Two studies of the potential of drought preconditioning to enhance deep root production in seedlings of western larch (*Larix occidentalis*)

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Abstract

Climate change is increasing the frequency and intensity of drought in the western USA. Evidence suggests that drought preconditioning of plants may improve the survival of planted seedlings under dry conditions through enhanced water uptake by roots, but the mechanisms underlying enhanced survival under drought remain unknown. We tested whether the vertical distribution of roots in root plug cross-sections varied with drought preconditioning and seed source. We subjected western larch (*Larix occidentalis* Nutt.) seedlings from eight different provenances to Low (50-65% gravimetric water content), Moderate (65-75%), and High (≥75%) watering regimes in a nursery. We then investigated dry root mass across four root-plug sections, including the taproot and three lateral root cross-sections (top 1/3rd, middle 1/3rd, and bottom 1/3rd of root plugs). We also tested for carry-over effect of drought preconditioning on the mass of egressed roots observed among cross-sections of potting soil in a 30-day potted study. Root plug mass varied significantly ($P < 0.001$) with watering regime, root plug cross-section, and an interaction between watering regime and cross-section. Overall, seedlings that received less water produced lateral root plug cross-sections of greater mass, which coincided with taproots of less mass. In contrast to findings from the root plug study, the distribution of egressed root mass among cross-sections of potting soil did not vary with drought preconditioning. This is the first study to assess seedling root growth in response to drought preconditioning in western larch with a focus on the distribution of root mass in root plugs and egressed root mass among soil cross-sections. We expect this work to facilitate future efforts to improve drought hardiness of western larch seedlings. Future improvement of western larch seedlings will require investigation into whether altered root plug mass distribution translates to improved seedling performance in outplanting trials.

Keywords

Biomass partitioning; Conifer; Potted study; Root egress; Seedling root plug; Stress memory

Contents

1 Introduction

The frequency and intensity of drought conditions in the western USA are increasing as regional mean air temperatures rise and precipitation patterns become more erratic (Fettig et al. 2013; USGCRP 2018). Consequently, plants are increasingly subjected to levels of drought stress that exceed conditions to which they are adapted (Nicotra et al. 2010; USGCRP 2018). High temperatures are expected to increase soil moisture deficits and lead to increased drought-induced mortality of planted seedlings with consequences for forest productivity, regeneration, and restoration across the western USA (Karl et al. 2009; Fettig et al. 2013; Clark et al. 2016).

The probability of seedling survival, whether natural or planted, is related to the ability of roots to contact plant-available water in the soil (Day et al. 1975; Grossnickle 2005). As drought coupled with warmer temperatures limits the establishment of planted seedlings, and climate change leads to hotter, drier conditions, forest managers will look to forest nurseries for seedlings with enhanced drought hardiness. One way forest nurseries may achieve enhanced seedling drought hardiness is by optimizing seedling root systems for greater water uptake by growing deeper into the soil following outplanting (Valliere et al. 2019). Drought preconditioning has been shown to increase seedling drought tolerance and survival for some species (van den Driessche 1992; Valliere et al. 2019). Enhanced seedling performance following drought preconditioning may result from greater seedling access to deep soil water due to altered biomass partitioning that yields increased production of root tissue deeper in the soil (Burdett 1990). Drought preconditioning may furthermore aid forest nurseries in the transition to water-conserving practices by reducing the amount of water nurseries use for plant production. However, information is lacking on the effect of drought preconditioning on the vertical distribution of root biomass in root plugs and egressed roots in soil.

In the case of western larch (*Larix occidentalis* Nutt.), an important timber species in western North America, it is unknown whether drought preconditioning promotes desirable (i.e., deep) root growth patterns associated with drought hardiness. It is also unknown whether western larch from different seed origins (provenances) vary in their responses to drought preconditioning. To close these knowledge gaps, we conducted two studies to address the following three questions.

1. Can drought preconditioning in the nursery growth phase increase the proportion of seedling roots deeper in root plugs?
2. Does the distribution of root mass among vertical root plug cross-sections vary by provenance in response to drought preconditioning?
3. Does drought preconditioning promote greater egressed root mass in deeper as opposed to shallower soil cross-sections?

2 Methods

Western larch seedlings from eight provenances spanning the species range were grown in a greenhouse in 91/130 Styroblock® containers (Beaver Plastics, Alberta, Canada) with cavity volumes of 130 ml. The provenances selected for this study included six woods-run bulk seedlots (provenance ID 5211, 27269, 39158, 39159, 39160, and 39264; Table 1) and two improved genetic families from an open-pollinated seed orchard (provenance ID 50106 and 50117). Each woods-run bulk seedlot represents seed from ≥ 50 maternal trees. Provenance locations and related 30-year climate norms from 1981 – 2010 derived from Climate WNA (Wang et al., 2016) are shown in Table 1. Cavities were filled with Berger© BM8 growing media (Saint-Modeste, QC, Canada) amended with 7.9 g slow-release Osmocote® fertilizer (N = 15%, P = 9%, K = 12%) per liter soil media. Cavities were then topped with TARGET® Forestry Sand (no. 9992002; Burnaby, BC, Canada). Seeds were sown and cavities were well-watered for 6.5 weeks, at which time seedlings were subjected to Low (50%-65% saturated container weight), Moderate (60%-75% saturated container weight), and High (75%-100% saturated container weight) watering regimes for six months of growth using overhead irrigation. Seedlings were lifted eight months after seed sowing and stored at -2.2°C for one month. See Moler and Nelson (2021) for further details concerning preconditioning treatment conditions.

In January 2021, seven randomly selected replicate seedlings of each provenance and treatment combination were selected for root plug dissections (n = 168 seedlings). Dry root mass was investigated across four sections of each root-plug: three vertical cross-sections of equal length including lateral roots from the top 1/3rd, middle 1/3rd, and bottom 1/3rd of the root plug (each root plug measures approximately 12.7 cm in length, for three segments of 4.23 cm in length), and taproot tissue from the top to the bottom of the plug. Samples were dried in a force-draft oven for 72 hours at 65 °C before weighing each cross-section of oven-dry root mass to the nearest 10^{-4} gram using a Veritas® M214I balance (H & C Weighing Systems™).

Two of the eight provenances used in the root plug study were included in a potted root growth study. One provenance from a hot and dry location and another from a relatively cold and moist location were selected (provenance 39159 and 5211, respectively; Table 1). Three replicates from each of two provenances and three drought preconditioning treatments were planted in one-gallon pots (Nursery Supplies Inc.) filled with moistened Berger© BM8 growing media, arranged in a completely randomized design, and grown from January 29 – February 28, 2021, under natural lighting in greenhouses at the University of Idaho Franklin H. Pitkin Forest Nursery (46.7324° N, 117.0002° W). The study was initiated in a greenhouse with approximate nightly and daily temperatures of 7.2° C and 10° C, respectively, where night was defined as the period from one hour past sunset (approximately 6 pm) to sunrise (approximately 7 am). From February 9 until the end of the study on February 28, pots occupied a greenhouse with mean daily and nightly temperatures of 15.6° C and 13.8° C, respectively. Three seedlings were destroyed during processing, leaving a total of 15 seedlings and a sample size of n=2-3 per each of six experimental groups. All seedlings were watered to maintain pot weights at or above 70% gravimetric weight during the entire 30-day growth period. After 30 days of growth, all seedlings were cut at the root collar and the pots were divided with a saw into three vertical cross-sections of equal depths of 5.93 cm, yielding soil volumes of approximately 1804 cm^3 per cross-section. Roots were carefully separated from the soil medium and gently washed, then dried in a force-draft oven for a minimum of 72 hours at 65 °C. Oven-dry mass of new root tissue that egressed from the root plug during the 30-day growth period was weighed separately for each cross-section to the nearest 10^{-4} gram using a Veritas® M214I balance (H & C Weighing Systems™).

Table 1. Location and 30-year climate norms from the period 1981 – 2010 for provenances included in the study. MAT = mean annual temperature, MWMT = mean warmest monthly temperature, MCMT = mean coldest monthly temperature, MAP = mean annual precipitation, NFFD = number of frost-free days annually, RH = relative humidity.

Provenance	Latitude	Longitude	Elevation (m)	MAT (°C)	MWMT (°C)	MCMT (°C)	MAP (mm)	NFFD	RH (%)
5211	49.49	-119.19	1893	2.4	12.8	-7.0	799	153	70
27269	49.10	-115.32	1048	5.5	17.1	-6.0	594	175	60
39158	49.37	-119.65	1534	5.3	15.8	-4.9	543	219	80
39159	49.28	-119.30	985	4.5	16.1	-7.4	521	150	56
39160	49.86	-119.11	1411	3.9	14.6	-6.3	752	170	68
39264	50.12	-118.31	1243	3.7	14.3	-6.0	878	151	62
50106	48.10	-115.52	739	6.7	18.1	-4.2	383	169	53
50117	48.31	-115.35	1303	4.9	16.1	-5.4	556	167	61

For each seedling, proportional masses of seedling roots by cross-section were calculated by dividing cross-sectional mass of dried root tissue by the total mass of dried lateral- and tap-root tissue. Responses of proportional mass to two fixed effects (drought preconditioning and cross-sections), preconditioning level × cross-section interactions, and provenance specified as a random effect were assessed using generalized linear mixed-effect models (glmmTMB package in the R statistical environment, version 3.6.2). Beta distributions, which are appropriate for continuous proportional response variables (Douma and Weedon 2019), were specified for all models. Normality of model residuals was assessed using quantile-quantile plots. Significance testing of omnibus models with interaction terms were tested using type three ANOVA. Where interactions terms were non-significant ($P > 0.05$), additive models were tested using type-two ANOVA (car package). Likelihood ratio tests for difference between models with and without provenance were conducted using ANOVA. Tukey HSD was used to calculate post-hoc contrasts (emmeans package). Following PSU (2021), modeled estimated marginal mean values were back-transformed from logit-scale to the original probability-scale ($\boldsymbol{p}$) by applying equation (1), where $\boldsymbol{e}$ is the exponential function and $\varnothing$ is a logit-scale estimated marginal mean value.

$$(1)\ \frac{\boldsymbol{e}^{\varnothing}}{(\boldsymbol{e}^{\varnothing}+1)} = \boldsymbol{p}$$

Similarly, modeled treatment contrast estimates (CE) were back-transformed from the log-odds-scale to the original probability-scale ($\boldsymbol{p}$) as shown in equation (2) (PSU 2021).

$$(2)\ \frac{\mathrm{CE}}{(\mathrm{CE}+1)} = \boldsymbol{p}$$

3 Results

In support of the first research question, we found that drought preconditioning significantly increased the proportion of lateral root mass deeper in root plugs (Figure 1, Table 2). The greatest proportional mass of lateral roots occurred in the bottom root plug cross-section in the Low watering regime. The first, second, and third greatest incremental differences among cross-sections in lateral root plug proportional masses occurred in the High, Moderate, and Low watering regimes, respectively (Table 2). Notably, the proportional mass of each cross-section increased with water limitation (Figure 1, Table 2), which appeared to be at least partially attributable to a proportional decrease in taproot mass as water limitation increased (Figure 1).

Table 2. Post-hoc contrasts of three lateral root plug cross-sections. The proportion of root mass attributable to taproot is not shown. EMM is estimated marginal mean.

	Proportional Mass Contrasts	Estimate	SE	*t*	*P*
High Watering Regime	Middle – Top	0.364	0.058	9.89	< 0.0001
	Bottom – Top	0.495	0.056	17.55	< 0.0001
	Bottom – Middle	0.289	0.050	8.09	< 0.0001
	Proportional Masses	EMM		Lower CL	Upper CL
	Top	0.137		0.127	0.148
	Middle	0.220		0.208	0.233
	Bottom	0.298		0.284	0.312
Moderate Watering Regime	Proportional Mass Contrasts	Estimate	SE	*t*	*P*
	Middle – Top	0.330	0.054	9.14	< 0.0001
	Bottom – Top	0.489	0.052	18.39	< 0.0001
	Bottom – Middle	0.317	0.048	9.65	< 0.0001
	Proportional Masses	EMM		Lower CL	Upper CL
	Top	0.173		0.162	0.185
	Middle	0.255		0.242	0.269
	Bottom	0.353		0.338	0.367
Low Watering Regime	Proportional Mass Contrasts	Estimate	SE	*t*	*P*
	Middle – Top	0.317	0.051	9.07	< 0.0001
	Bottom – Top	0.438	0.050	15.58	< 0.0001
	Bottom – Middle	0.240	0.047	6.71	< 0.0001
	Proportional Masses	EMM		Lower CL	Upper CL
	Top	0.205		0.193	0.218
	Middle	0.291		0.277	0.305
	Bottom	0.360		0.345	0.375

We did not find support for the second research question, as provenance identity did not influence the effect of drought preconditioning treatment on biomass partitioning among root sections (Figure 2, $\chi^2 = 0.0$, $P = 1$). The estimated among-provenance standard deviation for mass proportion by cross-section was small (4.8e-6) in comparison to even the smallest estimated effects of treatment × root section interactions (Table 2), suggesting that proportional masses among provenances varied little relative to the magnitude of the fixed effects of interest. Likelihood ratio testing furthermore showed no difference between models with and without provenance as a random effect ($\chi^2 = 0.0$, $P = 1$).

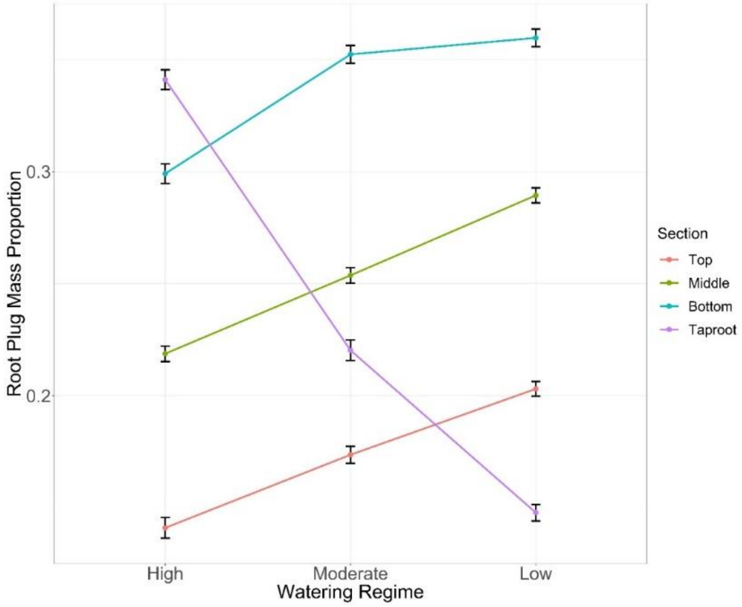

Figure 1. Root plug mass proportion in top, middle and bottom lateral sections across watering regimes. Error bars are standard error of the mean.

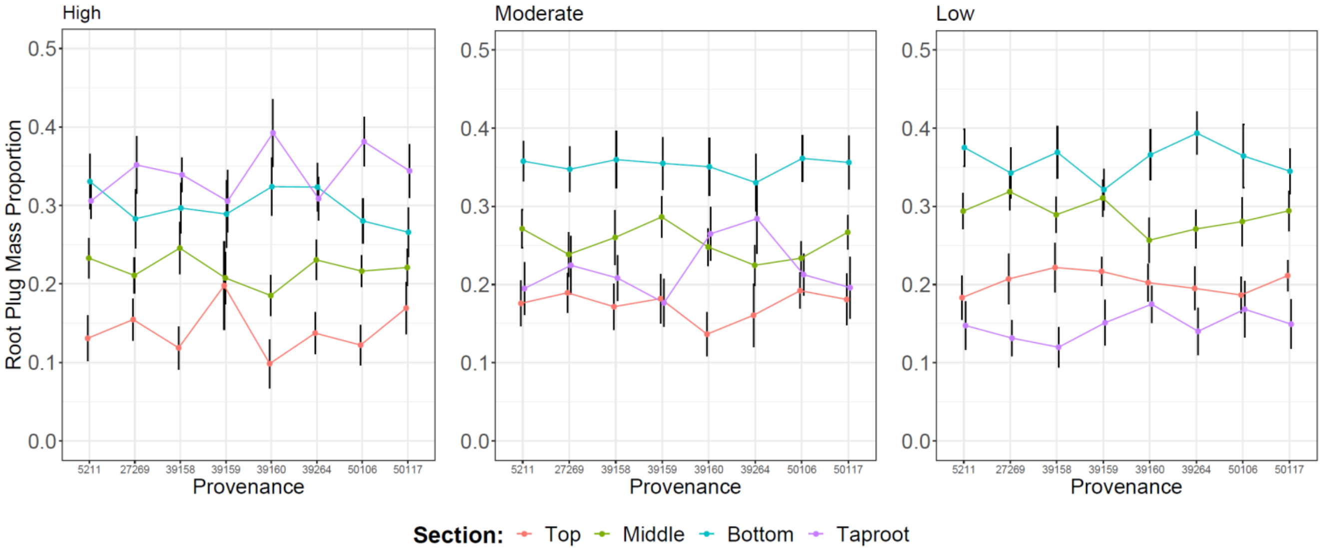

Figure 2. Root plug mass proportion by provenance across High, Moderate and Low watering regimes. Biomass partitioning varied among the eight provenances by section, but responses of biomass to drought preconditioning did not vary among provenances. Error bars are standard error of the mean.

We did not find support for the third research question as there was neither an interaction between root section and drought preconditioning treatment (χ^2 = 0.94, *P* = 0.92) nor an effect of drought preconditioning treatment alone (χ^2 = 0.61, *P* = 0.74) on root egress following the 30-day potted experiment. Nonetheless, significant differences in egressed root mass were found among soil cross-sections (χ^2 = 14.35, *P* = 7.63e-4). The greatest proportion of new root tissue egress was observed in the deepest soil cross-section (Figure 3).

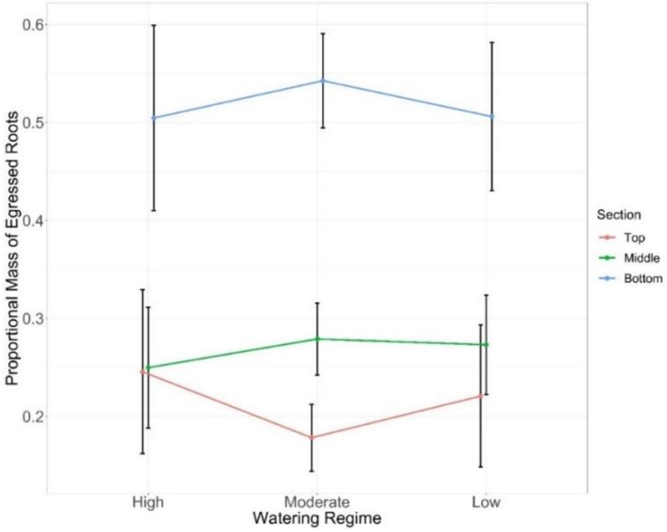

Figure 3. Proportional mass of egressed roots by watering regime and section from 30-day egress study. Error bars are standard error of the mean.

4 Discussion

In the root plug study, both the Moderate and Low watering regime drought preconditioning treatments increased the proportion of root mass contained in deep lateral roots versus taproots and upper lateral roots. Results from the root plug study thus suggest that drought-preconditioned western larch seedlings might be better able to access water deep in the soil by having a greater proportion of lateral root mass in the lowest root plug cross-section. We suspected that enhanced root mass in deeper plug cross-sections would correspond to an increased proportion of roots in deeper cross-sections of soil media, but results from the potted study revealed no effect of drought preconditioning on the distribution of egressed root mass among soil media cross-sections. Instead, the greatest proportion of egressed lateral root mass occurred in the lowest cross-section irrespective of preconditioning watering regime. If the morphological response of developing roots in deeper root plug cross-sections had corresponded to the depth of egressed roots, we might anticipate that drought preconditioning should increase seedling survival and growth under drought (Walter et al. 2013). Instead, results from the potted study suggest that drought preconditioning did not influence the snapshot of vertical distribution of egressed roots in pots described here. In both experiments in the present study, the root mass included in each vertical cross-section was the mass of all roots present in the section irrespective of where the roots originated. This design was chosen to describe the vertical root distribution we can expect of drought preconditioned *L. occidentalis* immediately after outplanting, which we expect to influence the earliest stages of seedling establishment. The contrasting results from the root plug and potted studies described here show that during water limitation, root growth distribution was strongly shifted to favor the proliferation of roots in a deeper location in the soil, but that once water limitation was relieved (i.e., in the potted study), new root production was no longer affected by prior exposure to water limitation. In other words, western larch seedlings did not express a memory of water limitation (*sensu* Yakovlev et al. 2011) as expressed through root egress when grown with ample water. Instead, new root growth occurred most abundantly in the deepest soil media cross-sections for all preconditioning treatments, and that trend was undoubtedly influenced by the Styroblock® container (Moreno 2000).

In contrast to results in the present study, a study of drought preconditioning using *Eucalyptus globulus* (Labill.) reported that severely water-limited seedlings exhibited significant reduction in root mass following a 36-day drought treatment (Guarnaschelli et al. 2003). While the Guarnaschelli et al. (2003) eucalyptus preconditioning treatment took place over only 36 days and began four months after germination, preconditioning of western larch in the present study was conducted for six months and began 6.5 weeks after germination. Drought regimes applied to eucalyptus seedlings in Guarnaschelli et al. (2003) were based upon the number of days elapsed between watering, whereas the present study maintained seedling drought status according to daily assessment of gravimetric weight. Garrity et al. (1983) implemented a drought stress conditioning treatment on grain sorghum (*Sorghum bicolor)* by reducing irrigation levels from 100% to 0% in six increments. Dry matter production and yield of grain sorghum varied and was found to depend on the timing and intensity of drought application, where application of drought stress early in the life cycle of sorghum increased the resiliency of the plant to drought during later stages of growth and improved plant yield (Garrity et al. 1983). Both studies (Garrity et al. 1983 and Guarnaschelli et al. 2003) suggest that the severity and duration of water limitation strongly influence the response of plant morphology to drought conditioning.

The present study found no interactive effect of provenance and watering regime on root plug mass among soil cross-sections. Similarly, Sloan et al. (2020) found that the effect of watering regimes on performance of *Populus tremuloides* (Michx.) seedlings did not vary with seed source, and the authors thus concluded that watering regimes may be useful for producing drought tolerant nursery stock for drought-prone sites irrespective of seed source. Semerci et al. (2017), on the other hand, found a highly significant interactive effect of watering regime and seed source latitude on drought stress of Scots pine (*Pinus sylvestris* L.) seedlings*,* suggesting that efforts to improve drought hardiness of Scots pine should prioritize the use of provenances that are most amenable to improvement via drought preconditioning. Though the present study did not demonstrate an interaction between watering regime and provenance, the results of Semerci et al. (2017) suggest that inclusion of western larch seed sources from across a wider range of environmental gradients may have revealed significant interactive effects of provenance × watering regime on root plug mass distribution.

In the 30-day egress study described here, while the highest proportion of root tissue mass occurred in the lower cross-section of the root plugs, drought preconditioning watering regime did not affect this trend. In a study of *Pinus halepensis* (Mill.) seedlings*,* Atzmon et al. (2003) found that it was not possible to predict the performance of a provenance under high stress conditions based on assessments of seedling performance conducted under mild conditions. Accordingly, accurate assessment of possible effects of drought preconditioning on plant performance under stress may require exposure of western larch seedlings to drought stress following drought preconditioning. Future studies should aim to mimic the conditions a seedling would experience in the field to assess how drought preconditioning and provenance might influence the resilience of seedlings to drought.

5 Conclusion

Improving plant survival under drought conditions is essential for successful reforestation and restoration in the present era of rapid global climate warming (Valliere et al. 2019). Importantly, this study illustrates that observations made during exposure of plants to controlled abiotic stress may not be indicative of plant behavior once plant stress is relieved. More research appears to be warranted to better understand the conditions under which plants express a memory of past stress, and how best to stimulate plant stress memories for plant improvement (Yakovlev et al. 2011). The present study does not lead us to recommend the selection of western larch provenances based on root biomass partitioning responses to drought conditioning. However, as geographic variation in plant climate hardiness is commonly detected (Carvalho et al. 2017; Semerci et al. 2017), provenance may yet be found to play an important role in western larch seedling responses to drought preconditioning. Common garden studies including numerous provenances and genetic families with investigations into plant-water relations and plant survival under water limited conditions would provide the most effective study system for improving drought hardiness in western larch seedlings. Finally, field trials in natural water-limited environments should be conducted with the aim of discovering drought preconditioning regimes that result in high seedling survival without sacrificing desirable above ground growth traits.

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