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

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Abstract Larch tree species (*Larix* Mill.) are both ecologically and commercially valuable in their native range and are the focus of many restoration, afforestation, and commercial reforestation efforts in the boreal forests of the northern hemisphere. Land use change, shifting climate, and poor natural regeneration are making it increasingly difficult to establish the species; therefore, artificial regeneration is critical to ensure this timber species maintains its productive role on the landscape. New stocktypes are continually being developed to aid target seedlings for difficult sites, and critical, non-confounding evaluations of them are needed for target seedling development. This research evaluates the effect of container parameters on potential target seedlings. It examines tolerance thresholds of western larch (*Larix occidentalis* Nutt.) with respect to moisture and temperature status in the rhizosphere during early establishment. A suite of morphological measurements was used to assess seedling quality and relative performance following transplant. Modifying a commercially available container developed four distinct stocktypes of 111, 143, 175 and 207 ml that were paired with a volume-dependent nutrient regime at two culturing densities. Seedling phenotype was affected to a greater extent by container density than by container volume. Despite changes to container volume, root:-shoot were found to be similar, indicating benefits of a tailored nutrient regime during nursery culture. Simulated field trials revealed that a low density growing arrangement improved 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 %, warranting the testing of earlier outplanting windows for this species. This

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evaluation of stocktype performance contributes to a greater body of work with this species and its congeners, which will ultimately benefit reforestation and afforestation efforts alike.

Keywords Regeneration · Container seedling · Seedling quality · Stocktype · Simulated field performance

Introduction

The deciduous conifer western larch (*Larix occidentalis* Nutt.) is among the species relevant to contemporary industrial forest operations and afforestation and restoration efforts alike. It is a valued timber species and during the first century of growth is the most productive and fastest growing among sympatric evergreen conifers (Schmidt et al. 1976). The current range is limited to intermountain areas of Western North American, specifically within the Cascade and Rocky Mountain regions of Canada and the USA. As a seral species, it is threatened in its native range by environmental shifts to warmer and drier climates. Intensive land-use including fire exclusion resulting in less frequent and higher intensity forest fires over the last century has limited stand survival and regeneration potential of larch, particularly in the northern Rocky Mountains (Smith and Fischer 1997). An additional challenge is posed by the rate of climate change; which could outpace the species' ability to adapt in certain areas without human intervention (Rehfeldt et al. 2004; Tchebakova et al. 2005; Rehfeldt and Jaquish 2010). Therein lies the necessity to aid in regenerating western larch stands both for current ecological and industrial production objectives, as well as for mitigating the effects of climate restricted regeneration over the next century (Spittlhouse and Stewart 2004). It is likely that much of the establishment efforts for western larch within and outside of its current and historic ranges will be accomplished through artificial reforestation. This is also true for other larch species in China that face poor natural regeneration and are being planted on difficult sites (Wang and Zhang 1992; Liu 1997; Liu et al. 2012).

Increasingly, the dialogue among scientists as well as forestry and restoration professionals is also taking into account the role that artificial regeneration will have in afforestation or assisted migration efforts aimed at moving commercial tree species into future ranges (Parker et al. 2000; Pedlar et al. 2012). There is demand for a greater diversity of native plant stock and the necessity to gain insight into the functional ecology of many species while establishing links between field performance and nursery culture (Valladares and Sanchez-Gomez 2006). Relevant to the production of planting stock for these endeavors, Oliet and Jacobs (2012) posit that past work has clearly demonstrated no universal or ubiquitous relationship between seedling attributes and planting response across biomes or species. Further, the past half century of research in the area of seedling production has made clear that desirable seedling characteristics can be achieved by carefully selecting culturing methods (Grossnickle 2012); and yet there is still room to define species specific responses to propagation parameters and to gauge the associated success rates when subject to field conditions.

An abundant variety of container types and configurations are available to growers for producing seedlings and many have been incorporated into past research. Important to note, however, is that containers with different parameters or unique features were often tested against each other in the same study, which can introduce some confounding during the evaluation of the resulting seedlings (as outlined by Pinto et al. 2011a). 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. Dominguez-Lerena et al. 2006; Simpson 1991; Lamhamed et al. 1997; 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 the highlighted work. This study aimed to minimize such potential sources of confounding when investigating volume and growing density as independent or 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 cell diameter. Cell diameter contributes exponentially to container volume and previous studies have found that, given equal volumes, containers with larger cell diameters result in better seedling growth, with density as factor in these findings (Hocking and Mitchell 1974; Dominguez-Lerena et al. 2006). To further reduce confounding in this study, we eliminated the changing cell diameter through taper by selecting a container (Jiffy® forestry pellet; Jiffy Products of America, Inc., Norwalk, OH) with uniform diameter. Each expanded container provided a cylindrical tube, filled with a homogenous mixture of sterilized peat moss that could be easily adjusted to desired experimental volume without compromising the integrity of the containment system or altering media density.

Changes in environmental conditions during nursery culture, specifically to container variables (i.e. diameter, depth, volume, and seedling density), can have significant impacts on post-transplant performance. 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. 2006; Pinto et al. 2011a). Root system quality, specifically root growth potential, is central to seedling establishment following transplant (Davis and Jacobs 2005). Among many contributing factors, relatively low temperatures and moisture in the rhizosphere can provide the most significant limitations to root growth and hinder the seedling establishment process (Grossnickle 2005). Thus, examining the response under controlled conditions ranging from stressful to lush can provide a valuable assessment of true field performance.

The aim of this study was to determine the relative contribution of container volume (as influenced solely by depth) and density to seedling growth and development to improve field performance of western larch through site-specific stocktype optimization. The limiting factors at an outplanting site will ultimately influence whether stocktype differences persist after transplant. Thus, we hypothesized that initial differences in seedling phenotype would be transient and disappear quickly when seedlings were subjected to optimal post-transplant conditions (i.e. ample water and warm rhizosphere temperature). Conversely, suboptimal post-transplant conditions (i.e. low moisture and cold rhizosphere temperature) would result in the persistence of initial stocktype differences. We used two experiments, conducted the year after nursery culture, to isolate the effects of varying levels of (a) rhizosphere moisture, (b) temperature, and (c) the residual effects of stocktype on seedling establishment potential.

Materials and methods

This study used a mixture of western larch seeds collected from intermountain sites throughout Idaho, Oregon, and Washington, USA. Seedlings were grown in an open sided greenhouse at the University of Idaho Pitkin Forest Nursery (UIPFN) in Moscow, Idaho (46°43′N, 117°00′W) using Jiffy® forestry pellet containers under natural light conditions. The 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 in: 207, 175, 143 and 111 ml, respectively. Each 2 cm decrease in plug depth correlated with a 15 % decrease in total plug volume. After depth adjustments, the containers were placed in Jiffy® air trays (six × six 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 used a randomized complete block design (RCBD) with a factorial treatment structure (four volume treatments × two density treatments × five 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.

Nursery culture

Seeds were sown into the adjusted containers on 1 June 2010 and cultivated using an adaptation of the Dumroese (2009) protocol. Using a digital balance, and with bi-monthly calibrations, irrigation was determined by gravimetric water content (GWC) 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. Nutrient solutions of Peters® Professional Conifer Starter™, Grower™, or Finisher™ (The Scotts Company, Marysville, OH) along with calcium nitrate and Soluble Trace Element Mix (STEM) were applied to seedlings depending on the respective growing phase. A target N was established for the 207 ml containers at 56 mg N^{-1} and proportionally reduced for the other volumes resulting in 48, 39, 31 mg N^{-1} for the remaining experimental volumes, respectively. The calculations for this estimate of total N is based on five applications of starter, nine applications of grower, and four applications of finisher per container treatment (n = 432) administered weekly over an 18 week period encompassing rapid growth through early hardening phases. After 15 October 2011, seedlings received only periodic water application to maintain plug moisture around 75 % GWC. Terminal buds formed in late October and needles began to senesce indicating a dormant state. On 1 February 2011, senesced needles were removed by hand, then seedlings were lifted and placed into plastic bags for cold storage (−3 to 0 °C) for 5 months.

Sampling

The morphological characteristics of height (HT), root-collar diameter (RCD) were assessed on every seedling grown for the nursery culture phase of this study (n = 1,080). Additionally, using destructive sampling techniques, root volume (RV), root dry mass (RDM), and shoot dry mass (SDM) were measured on a subset of seedlings (n = 200) post-harvest, on 31 January 2011. After carefully washing root systems free of all peat media, RV was determined by water displacement (Harrington et al. 1994) and biomass was measured after separated roots and shoots were dried at 60 °C for 72 h. Using RDM and SDM values, the ratio of root-to-shoot (R:S) biomass was evaluated for all destructively sampled seedlings (n = 200).

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 the destructive harvest. Each variable represents total growth after the first growing season. 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 $\alpha = 0.05$. Due to large sample sizes, there were circumstances where the main effect yielded no statistical significance, yet significant differences existed among individual parameters. Because of the discrepancy, these values are omitted from the results section, but are displayed in data tables and considered in the discussion.

Simulated field trials

Moisture regime

A subset of seedlings representing all treatment combinations (n = 120) were obtained from the nursery culture portion of this study on 2 July 2012, from cold storage. Seedlings were transplanted directly into 5.05 L TP 430 "Long Pots" (Stuewe and Sons, Inc., Tangent, OR), which contained a 2:1 of sterilized sand (particle size between 0.25 and 3 mm) and vermiculite growing media. Moisture regime treatments were carried out on each stocktype, representing all density and depth configurations. For this, the experiment employed three different moisture treatments: High (100 % field capacity [FC]), medium (50 % of FC), and low (10 % of FC). Each treatment was randomly assigned to one seedling and all treatments were applied four times for the duration of the experiment. As a result seedlings received precisely 4,400, 2,200, or 440 ml for high, medium, or low moisture treatments, respectively. Water was applied at transplant, and then tapered throughout the growing season. The experimental duration was 4 months, thus tapered water application occurred after a 2, 3, 4 week interval with a final harvest taking place after an 8 week dry period. The range of western larch is spread over an area where precipitation varies widely, however, the summer drought period is common (McKenzie et al. 2003). Thus, our FC treatments were designed to emulate different annual precipitation and the increased period without moisture late in the growing season.

The experiment was established as a RCBD with a factorial treatment structure (four volume treatments × two density treatments × three FC treatments).

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. Prior to transplant, seedling status was evaluated through HT, RCD, and RV measurements. Less than 30 min elapsed between seedling removal from cold storage and placement into regulated rhizosphere conditions. A subset of seedlings representing all treatment combinations (n = 80) were transplanted into 2.83 L TP 414 "Tall Ones" (Stuewe and Sons, Inc. Tangent, OR) on 2 July 2012. 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. For the water bath, Coleman® 100 quart standard coolers were modified by removing the original lids and 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. The water baths maintained simulated rhizosphere temperatures of 10 and 20 °C, respectively. Temperatures were monitored in real-time using calibrated digital aquarium thermometers with probes that were inserted 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 or 20 °C media temperatures. Regional soil temperatures in western larch stands in the Northern Rocky Mountains were recorded late last century as varying annually between 5 and 15 °C at a 50 cm (Schmidt et al. 1976). Thus, our study temperatures, aimed to capture a similar range but with consideration given to shallower rooting depths and a wider geography.

The experimental duration was 6 weeks, followed by a destructive harvest. The experiment had five blocks and thus five repetitions of each temperature. It was established in a RCB split-plot design (four volume treatments × two density treatments × two temperature treatments) with volume and density as whole-plot factors and temperature as the split-plot factor.

Sampling and statistical analysis

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. Measurements reflect post-transplant growth 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. Each variable represents the growth that occurred from the time of transplant into the simulation, to the time of harvest. 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

An interaction between the container volume × growing density variables was not significant. However, volume and density, respectively, had significant effects on morphological characteristics which differed in response to those treatments (Table 1). Specifically, HT was greater for seedlings grown at high density ($p = 0.0002$). Conversely, RCD was lower for seedlings cultivated at high density ($p = 0.0125$). With respect to HT,

Table 1 Morphological growth characteristics of western larch seedlings following nursery culture in 111, 143, 175 and, 207 ml stocktypes and at high and low densities (224 and 112 seedlings m^{-2}, respectively)

	Height (cm)	RCD (mm)	RV (cm^3)	RDM (g)	SDM (g)	R:S[a]
Container volume						
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						
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

Means for measured response variables: height, root-collar diameter (RCD), root volume (RV), root dry mass (RDM), shoot dry mass (SDM) and the associated standard error are reported

Different letters indicate significant differences ($\alpha = 0.05$); n = 270 for container volume treatments, and n = 360 for tray density treatments

[a] Root:Shoot (R:S) values were calculated using dry mass

seedlings were similar among the tested container volumes ($p = 0.5050$). Seedlings cultivated in the 111 ml containers had smaller RCD than those cultivated in the 207 ml containers ($p = 0.0125$).

Across container volume and density treatments, seedling RV was found to be similar ($p > 0.05$ and $p = 0.0858$, respectively). SDM of seedlings cultivated in the 207 ml containers were higher than of those produced in the 111 ml containers ($p = 0.0104$). RDM was greater for seedlings cultivated in the 207 ml than for two smallest container sizes (111 and 143 ml) ($p = 0.0009$ and $p = 0.0485$, respectively). Seedlings cultivated at low density had more RDM than those at high density ($p = 0.0182$). Seedling R:S was similar across container volumes ($p > 0.05$), however, those grown at low density had higher value than those at high density ($p = 0.0014$).

Simulated field trials

Moisture regime

Data analysis indicated no two- or three-way interactions among volume, density, and moisture regime in this experiment; however, individual treatment effects were present (Table 2). Seedlings receiving the low water treatment produced less RV growth than those receiving the medium water treatment ($p = 0.0078$), with the exception of the 207 ml stocktype which responded (Fig. 1). With respect to nursery culture, seedlings grown at low density had greater RV ($p = 0.01$) than those grown at high density, irrespective of moisture regime.

Rhizosphere temperature

Data analysis indicated no three-way interaction among volume, density, and rhizosphere temperature treatments in this experiment; however, a two-way interaction between volume and temperature was detected for SDM ($p = 0.0386$). Seedling RV was higher for seedlings transplanted into 20 °C medium temperature, than for those transplanted into

Table 2 Morphological characteristics of containerized western larch seedlings following a simulated outplanting

	Height (cm)	RCD (mm)	RV (cm^3)	RDM (g)	SDM (g)
Container volume (ml)					
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					
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					
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

Container volume, tray density and watering regime treatments (% field capacity, FC) were evaluated in the moisture regime experiment. Measured response variables: height, root-collar diameter (RCD), root volume (RV), root dry mass (RDM) and shoot dry mass (SDM) and the associated standard error are reported

Different letters indicate significant differences ($\alpha = 0.05$); n = 40 for moisture regime treatments

10 °C medium temperature ($p = 0.0327$) (Table 3). Seedlings grown at high density during nursery culture had less RDM than those grown at low density after transplant into the 10 °C medium temperature ($p = 0.0028$).

Discussion

Nursery culture

Practitioners have noted a correlation between container cell density and rooting volume; concluding that bigger containers will often facilitate greater survival (Endean and Carlson 1975; Carlson and Endean 1976). Similarly, other studies examining which container variables have the greatest influence on seedling phenotype and subsequent field performance found the response to be species-dependent (Dominguez-Lerena et al. 2006; Pinto et al. 2011a, b). Examining the separate and combined effects of container parameters—specifically cell density and rooting volume—is important for optimizing greenhouse production space (Aphalo and Rikala 2002), while maximizing seedling quality of any species in production.

When provided with ample rooting medium, water, and fertilizer, space-related resources appear to be the limiting factors in western larch seedling development. At high density, RCD and RDM values were significantly lower than at low density; leading us to suspect that seedling height growth occurred at their expense. The exceptional HT growth and response to higher growing densities aligns with results reported for *Betula pendula* (Aphalo and Rikala 2002) and sympatric conifers including *Pseudotsuga menziesii* (Timmis and Tanaka 1976) and *Picea glauca* (Scarratt 1972). Additionally, this builds on findings discussed in Landis et al. (1990), where seedlings are described as responding to wider spacing and bigger container volumes with larger growth characteristics. Our

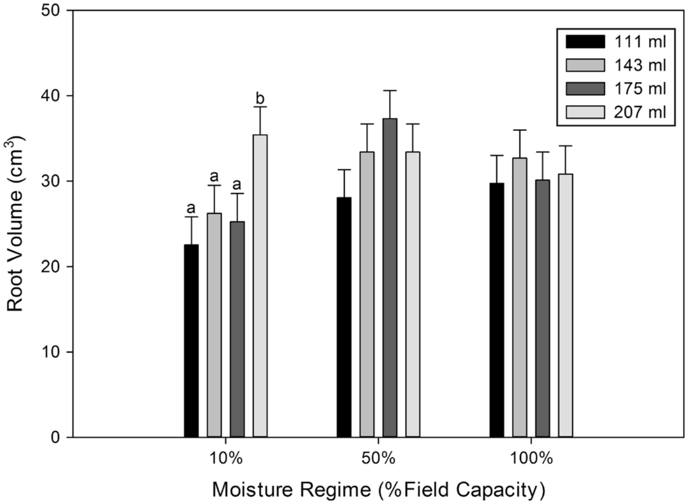

Fig. 1 Western larch root volume (mean ± SE) in cm^3 as influenced by container volume and field capacity treatment (post-transplant) in a simulated outplanting experiment. *Different letters* indicate significant difference ($\alpha = 0.05$) between 207 ml and all smaller volume stocktypes subject to the 10 % moisture regime treatment ($n = 10$). No significant differences in RV were present for stocktypes receiving other moisture regime treatments

Table 3 Morphological growth characteristics of western larch seedlings following a simulated outplanting experiment

	Height (cm)	RCD (mm)	RV (cm^3)	RDM (g)	SDM (g)
Container volume (ml)					
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					
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					
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

Container volume, tray density and rhizosphere temperature treatments were evaluated in the rhizosphere temperature experiment. Measured response variables: height, root-collar diameter (RCD), root volume (RV), root dry mass (RDM) and shoot dry mass (SDM) and the associated standard error are reported

Different letters indicate significant differences ($\alpha = 0.05$); $n = 40$ for rhizosphere temperature treatments

findings diverge slightly, in that western larch seedlings did not respond to increased volume with significantly greater growth, and did respond to lower density with greater growth but only in belowground biomass.

The difference in RCD and RDM could be explained by greater root density or the observed metacutization of roots that appeared to occur more markedly among seedlings derived from the low density arrangement. Therefore it is important to consider the belowground response of seedlings to growing density and materials from 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. 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 1986). Chapman and Colombo (2006) have shown significant differences in root proliferation among seedlings grown in air-pruning and non-air-pruning containers. In the same study, air-pruning plugs required mechanical separation due to extensive inter-rooting, which can result in significant reductions of root mass. In our study with larch seedlings, inter-rooting did not occur and airflow may have instead facilitated increased lignification and suberization in the roots of seedlings.

Interestingly, measures of seedling RV remained static between the two density treatments despite significant differences in RDM. Examined from the standpoint of shading, past work (Vance and Running 1985) tells us that lower light levels in the high density treatment (i.e. shading) should have facilitated height growth while not limiting diameter and root dry weight. Canopy density is known to alter whole seedling growth through shading (Aphalo and Ballare 1995); more specifically, shoot phenotype and rate of root growth are affected by altering photosynthetically active irradiance (PAR) and light quality (Aphalo and Rikala 2002). The western larch seedlings in our study appear to have less mass accumulation in their roots as they are subject to increased growing density, where resources are more likely being allocated to shoot growth for improved photosynthetic capabilities. The differences in biomass accumulation correspondingly changed the R:S significantly between density treatments.

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). Also, R:S balance is a predictor of seedling tolerance to planting stress or “drought avoidance potential” (Grossnickle 2005, 2012); thus, 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, suggesting a relatively similar phenotypic response to culturing despite available rooting medium. Previous studies evaluating optimal container selection (Pinto 2005; Pinto et al. 2011b) have used a tailored culturing regime—with nutrient and irrigation rates proportional to container type—to better determine container effects on seedling success following transplant. Larch seedlings in this study were grown using a nutrient regime tailored to container size, and this likely contributed to the similar R:S values among the four tested stocktypes.

Differences in seedling phenotype existed when there was a 30 % or more change in container volume (Table 1); 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, a physical limit is imposed on the roots which in turn reduces water and mineral uptake capacity, thus negatively impacting overall plant development (Tschaplinski and Blake 1985; Will and Teskey 1997). Because container width was constant 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 unit area) and reduced container volume can decrease 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 (Landis et al. 1990). 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 a worthwhile investment; particularly if it results in a more robust seedling, could exhibit greater morphological growth potential and thus presumably greater survival following outplanting.

Simulated field trials

Moisture regime

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 moisture regime received 10 times the amount of water throughout the growing season as those subject to the low moisture regime (4,440 vs. 440 ml, respectively). The low moisture 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 moisture regime actually grew larger root systems than seedlings assigned the high moisture regime (Table 2). 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 moisture regime) did not provide a belowground advantage to growth but assisted shoot development, as indicated by measures of SDM. Under optimal moisture conditions, new root growth is not required because the root system is sufficient for transporting water to the shoot system in order to meet transpirational demand (Simpson and Ritchie 1997).

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 to produce equally robust seedlings, despite variation in stocking size. After transplant seedlings subject to the medium and high moisture regimes did not display growth differences attributable to container size. However, container size was a factor among seedlings subject to the low moisture regime, where the largest container resulted in the most growth. We know that larger container volumes tend to provide more water and nutrient availability, along with more space for root development post-transplant, resulting in better seedling growth and survival (Hsu et al. 1996; Matthes-Sears and Larson 1999) after planting (Dominguez-Lerena et al. 2006; Grossnickle 2005). We found that western larch seedlings growing in the 207 ml container outperformed other sizes (Fig. 1); even outperforming the same stocking grown in wetter conditions. The finding supports past work with sympatric conifers of western North America that suggested performance could 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, 2012).

Drying 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). Additionally, increased length of the root plug is known to provide an advantage to seedlings transplanted into dry edaphic conditions (Chirino et al. 2008; Pinto et al. 2012). Many site limitations can affect root growth potential, but ultimately, true potential will depend on the stocktype quality. In this simulation, the advantage illustrated by the performance of the 207 ml stocktype was greater RV than in smaller stocktypes. High quality, large stocktypes are suggested perform better on harsh, dry Mediterranean planting sites (Villar-Salvador et al. 2012), therefore, we believe this stocktype, based on its performance, has potential for further testing on moisture-limited sites.

Other research has found that RV can be directly correlated with outplanting success in some conifers (Rose et al. 1991a, b) and that a larger root volume is associated with higher RGP and increased capacity for water uptake (Carlson 1986). Independent of their assigned moisture 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 between 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 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

Rhizosphere temperature had no effect on seedling survival; however, it did hinder root growth at the lower temperature (10 °C). This suggests that seedlings were viable for growth and survival following transplant despite differences in root growth response to rhizosphere temperature. Also, note that experimental temperatures were only limited to 10 °C; however, field conditions could be much more limiting and have a greater effect on seedling growth (Lopushinski and Max 1990). Experimental temperatures were selected to encompass productive root growth conditions for conifer species (*Pseudotsuga menziesii*, *Tsuga heterophyla*, and *Picea* spp.) in northern forests, which have been demonstrated to be at temperatures near 10 °C or greater (Jacobs et al. 2008). In real field conditions, edaphic factors are linked with seasonal weather patterns, which in the Pacific and Inland Northwest include a distinct summer drought period (Waring and Franklin 1979). In the spring, when western larch seedlings are often outplanted, rhizosphere temperature increases over time; while in a fall planting scenarios those temperatures decrease gradually. 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 (Spittlhouse and Stewart 2004; Rehfeldt and Jaquish 2010).

The absence of growth differences among measures of HT, SDM, and RCD in response to rhizosphere temperature corresponds to 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 fully evaluate the effects that temperature had on root growth. However, 6 weeks sufficient in evaluating the period immediately following transplant, during which root growth is most vital to seedling survival (Grossnickle 2005). Low density cultural effects carried over into post-transplant seedling performance, with greater RDM growth in response to both temperature treatments. Additionally, seedlings cultured at low density had a higher R:S prior to transplant. In this case, the higher R:S correctly predicted superior outplanting performance when compared to seedlings cultured at high density which had lower corresponding R:S.

Conclusions

Because of land use changes, shifting climate, and increasing afforestation efforts on disturbed landscapes, restoration efforts incorporating western larch will likely be targeting harsher and drier conditions in this species' former range. An initial investment into a higher quality seedling, while having options to select stocktype size to meet site (e.g. rocky or shallow soils, presence/absence of competing vegetation, animal browse, etc.) and cost constraints (e.g. seed availability, containers and growing medium, water, fertilizer, etc.), will be useful. This study demonstrates that when culturing protocols are tailored to container volume, and high quality seedlings are produced, the advantages of stocktype size are somewhat reduced (within the tested range of this experiment). However, compared to smaller options, a container volume of 207 ml appears to greatly improve seedling establishment following transplant into dry edaphic conditions, particularly in the area of root egress. Yet, larger stocktype sizes may confer an advantage on dry sites as have been observed and suggested with other species in drier habitats (Villar-Salvador et al. 2012).

The findings of these experiments confirm only a portion of the hypothesis in which we expected stocktype differences by volume, and for those differences to persist given challenging or "harsh" outplanting scenarios. In optimal post-transplant conditions, the response of western larch seedlings was relatively homogeneous in terms of seedling root and shoot growth. We attribute these results to production methods in which nutrient regimes were tailored to container parameters, facilitating a similar growth potential among seedlings following transplant. 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. To further refine target seedlings for larch and the anticipated future establishment scenarios, additional investigations are encouraged to incorporate planting scenarios that may have been considered too extreme in the past, specifically, fall planting, earlier spring planting, and higher elevation planting locations.

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