrangement may be seen as a worthwhile investment; particularly if this practice is advantageous to larch seedling survival snd growth following outplanting.

Simulated Field Trials

Watering Regime

For experimental watering regimes, the contrasting amount of water, in addition to the increasing interval between irrigation was designed to simulate a broad spectrum of soil moisture across treatments. Seedlings that were subject to the high watering regime received 10 times the amount of water throughout the growing season as those subject to the low watering regime (4,440ml vs. 440ml, respectively). The low treatment provided the equivalent of just under a pint (US) of water applied to container media and the high provided over a gallon.

The low watering regime expectedly limited seedling root growth, as determined by measures of RV. Though not statistically significant, a trend in the data indicates that seedlings subject to the medium watering regime actually grew larger root systems than seedlings assigned the high watering regime (Table 2.6). It is known that planting stress does not occur when recently transplanted seedlings have ample soil water and thus can meet atmospheric demand (Grossnickle 2005). In this experiment, a threshold for growth was displayed only among seedlings from the driest treatment. Additionally, excess water (high watering regime) did not provide a belowground advantage to growth but assisted shoot development, as determined by measures of SDM. Under optimal moisture conditions, new root growth is not required because the root system is sufficient in transporting water to the shoot system in order to meet transpirational demands (Simpson and Ritchie 1997).

After transplant seedlings subject to the medium and high watering regimes did not display growth differences attributable to container size. However, container size was a factor among seedlings subject to the low watering regime, where the largest container resulted in the most growth. The 207 ml container outperformed other sizes (Figure 2.1); even outperforming stocktypes in wetter soil conditions. Past research has shown that differences in seedling performance can be affected by container type during drought conditions and that larger containers often result in greater survival and growth (Amidon et al. 1982; Pinto et al. 2011b; Pinto 2012). For this study, seedlings were produced to have proportional changes in morphological status across container sizes, with the intention of limiting differences in physiological status at the time of transplant. Essentially the aim was equally robust seedlings, despite variation in stock size.

Dry soil conditions are known to stimulate root egress (Taiz and Zeiger 2006) as transplanted seedlings grow roots in search of water needed to meet transpirational demands (Grossnickle 2005). Also, increased length of the root plug is known to provide an advantage to seedlings transplanted into dry edaphic conditions (Chirino et al. 2008; Pinto 2012). Many site limitations can affect root growth potential, but ultimately, true potential will depend on the stocktype quality. In this simulation of field conditions, the advantage illustrated by the performance of the 207 ml stocktype was greater root proliferation than that measured among smaller stocktypes. This finding warrants further testing of this stocktype through outplanting trials on moisture-limited sites.

Other research has found that RV can be directly correlated with outplanting success in some conifers (Rose et al. 1991 a,b; 1997) and that a larger root volume is associated with higher RGP and increased capacity for water uptake (Carlson 1986). Independent to their assigned watering regime, seedlings grown at low density during nursery culture were found to have greater RV than those grown at high density. Prior to transplant, these seedlings were destructively sampled and no difference in RV was found among density treatments; however, RDM was larger in seedlings from the low-density arrangement. The increased RDM can be correlated to the greater root development achieved after transplant and may be related to the anecdotally observed metacutization mentioned previously. This finding suggests that producing seedlings at lower density during nursery culture can be advantageous to root development following transplant.

Rhizosphere Temperature

The lower rhizosphere temperature (10°C) hindered root growth when compared to seedlings growing in the higher (20°C) temperature. However, seedling survival was 100% at the conclusion of the experiment. Aside from differences in growth rate seedlings appeared equally viable for continued growth. In real field conditions, edaphic factors are evolving with seasonal weather patterns. In the spring, when western larch seedlings are often outplanted, rhizosphere temperature increases over time. In a fall planting scenario, rhizosphere temperature would decrease over time (Schmidt 1976). The high survival and presence of root growth during this simulation warrants future trials with expanded outplanting windows for this species; particularly as shifts in the climate become more influential on seasonal weather patterns (Rehfeldt and Jaquish 2010).

Rhizosphere temperature did not result in differences among seedling HT, SDM or RCD growth after transplant. This corresponds previous findings in a study by Alvarez-Uria and Korner (2007), in which rhizosphere temperatures from 6 to 23°C did not have a significant effect on shoot growth of several sub-alpine conifer and broadleaf species during a 10-week experimental period. This experiment was only 6 weeks long; a duration that may have been too short to capture the effects of temperature on seedling shoot growth. However, 6 weeks captures the time after transplant during which root growth is most vital to seedling survival (Grossnickle 2005).

When examining residual effects of nursery culture, the low density growing arrangement resulted in greater RDM growth for seedlings after transplant. The increased RDM growth occurred under both rhizosphere temperatures. Additionally, those same seedlings had a higher R:S prior to transplant. As mentioned previously, R:S can be a predictor of post-transplant performance. In this case it implied the potential for superior outplanting performance compared to seedlings with lower R:S values, which were grown at high density.

Conclusions

Prior to transplant it was hypothesized that subjected to optimal conditions (i.e., ample water and warm rhizosphere temperature), initial differences in seedling phenotype after nursery culture would be transient and disappear quickly; and that suboptimal conditions (i.e., low moisture and cold rhizosphere temperature) would result in the persistence of initial stocktype differences. The response of western larch seedlings after transplant was a relatively homogeneous expression of seedling root and shoot growth, despite watering regime and despite rhizosphere temperature. Contributing to these results were stockypes produced with nutrient regimes tailored to container parameters, which generally means a high quality seedling.

Typically, manipulation of growing density at a container nursery targets maximizing the number of seedlings produced per area^2. This study suggests that increased spacing (lower growing densities) during the propagation of containerized western larch seedlings will result in favorable performance following transplant. Further, this study demonstrated that producing a desirable, quality seedling phenotype is possible across the range of tested container sizes. However, when compared to smaller options, a container volume of 207 ml appears to greatly improve seedling establishment following transplant into dry edaphic conditions.

Drawing from a larger body of research will greatly benefit target seedling production, even from work with congeners of a species of interest. For instance, in a recent study, Pinto et al. (2012) examined seedling growth and survival when subject to water stress enhanced by vegetative competition. Ponderosa pine seedlings were produced in three stocktypes that differed only in size and planted into sites with varying degrees of competition. The study found that mortality was lower among larger stocktypes on sites where competition and water stress was the highest. Piecing together information from these studies suggests that in preparation for outplanting on challenging a site, where competition is high and moisture is limited, a desired species should be grown in a larger container and produced at a lower density during nursery culture.

In this study, lowering density halved the number of seedlings produced per m^2 and each container depth – in descending order – reflected a 15% decrease in materials (peat and fertilizer) used to cultivate seedlings throughout the growing season. This presents the possibility for seedling growers to evaluate cost savings through manipulating materials consumption with reduced concern for loss of quality. While the performance of larch seedlings during simulated field trials evaluated the quality of these seedlings, gaining a better understanding of the influence of nursery cultural practices on seedling phenotype will need to be accomplished through true field trials. Further research into optimizing stocktype selection for western larch in relation to a variety of field conditions will greatly benefit target seedling production.

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Table 2.1 Total nitrogen (N) application by container volume during western larch nursery culture.

Container Volume (ml)	Total N (mg) Seedling^{-1}
207	56
175	48
143	39
111	31

*estimate based on 5 applications of starter, 9 applications of grower, and 4 applications of finisher per container treatment (432 seedlings),

Table 2.2 Assigned field capacity treatments for western larch seedlings for the duration of the simulated field experiment. Respective amount of water and total water applied to n=40 (per low, medium, or high treatment) seedlings over a four month growing period. Also included is the irrigation schedule for the watering regime treatments; each seedling was watered once on the listed calendar dates.

Treatment	Low	Medium	High
% Field Capacity (FC)	10%	50%	100%
H_2O per Irrigation Event (mL)	110	550	1100
Total H^2O Application (mL)	440	2200	4400
Irrigation Frequency		**Irrigation Interval**	
17-Jun (Transplant)		Initial	
1-Jul		2 weeks	
22-Jul		3 weeks	
19-Aug		4 weeks	
14-Oct (Harvest)		8 weeks	

Table 2.3 Western larch seedling morphological growth response to container volume, growing density, and their interaction.

Nursery Culture	**P-values**		
	Volume	**Density**	**Volume×Density**
Root Volume	0.2893	0.0858	0.9776
Root-collar Diameter	0.0082*	0.0878	0.7924
Height	0.5693	0.0002*	0.7213
Shoot Dry Mass	0.0841	0.8738	0.466
Root Dry Mass	0.0100*	0.0182*	0.8691
Root:Shoot	0.2614	0.0014*	0.1558

*indicates significance (α=0.05)

Table 2.4 Morphological growth characteristics of western larch seedlings following nursery culture. Statistical means for measured values and the associated standard error; height, root-collar diameter (RCD), root volume (RV), root dry mass (RDM), shoot dry mass (SDM) and Root:Shoot (R:S) were measured.

Container Volume (ml)	**Height (cm)**		**RCD (mm)**		**RV (cm^3)**		**RDM (g)**		**SDM (g)**		**R:S**	
111	25.05 (0.91)	a	4.99 (0.14)	a	5.8 (0.38)	a	1.35 (0.09)	a	2.48 (0.17)	a	0.53 (0.03)	a
143	26.46 (0.91)	a	5.33 (0.14)	ab	6.35 (0.39)	a	1.53 (0.09)	a	2.81 (0.17)	ab	0.65 (0.03)	a
175	25.52 (0.91)	a	5.29 (0.14)	ab	6.65 (0.38)	a	1.58 (0.09)	ab	2.84 (0.17)	ab	0.60 (0.03)	a
207	26.59 (0.91)	a	5.49 (0.14)	b	6.77 (0.38)	a	1.79 (0.09)	b	3.11 (0.17)	b	0.60 (0.02)	a
Tray Density	**Height (cm)**		**RCD (mm)**		**RV (cm^3)**		**RDM (g)**		**SDM (g)**		**R:S**	
High	27.61 (0.63)	a	5.09 (0.1)	a	6.06 (0.28)	a	1.49 (0.07)	a	2.80 (0.12)	a	0.52 (0.02)	a
Low	24.2 (0.66)	b	5.46 (0.1)	b	6.72 (0.27)	a	1.67 (0.06)	b	2.82 (0.12)	a	0.62 (0.02)	b

*different letters indicate significant differences (α=0.05); n=270 for container volume treatments, n=360 for tray density treatments.

Table 2.5 Western larch seedling morphological growth response to container volume, growing density, and watering regime treatments and their interactions.

Water Availability	**P-values**						
	Volume	**Density**	**Water**	**Volume×Density**	**Density×Water**	**Volume×Water**	**Water×Volume×Density**
Root Volume	0.3492	0.0100*	0.0259*	0.9453	0.5409	0.1467	0.4732
Root-collar Diameter	0.9549	0.4983	0.0773	0.4001	0.1909	0.902	0.8657
Height	0.2148	0.5733	0.3239	0.8568	0.7427	0.8106	0.9528
Shoot Dry Mass	0.0338*	0.0972	0.0014*	0.3707	0.5058	0.8506	0.8547
Root Dry Mass	0.3653	0.0643	0.0106*	0.9902	0.3823	0.5901	0.9621

*indicates significance (α=0.05)

Table 2.6 Morphological characteristics of western larch seedlings following the watering regime experiment. Values are statistical means for measured values (time 1 – time zero) and the associated standard error; container volume, tray density and watering regime treatments (% field capacity, FC) were evaluated by measures of height, root-collar diameter (RCD), root volume (RV), root dry mass (RDM) and shoot dry mass (SDM).

Container Volume (ml)	**Height (cm)**		**RCD (mm)**		**RV (cm^3)**		**RDM (g)**		**SDM (g)**	
111	7.06 (1.11)	a	1.45 (0.14)	a	26.75 (2.53)	a	5.88 (0.43)	a	5.09 (0.27)	a
143	10.07 (1.11)	a	1.52 (0.14)	a	30.76 (2.53)	a	6.41 (0.43)	a	5.78 (0.27)	ab
175	9.50 (1.11)	a	1.40 (0.14)	a	30.88 (2.53)	a	6.97 (0.43)	a	6.05 (0.27)	b
207	9.76 (1.11)	a	1.46 (0.14)	a	33.2 (2.53)	a	6.47 (0.43)	a	6.17 (0.27)	b
Tray Density	**Height (cm)**		**RCD (mm)**		**RV (cm^3)**		**RDM (g)**		**SDM (g)**	
High	8.78 (0.79)	a	1.41 (0.1)	a	27.04 (1.79)	a	6.03 (0.3)	a	6.01 (0.19)	a
Low	9.41 (0.78)	a	1.50 (0.1)	a	33.75 (1.79)	b	6.84 (0.3)	a	5.54 (0.19)	a
FC Treatment	**Height (cm)**		**RCD (mm)**		**RV (cm^3)**		**RDM (g)**		**SDM (g)**	
10%	9.33 (0.76)	a	1.23 (0.12)	a	27.33 (1.74)	a	5.86 (0.28)	a	5.22 (0.21)	a
50%	8.35 (0.75)	a	1.62 (0.12)	a	32.85 (1.74)	b	6.74 (0.27)	a	5.92 (0.2)	bc
100%	9.62 (0.76)	a	1.51 (0.12)	a	31.00 (1.74)	ab	6.70 (0.28)	a	6.18 (0.21)	c

*different letters indicate significant differences (α=0.05); n=40 for watering regime treatments

Table 2.7 Western larch seedling morphological growth response to container volume, growing density, and rhizosphere temperature treatments and their interactions.

Rhizosphere Temperature	P-values						
	Volume	Density	Temperature	Volume×Density	Density×Temp.	Volume×Temp.	Volume×Temp.×Density
Root Volume	0.1911	0.2243	0.0327*	0.2345	0.466	0.4028	0.4702
Root-collar Diameter	0.2199	0.4883	0.4725	0.6432	0.4098	0.4807	0.2949
Height	0.6855	0.5297	0.0929	0.3343	0.9719	0.8507	0.9877
Shoot Dry Mass	0.2907	0.8799	0.1089	0.5454	0.4832	0.0386*	0.7254
Root Dry Mass	0.4002	0.0004*	0.0601	0.6432	0.4841	0.1944	0.6347

*indicates significance (α=0.05)

Table 2.8 Morphological growth characteristics of big sagebrush seedlings following the rhizosphere temperature experiment. Values are statistical means for measured values (time 1 – time zero) and the associated standard error; height, root-collar diameter (RCD), root volume (RV), root dry mass (RDM) and shoot dry mass (SDM) were measured.

Container Volume (ml)	Height (cm)		RCD (mm)		RV (cm^3)		RDM (g)		SDM (g)	
111	4.78 (1.12)	a	0.84 (0.16)	a	3.55 (0.49)	a	2.15 (0.2)	a	3.10 (0.23)	a
143	4.66 (1.08)	a	0.61 (0.15)	a	2.21 (0.47)	a	2.17 (0.19)	a	3.57 (0.22)	a
175	6.21 (1.09)	a	0.99 (0.15)	a	2.46 (0.47)	a	2.54 (0.19)	a	3.65 (0.22)	a
207	4.68 (1.17)	a	0.61 (0.17)	a	3.19 (0.52)	a	2.32 (0.21)	a	3.29 (0.24)	a
Tray Density	**Height (cm)**		**RCD (mm)**		**RV (cm^3)**		**RDM (g)**		**SDM (g)**	
High	4.66 (0.92)	a	0.70 (0.13)	a	2.49 (0.41)	a	1.82 (0.16)	a	3.38 (0.19)	a
Low	5.51 (0.87)	a	0.83 (0.13)	a	3.22 (0.38)	a	2.73 (0.15)	b	3.42 (0.18)	a
Temperature	**Height (cm)**		**RCD (mm)**		**RV (cm^3)**		**RDM (g)**		**SDM (g)**	
10°C	4.14 (0.85)	a	0.7 (0.13)	a	2.26 (0.39)	a	2.06 (0.16)	a	3.20 (0.18)	a
20°C	6.03 (0.78)	a	0.83 (0.12)	a	3.45 (0.36)	b	2.49 (0.15)	a	3.61 (0.17)	a

*different letters indicate significant differences (α=0.05); n=40 for rhizosphere temperature treatments.

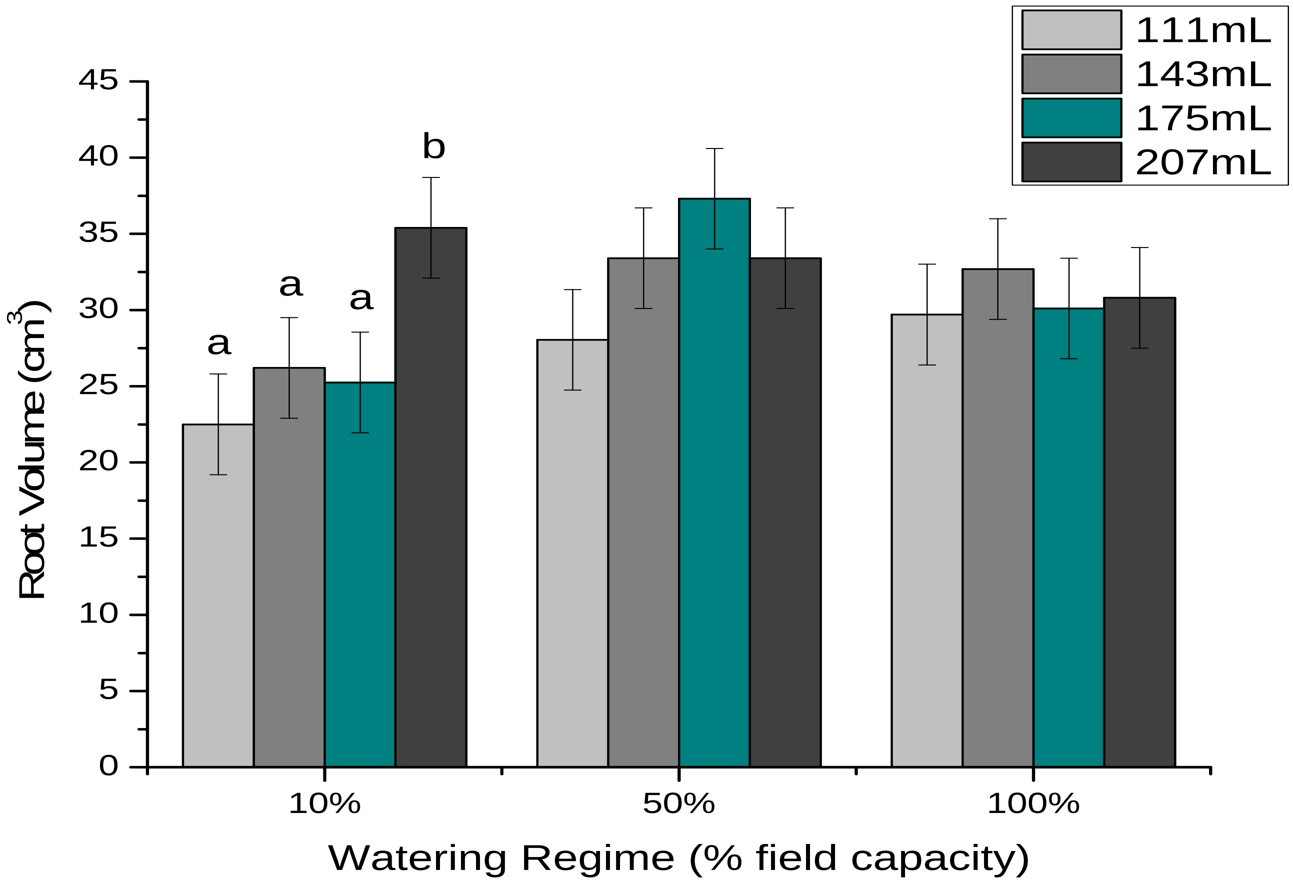

Figure 2.1 Western larch root volume (mean ± SE) in cm^3 as influenced by container volume (via depth) and field capacity treatment (post-transplant). No significant differences were found in this analysis ($\alpha = 0.05$); n=10 for each density and field capacity treatment combination