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**YEAR:**  **3** of  **4**

**PROJECT TITLE:** Intraspecific hydraulic responses of commercial tree seedlings to nursery drought conditioning

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| **PROJECT DESCRIPTION:** Planting seedlings is the most common form of plantation establishment across much of forest industry lands in the USA. Planting reduces uncertainties of natural regeneration, provides the ability to introduce elite genetics, and assists with establishing desirable tree species and density. In 2018, forest nurseries produced over 1.19 billion seedlings that were planted across approximately 2.2 million acres, roughly equating to approximately $712 million invested by forest industry and governments. Various interacting factors influence seedling survival and growth following planting including the physiological potential to withstand environmental stresses and production of new roots with desirable characteristics to avoid moisture limitations. We may be able to select intraspecific phenotypes for physiological properties and root traits that allow for greater water uptake and transport and resistance to water limitations, yet little work has been done to advance understanding of drought-conditioning in commercial tree species.Our multi-institution project (University of Idaho, Purdue University, and Oregon State University) examines drought-related physiological parameters (e.g., stem and root hydraulics, resistance to cavitation) and root morphological traits of various genotypes of western larch, black walnut, and coastal Douglas-fir from diverse maternal tree climates across each species’ native range. Seedlings will first be drought-conditioned in the nursery using different watering regimes to produce a range of phenotypes. Seedlings will then be exposed to additional drought conditions to test the hypothesis that more extreme drought-conditioned phenotypes will have greater resistance to loss of xylem functionality and root system architecture that encourages water scavenging (i.e. longer and thicker roots, greater root branching).Results from the research will help inform reforestation efforts of CAFS members by targeting specific genotypes and drought conditioning treatments that will confer greater seedling survival and growth following outplanting. |
| **HYPOTHESES or OBJECTIVES:** The objective of this project is to examine seedling physiology and root system architecture in response to nursery-induced drought conditioning intensity and subsequent drought. These factors will be examined across three commercially important tree species in the United States (coastal Douglas-fir, western larch, and black walnut) and for seed sources from different maternal tree environments. |
| **METHODS:** Nursery Experimental DesignSeed sources were obtained from maternal tree environments across each species’ native range. Seedlings from the different sources were then grown in containers in greenhouses in 2020. Black walnut seedlings were grown in greenhouses at Purdue University. Western larch and Douglas-fir seedlings were grown in greenhouses at the University of Idaho. The experiments used three sources of black walnut seed (Kansas, Indiana, and Maryland), eight sources of western larch seed (spanning a parent-tree climate across southern Interior British Columbia and two seed orchard seed sources from Montana), and three sources of Douglas-fir seed (Coast Range, Inland and Cascade Foothills). Trays of seedlings from a single seed source were randomly assigned to different drought-conditioning treatments, resulting in factorial combinations of seed source × drought-conditioning. The drought conditioning treatments were (1) no water stress (control), (2) moderate water stress (moderate water), and (3) high water stress (low water), although the exact watering prescriptions differed by species due to differences in physiological responses to water limitations and to minimize the chance of seedling mortality. Western larch seedlings were slowly acclimated to the moderate and low water treatments starting the sixth week after sowing and stepping down soil moisture on a weekly basis. By the sixteenth week, the control treatment had seedlings drop to 70% of saturated weight before completely rewatering to saturation. The moderate treatment maintained seedlings between 60 and 75% of saturated weight, while the low treatment maintained seedlings between 45 and 60% of saturated weight. Moderate and low water treatments were watered to saturation on a monthly basis to leach salts from the controlled-release fertilizer. Douglas-fir seedlings did not have controlled-release fertilizer mixed with the soil medium, therefore the seedlings were fertilized using liquid fertilizer and had to be watered to complete saturation at each watering to ensure the same amount of fertilizer was applied to seedlings on a weekly basis. The Douglas-fir control treatment allowed moisture to decrease to 70% before watering to saturation, while the moderate treatment dried down to 60% of saturated weight, and the low treatment dried down to 50% of saturated weight. Black walnut drought conditioning treatment were applied using a subirrigation system seven weeks after sowing. By the third week, the control treatment allowed seedlings to drop to a range of 85-95% of saturated weight before watering them for one hour through subirrigation. For the moderate and low treatments, seedlings were watered for 10 and 20 minutes once they reached a range of 75-85% and 55-65% of saturated weight, respectively.Seedling Hydraulic ConductanceRandom seedlings were selected for periodic measurement of height and diameter throughout the greenhouse phase of the experiment. Additional random seedlings were selected from each seed source × drought treatment combination each month to measure gas exchange, plant moisture stress, and biomass allocation. In mid-July 2020, 10 seedlings of each seed source within the control treatment were sent to Oregon State University to measure root hydraulic conductance (k, expressed per unit root volume). The k of roots was measured by inserting the whole root system into a custom made “vacuum canister”, which was attached to a vacuum pump (to produce the suction of water from the roots) and connected to a water reservoir placed on top of an analytical balance (to measure the change per unit time of water mass/volume). The initial “native” k (knat) was determined as the first measurement immediately after sample collection. After knat determination, segments were soaked under vacuum for 72 hours to refill embolized tracheids/vessels. After removing tracheid/vessel embolisms through this process, the maximum k (kmax) was measured using the procedure outlined previously. The vulnerability to cavitation curves of the whole root system was determined after measuring k on roots that were subjected to increased pressure (up to 4 MPa) using a pressure chamber. Additional seedlings were sent to OSU at the end of the drought experiments to measure root hydraulic conductance for the different seed sources and drought treatments. These seedlings included all three drought conditioning treatments and three families per species. Simulated Outplanting and Three-Dimensional Analysis of Root Growth. At the end of the nursery experiment, a random selection of seedlings were sent to Purdue University for the Controlled Environment Phenotyping Facility phase of the project in spring 2021 to analyze post-planting root growth dynamics and architecture. The Controlled Environment Phenotyping Facility is equipped with X-ray computed tomography technology that allows the visualization and assessment of the root systems of plants nondestructively. To our knowledge this technology is being used for the first time for tree species in our experiment. Nine seedlings were potted into large pots (11L, 45cm tall), that allow periodic root growth assessment for six weeks. Seedlings were then scanned with an X-ray CT scanner to examine the development of post-planting root system architecture every two weeks after transplanting for a total of three scans per seedling. The resulting data is thousands of images per seedling that will be segmented together to form a single 3D point cloud model of the root system architecture for each of the three scanning time points. Data post-processing is currently underway. In order to study the root growth dynamics and architecture of our seedlings, we developed models that can extract quantitative data from the point cloud models. After six weeks, gas exchange, whole plant transpiration and plant moisture stress were evaluated for each seedling under drought conditions and two days after being watered. Net photosynthesis (Anet) and stomatal conductance (gs) measurements were taken on the most mature and fully expanded leaves of each seedling with a Li-Cor 6400XT (Licor Inc., Lincoln, NE, USA). To measure seedling whole seedling transpiration (Eplant) the container was sealed around the seedling with plastic wrapping foil to minimize water lost through evaporation directly from the media instead of leaf transpiration. Container weight and water potential were measured before sunrise, 9am and noon. The exact time of measurement was registered for each seedling (t1: predawn, t2: morning, t3 noon). Four containers with the same media and similar moisture composition as the ones containing the seedling were weighted at the same time as a control for the loss of water through evaporation independent of the seedling. Measurements were taken under full sunlight and at 24 ºC. Eplant was calculated as Eplant = ΔWt3-t2/ Δt t3-t2 ·Δ Ψ t3-t1· Leaf area. Where ΔWt3-t2 is the difference in weight between measurements at noon and morning, Δt t3-t2 is the time in seconds between measurements at noon and morning. Then, seedlings were harvested and frozen for further measurements in the laboratory. Leaf and root surface areas were measured with a LI-3100C area meter and a Winrhizo system, respectively. Total and new growth dry weights were measured for roots, stems and leaves. Field Outplanting ExperimentSeedlings from each of the seed sources and nursery drought conditioning treatment were planted on field sites in the three respective regions. Douglas-fir seedlings were planted first in February 2021 in a completely randomized block design at three sites that match each of the three seed sources. Western larch seedlings were planted using the same experimental design at two sites with contrasting environmental conditions. One site was a harsh lower elevation, western aspect site and a higher quality mid-elevation eastern aspect site. Each seed source and drought conditioning treatment was planted in a plot with 12 seedlings spaced 5 feet apart, with multiple blocks within each site. Black walnut seedlings were planted in a split-plot design where the whole plot factor was irrigation vs. no irrigation and the split-plot factor was seed source × drought conditioning treatment. Each plot contained 12 seedlings spaced 5 feet apart. Soil temperature and moisture conditions were monitored throughout the year at a depth of 15 cm. Plant moisture stress was measured in mid-August, during the driest part of the growing season, and seedling survival and growth were measured in mid-October. The complete root system was excavated for two seedlings per plot and block and their maximum rooting depth was measured in the field. Seedlings were stored in a freezer for further analysis of their root system characteristics such as total length of the different diametrical classes and dry weight of the root plug and egressed new roots. Two review papers were developed in support of this project. The first study, led by Dr. Ehren Moler and Dr. Andrew Nelson at the University of Idaho, reviewed different approaches for combining genetic and cultural tools to solve challenges associated with seedling quality improvement. Findings from this review suggest that plant improvement can be optimized to harness root trait variation due to genetics, maternal effects, and nursery cultural regimes for enhanced outplanting survival and growth. The second paper, led by Dr. Andrei Toca and Dr. Douglas Jacobs at Purdue University, is a synthesis of the available research on how environmental conditions affect root system development. Specifically, we reviewed the effects of nutrient and water availability, light (shading and photoperiod reduction), soil temperature, and their interactions on seedling root system development and architecture. Based on this synthesis, we then determined the relative importance of these environmental variables for root development regulation and discussed the implications of this synthesis for forestry management. |
| **MAJOR FINDINGS:** Our previous reports focused on nursery-phase of the experiment, simulated outplanting to study three-dimensional root growth and the field experiment. Year 3 focuses on seedling morphological and physiological acclimation in response to drought preconditioning during simulated outplanting. Major finding include:* One of our key results shows that biomass allocation is a key component in determining the water uptake capacity and gas exchange of seedlings, even during the early stages of establishment. For all three species, new root allocation was positively correlated to whole plant transpiration rate (Eplant) under drought conditions and optimal watering (Fig. 1A, C, E). Similarly, an increase in new root allocation was associated with higher net photosynthesis (Anet) in black walnut and Douglas-fir (Fig. 1B, D, F). Although the relationship between new root allocation and Anet was not significant in Western larch, seedlings with higher new root allocation had higher Eplant, which translates into a higher amount of carbon assimilation over the three-hour period that Eplant was measured.
* We found that the gradient in drought stress during the first year of growth yielded different morpho-physiological responses in the subsequent growing season drought for all species.
* Western larch showed an increase in allocation patterns towards new egressing roots (Fig 2A, P<0.0001), which allowed for higher water uptake as indicated by a higher whole seedling transpiration rate (P= 0.0082).
* Douglas-fir seedlings had an earlier bud bread in response to previous exposure to drought (P<0.0001) accompanied by an increase in new foliage allocation (Fig 2D, P<0.0001) and net photosynthesis (P=0.0002), while no effects were detected in root allocation (Fig 2C, P=0.2679).
* Finally, black walnut did not show any changes in biomass allocation (Fig 2E and 2F) or physiology in response to drought preconditioning.
* Our results suggest the presence of a drought memory in that early drought stress modified seedling responses to subsequent drought experiences when controlling for initial seedling morphology resulting from the first season drought.
* These results also highlight the importance of species ecology and provenance of the seed in the formation of drought memory and its impact on the morpho-physiological acclimation responses to subsequent drought stress.

 **Figure 1:** Correlations among new root mass allocation and whole seedling transpiration rate (Eplant; leftside) and photosynthesis rate (A; right side) for Western larch (A, B), Douglas-fir (C, D), and western larch (E, F) across all provenances and treatments, under second-year growing season drought (○) and after watering (●). Statistical analysis values on the lower right side of each graph correspond to second year drought conditions and upper left for the same correlation after watering the seedlings to optimal levels. **Figure 2:** Post-planting new root (left) and leaf (right) mass allocation response to drought stress (Control, Moderate and High) during the first year of growth across species and provenances. Data are estimated marginal means and standard errors of the mean. |
| **DELIVERABLES:**  * Species-specific protocols for drought conditioning western larch, Douglas-fir, and black walnut. Regimes vary by species due to internal physiological tolerance to drought.
* Novel approach to assessing root development non-destructively based on Computed X-ray tomography that enables the quantitative analysis of root system architecture of forest tree species as roots develop. Currently our model is capable of total root length, rooting depth, root tips, root junctions and root dispersion angles.
* Review paper accepted for a special issue in New Forests: Toca, A., Moler, E., Nelson, A., Jacobs, D.F., 2022. Environmental conditions in the nursery regulate root system development and architecture of forest tree seedlings: a systematic review. New For. 53, 1113–1143. <https://doi.org/10.1007/s11056-022-09944-8>
* Review paper accepted for a special issue in New Forests: Moler, E.R. V., Toca, A., Jacobs, D.F., Nelson, A.S., 2022. Root system adaptations represent untapped opportunities for forest tree seedling improvement. New For. 53, 1069–1091. https://doi.org/10.1007/s11056-022-09917-x
* Toca, A. Moler, E., Nelson, A.S., and Jacobs, D.J. 2022. X-ray computed tomography opens new horizons for root system architecture analysis in forest tree seedlings. 8th International Symposium on Physiological Processes in Roots of Woody Plants. State College, PA. (Oral presentation)
* Edmond, V., Nelson, A.S., Moler, E., 2022. Intraspecific root trait variability of western larch (Larix occidentalis) seedlings in response to drought. Society of American Foresters Convention. Baltimore, MD. (Oral presentation)
* Edmond, V., Nelson, A.S., Moler, E., Intraspecific root trait variability of western larch (Larix occidentalis) seedlings in response to drought. The Reforestation Pipeline in the Western United States Conference. Missoula, MT. (Oral presentation)
* Nelson, A.S., Moler, E.R.V., Toca, A., Jacobs, D., Gonzalez-Benecke, C., Pinto, J., and McDonough, T. 2023. Drought conditioning to improve outplanting performance: Perspectives from tree seedlings and applications to rangeland plants. Society for Range Management Meeting, Rangeland Technology and Equipment Council Symposium: Beyond direct seeding: the next frontier of landscape scale vegetation restoration. Boise, ID. February 15. (Oral presentation)
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| **MEMBER COMPANY BENEFITS:** Results may help reduce reforestation costs associated with replanting failed plantations by tailoring phenotypes to match a broad range of site conditions across three major forestry regions of the US. Results may also help inform tree improvement programs by integrating genotypes into seed orchards and breeding programs that produce physiological and root phenotypes that enhance seedling establishment.The X-ray computed tomography technology presented in this project will open new opportunities to generate data on dynamic responses of root systems to nursery treatments, environmental conditions and localized availability of soil resources in forest tree species. This knowledge will open new horizons for selecting root traits expected to enhance seedling performance under environmental stress such as drought. |
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