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YEAR: 1 of 3

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:

Seed 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.

Random 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.

Additional 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. 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 model of the root system architecture for each of the three scanning time points. Data post-processing is currently underway. After six weeks, gas exchange and plant moisture stress were evaluated for each seedling. Then, seedlings were harvested and frozen for future evaluation of root and leaf surface areas, biomass allocation across root stem and leaves as well as new growth biomass allocation.

Seedlings 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. Seedlings will be monitored during the 2021 growing season for growth and survival.

MAJOR FINDINGS:

- Completion of nursery-phase of project:
  - Seedling height and diameter decreased with increasing drought conditioning intensity.
  - Roots of Douglas-fir control seedlings showed lower vulnerability to cavitation ($P<0.001$),(measured as the xylem water potential at which 50% loss of hydraulic conductance occurs, Ψ_{50}) among the three species
    - Western larch -1.41 Mpa
    - Douglas-fir -1.99 MPa
    - Black walnut -1.50 MPa
  - After finishing nursery stage: For Black Walnut, there was no difference in *knat* across seed sources ($P=0.468$), and roots subjected to moderate or extreme drought showed reduced *knat* ($P=0.0028$). For Douglas-fir, *knat* was there was no effect of watering treatments ($P=0.998$), but a significant difference across seed sources was observed, being Cascade Foothills the seed source with higher *knat* ($P=0.0009$) both with the lowest root volume ($P<0.001$). Western Larch showed the largest *knat* ($P<0.001$), but the smallest root volume ($P<0.001$).

  - Vertical distribution of fine roots within western larch seedlings was significantly greater for the moderate and high drought stress treatments compared to the control, while the control seedlings had greater root allocation to the taproot.
    - High: 0.44 g bottom, 0.35 g middle, 0.25 g upper, 0.17 g taproot

  - Moderate: 0.47 g bottom, 0.34 g middle, 0.23 g upper, 0.28 g taproot
  - Control: 0.30 g bottom, 0.22 g middle, 0.15 g upper, 0.34 g taproot
- New root growth production under optimal growing conditions in mist chambers was significantly greater with increasing drought conditioning:
  - High: 56 new roots, 11.6 cm new root length, 0.08 g new root biomass
  - High: 48 new roots, 9.9 cm new root length, 0.06 g new root biomass
  - High: 31 new roots, 7.0 cm new root length, 0.03 g new root biomass

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.
- Phenotyping facility protocols to examine 3D root system architecture development following drought conditioning

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.