John Van Gundy student scholarship awarded for 2023

The proposals were reviewed by the Evaluations Committee, composed of former board members Bryan Donner, Cyndi Smith, and Kathy Tonnessen, and Nutcracker Notes *editor and associate director Bob Keane. We are pleased to announce the 2023 recipient of the John Van Gundy student scholarship, administered by the WPEF:* ***JESSICA HARRIS****, an MS student with Dr. Danielle Ulrich of the Faculty of Biological Sciences at Montana State University.*

Assessing within species variation of seedling growth and physiological traits in Whitebark Pine (*Pinus albicaulis*) across climatic gradients

Background, Objectives, and Justification

Over recent decades, Whitebark Pine (*Pinus albicaulis*: WBP), has seen radical population declines due to white pine blister rust (*Cronartium ribicola*: WPBR), mountain pine beetle (*Dendroctonus ponderosae*: MPB), understory habitat encroachment, and climate change-induced drought. While WPBR is the leading threat to WBP, higher global temperatures will increase the frequency, duration, and severity of drought (Allen *et al.* 2010, Chmura *et al.* 2011, Cartwright *et al*. 2022). To compound upon this, drought is also a precursor to WBPR infection and MPB infestation (Tomback *et al*. 2022). These threats have resulted in WBP being listed as "threatened" via the US Endangered Species Act in 2021 (Tomback *et al*, 2022). The loss of WBP has serious ecological repercussions for high-elevation forests throughout western North America. WBP is a long-lived and slow-growing species that is a keystone and foundational species. WBP seedlings are often the first tree species to establish in the subalpine tree line ecosystem despite the nutrient-poor soils, short growing seasons, and extremely cold, windy, and bright conditions common to this environment (Tomback *et al.* 2001). Accordingly, WBP seeds are a vital food source for endangered species such as Grizzly Bear and Clark's Nutcracker. WBP also creates a suitable microclimate for the regeneration of younger and more shade-tolerant plant species (McLane and Aitken 2012).

To restore and conserve WBP, USFS restoration efforts have focused on outplanting rust-resistant seedlings. These efforts require research that identifies which families have the greatest chance of successfully surviving in those areas. One major factor that determines which families should be outplanted include their capacity to overcome the bottleneck of seedling establishment. This capacity is demonstrated via traits such as seedling growth, biomass, phenology, and carbohydrate traits. During early life stages (< 2 years), WBP is especially vulnerable to mortality due to both abiotic and biotic stressors. Importantly, climate-induced drought has been identified as potentially the greatest limiting factor for other high-elevation pine seedlings with drought resulting in shorter individuals, lower leaf area, altered germination and establishment rates, and altered non-structural carbohydrate dynamics (Broderson *et al*. 2019, Smithers and North 2020). To ensure that outplanted seedlings can survive into reproductively mature adults, identifying which families have the highest rates of seedling establishment (due to optimal drought resistance and response) is imperative for successful cost- and time-effective restoration efforts.

My research fills this knowledge gap by identifying the intraspecific (within species) variation in seedling growth (leaf area, root biomass, etc.), phenology (emergence, establishment, survival rates), and drought resistance (non-structural carbohydrates: NSCs) within 50 WBP families. These WBP families originated from locations spanning the east-west precipitation gradient of the Cascades Mts from southeastern Oregon, northern Washington, and the Coastal and Northern Rocky Mountains in British Columbia. Objectives of this study are to: 1) identify which families have the highest rate of establishment, 2) investigate whether successful establishment rates are correlated with specific USFS seed zones or British

Columbia Forest Service (BCFS) locations and 3) determine a timeframe for how many months were required for successful families to reach the emergence and establishment stages, 4) identify correlations between establishment and survival with growth, phenology, and drought resistance traits, and 5) determine if precipitation and temperature are correlated with family-specific traits.

Study Plan and Methods

To accomplish these objectives, a greenhouse common garden experiment was implemented and seed from each of the 50 families (seed donated by USFS and the BCFS) was grown in the same controlled environment at MSU's Plant Growth Center. Methods included seedling growth (non-destructive and destructive measurements), phenology (seed counts for each development stage), and drought resistance (destructive harvest for NSC analysis). Non-destructive measurements (e.g., seedling height, stem diameter) were obtained monthly by taking images of seedlings using an iPhone for ImageJ analysis (Schneider *et al.* 2012) and manually measuring seedlings with a ruler or calipers. Destructive measurements (e.g., root surface area, root:shoot biomass) were obtained on eight seedlings per family at 4-, 6-, 8-, and 12-months post-sowing to obtain information for functional leaf and root traits, biomass, and NSCs. During this process, leaf trait data were obtained using iPhone imaging for ImageJ, root trait data were collected using a desktop scanner to obtain 3D root scanned images for Rhizovision analysis (Seethepalli and York 2020), and fresh and dry biomass values were collected using a scale for leaves, stems, and roots. Phenology data were obtained by conducting weekly seedling counts. During these counts, all seedlings were examined to designate the development stage (emerged, establishment stage 1 (cotyledons surrounding needle bud), and establishment stage 2 (needle burst)). A family was considered as successfully established if the family had an establishment rate of > 50% at nine months post-sowing (Overton *et al.* 2016). For drought resistance data, NSC samples were obtained post-destructive harvesting by following the Ulrich Lab protocol (adapted from Landhäusser *et al.* 2018).

Measures of Success

These measurements will be used to obtain information that is paramount for outplanting families that will succeed in the face of WPBR and climate change. Specific measures of success include ranking all families (1-50) (objective 1), USFS seed zones (1-8) and BCFS locations (1-5)(objective 2) for establishment success rate. Using statistical testing, the average date post-sowing for reaching the emergence and establishment stages will be obtained for each family (objective 3) and correlated with growth and drought resistance traits (objective 4). Lastly, a GIS model will be used to test for correlations between climate and successfully established families (objective 5). A small proportion of seedlings were harvested for NSC analysis as a cost-effective and training-efficient method for an MS thesis. Obtaining these measurements through a common garden study is one of the first steps towards identifying how climate change may impact WBP seedling growth traits and regeneration success in the field.

Literature Cited

Allen, CD, AK Macalady, H Chenchouni, D Bachelet, N McDowell, M Vennetier, T Kitzberger, A Rigling, DD Breshears, EH Hogg, P Gonzalez, R Fensham, Z Zhang, J Castro, N Demidova, J-H Lim, G Allard, SW Running, A Semerci, and N Cobb. 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. Forest Ecology and Management 259(4):660-684. https://doi.org/10.1016/j.foreco.2009.09.001

Brodersen, CR, MJ Germino, DM Johnson, K Reinhardt, WK Smith, LM Resler, MY Bader, A Sala, LM Kueppers, and G Broll. 2019. Seedling survival at timberline is critical to conifer mountain forest elevation and extent. Frontiers in Forests and Global Change 9. https://doi.org/10.3389/ffgc.2019.00009

Cartwright, C, RA Sniezko, M Murray, and I Reid. 2022. Whitebark pine genecology field trials in British Columbia: Age 5 results. Forest Ecology and Management 521:120419. https://doi.org/10.1016/j.foreco.2022.120419

Chmura, DJ., PD Anderson, GT Howe, CA Harrington, JE Halofsky, DL Peterson, DC Shaw,D. C., & JB St. Clair. 2011. Forest responses to climate change in the northwestern United States: Ecophysiological foundations for adaptive management. Forest Ecology and Management 261(7):1121-1142. https://doi.org/10.1016/j.foreco.2010.12.040

Landhäusser, SM, PS Chow, LT Dickman, ME Furze, I Kuhlman, S Schmid, J Wiesenbauer, B Wild, G Gleixner, H Hartmann, G Hoch, NG McDowell, AD Richardson, ARichter, HD Adams. 2018. Standardized protocols and procedures can precisely and accurately quantify non-structural carbohydrates. Tree Physiology 38(12):1764-1778. https://doi.org/10.1093/treephys/tpy118

McLane, SC, and SN Aitken. 2012. Whitebark pine (*Pinus albicaulis*) assisted migration potential: Testing establishment north of the species range. Ecological Applications 22(1):142-153. https://doi.org/10.1890/11-0329.1

Overton, EC, J Park, N Robertson, and A Eramian. 2016. Current practices for growing whitebark pine seedlings at the U.S. Department of Agriculture, Forest Service, Coeur d'Alene Nursery - Reforestation, Nurseries and Genetics Resources. Tree Planters' Notes 59(1):64-68. https://rngr.net/publications/tpn/59-1/current-practices-for-growing-whitebark-pine-seedlings-at-the-u.s.-department-of-agriculture-forest-service-coeur-d2019alene-nursery

Schneider, CA, WS Rasband, and KW Eliceiri. 2012. NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9(7):671-675. doi:10.1038/nmeth.2089

Seethepalli, A, and LM York. 2020. RhizoVision Explorer - Interactive software for generalized root image analysis designed for everyone (Version 2.0.3). Zenodo. http://doi.org/10.5281/zenodo.5121845

Smithers, BV, and MP North. 2020. Mechanisms of species range shift: Germination and early survival of Great Basin bristlecone pine and limber pine. Plant and Soil 457(1):167-183. https://doi.org/10.1007/s11104-020-04732-9

Tomback, DF, SF Arno, and RE Keane. 2001. Whitebark Pine Communities: Ecology and Restoration. Island Press.

Tomback, DF, and E Sprague. 2022. The National Whitebark Pine Restoration Plan: Restoration model for the high elevation five-needle white pines. Forest Ecology and Management 521:120204. https://doi.org/10.1016/j.foreco.2022.120204