WPEF student research grant 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 that* ***LOU DULOISY****, a PhD student with Dr. Danielle Ulrich of the Faculty of Biological Sciences at Montana State University, was chosen as this year's grant recipient.*

Evaluating physiological differences of closely related high-elevation five needle pines: *Pinus albicaulis* and *Pinus flexilis*

Background and Objectives

Whitebark pine (*Pinus albicaulis*: WBP*)*, is considered a keystone and foundational species in the Greater Yellowstone Ecosystem (GYE) and was recently listed as a threatened species under the Endangered Species Act. WBP thrives at the subalpine tree line and provides vital protection for other species, due to its tolerance of cold, dry, windy conditions. The large and nutritious seeds of WBP supply valuable food resources for wildlife, including the Clark's Nutcracker (*Nucifraga columbiana*) and Grizzly Bear (*Ursus arctos horribilis*) (Tomback *et al*. 2001). Previous research found that Grizzly Bear survival in the GYE is strongly linked to seed availability and production (Felicetti *et al*. 2003). WBP also provides vital watershed protection by regulating snowmelt runoff and soil erosion (Keen *et al*. 2020). Given their ecosystem services, WBP has a disproportionally large impact on ecosystem health. Limber pine (*Pinus flexilis*: LP), an early successional species, is tolerant of warm and dry conditions and generally occupies the lower tree line ecosystem. Although LP is a drought tolerant and shade intolerant species, it forms open canopy stands unlike the tree islands formed by WBP *(*Letts *et al*. 2009, Webster and Johnson, 2000). Despite these differences, WBP and LP can occupy similar geographic ranges in the northern Rocky Mountains. Current research predicts climate change will continue to affect both species (Hansen *et al*. 2021), though the underlying physiological mechanism are not fully understood.

WBP and LP differ by seed cone; identification of non-cone bearing trees is difficult and the lack of effective techniques hinders research in areas where both species are present (Alongi *et al*. 2019, Baumeister and Callaway 2006). Current species distribution models assume that WBP does not inhabit the warm and dry conditions at the lower tree line where LP is present. However, future climate scenarios fail to include juvenile trees, and previous research shows that juvenile WBP are able to regenerate in warmer and drier environments than their adult counterparts (Buermeyer *et al*. 2016).

The frequency, intensity, and duration of severe droughts and heat waves are projected to increase, affecting tree growth and survival, and increasing tree mortality (Allen *et al*. 2010, 2015). WBP has experienced decline due to the changing climate, mountain pine beetle (*Dendroctonus ponderosae*), blister rust (*Cronartium ribicola*), and fire exclusion (Brar *et al*. 2015, Macfarlane *et al*. 2013). As a result of the challenges associated with WBP and LP identification, limited research has been conducted examining the physiology of both species. This lack of research limits our ability to conserve and manage these forest ecosystems, considering changing climate. Understanding how physiological mechanisms contribute to species' range limits is important for assessing WBP and LP responses to a changing environment. Inherently, increasing our knowledge of the physiological mechanisms of both WBP and LP is critical to management of ecologically sustainable forests.

Given the importance of conserving forests, specifically WBP, a multi-proxy research approach is needed. To quantify physiological mechanisms in WBP and LP, we plan to measure gas exchange and water relations (photosynthesis, respiration, and transpiration) with *in situ* methods. However, *in situ* physiological measurements of plant responses to the environment cannot be applied retrospectively

(Siegwolf *et al*. 2022). As such, tree-ring carbon stable isotopes (δ13C) are crucial for understanding tree physiological responses over temporal and spatial scales (Cernusak and Ubierna, 2022). Additionally, a critical factor in understanding tree physiology is assessing the storage and allocation of carbon, by measuring non-structural carbohydrates (NSCs) in addition to gas exchange and δ13C (Sevanto *et al*. 2014, Cho *et al*. 2022). The objective of our study is to better understand WBP and LP physiology over a temporal scale to address the following questions: 1) Do seasonal patterns of leaf-level photosynthesis, respiration, and transpiration differ between WBP and LP*?* 2) Do tree-ring stable isotopes (δ13C) differ between WBP and LP*?* 3) Do seasonal patterns of NSCs differ between WBP and LP*?*

Study plans

To conduct this research, we will be visiting three sites located in the GYE. Our sites are previously established, with genetically identified WBP and LP (Alongi *et al*. 2019, Hansen *et al*. 2021). Each site will be visited once monthly to gather seasonal physiology data. At each site, we will measure photosynthetic rate and gas exchange (stomatal conductance) using a portable photosynthesis system from LICOR Biosciences (LI-6800) and water potential using a portable pressure chamber (PMS). Additionally, we will collect samples from all genetically identified trees, and all tissues (needles, branches, stems and roots) to measure NSC content throughout the duration of the study. We will measure both total NSC content, as well as patterns of allocation to different tissues. Once throughout the study, we will collect tree cores. Two 12-mm cores (20 cm depth) will be taken to quantify recent tree-ring radial growth and tree-ring δ13C. These cores will be cross-dated, and we will measure tree ring widths. Then, the last 50 years/rings of growth will be separated from each tree core using a razor blade. From each ring, alpha-cellulose will be isolated using a standard bleaching protocol (Rinne *et al*. 2005). The alpha-cellulose will be weighed and packaged in tin cups at Montana State University and analyzed for δ13C, using an Isotope Ratio Mass.

Measures of success

This study will improve our understanding of seasonal and long-term physiology of two cohabitating species, WBP and LP. The data collected will allow scientists and managers to establish a stronger understanding of WBP and LP physiology and prioritizes adaptive management in our changing climate. Due to WBP being a keystone and foundational species, management of WBP and LP forests is crucial for preserving valuable food resources, watershed protection, and species protection.

Literature Cited

Allen, CD, DD Breshears, and NG McDowell. 2015. On underestimation of global vulnerability to tree mortality and forest die-off from hotter drought in the Anthropocene. Ecosphere 6(8):art129. https://doi.org/10.1890/ES15-00203.1

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

Alongi, F, AJ Hansen, D Laufenberg, RE Keane, K Legg, and M Lavin. 2019. An economical approach to distinguish genetically needles of Limber from Whitebark Pine. Forests 10(12):12. https://doi.org/10.3390/f10121060

Baumeister, D, and RM Callaway. 2006. Facilitation by *Pinus flexilis* during succession: A hierarchy of mechanisms benefits other plant species. Ecology 87(7):1816-1830. https://doi.org/10.1890/0012-9658(2006)87[1816:FBPFDS]2.0.CO;2

Brar, S, CKM Tsui, B Dhillon, M-J Bergeron, DL Joly, PJ Zambino, YA El-Kassaby, and RC Hamelin. 2015. Colonization history, host distribution, anthropogenic influence and landscape features shape populations of white pine blister rust, an invasive alien tree pathogen. PLOS ONE 10(5):e0127916. https://doi.org/10.1371/journal.pone.0127916

Buermeyer, K, D Reinhart, and K Legg. 2016. Case study: Whitebark pine in the Greater Yellowstone Ecosystem. In Climate Change in Wildlands: Pioneering Approaches to Science and Management, AJ Hansen, WB. Monahan, ST Olliff, and DM Theobald, editors, pp. 304-326. Island Press/Center for Resource Economics. https://doi.org/10.5822/978-1-61091-713-1_15

Cernusak, LA, and N Ubierna. 2022. Carbon isotope effects in relation to CO_2 assimilation by tree canopies. In Stable Isotopes in Tree Rings: Inferring Physiological, Climatic and Environmental Responses, RTW Siegwolf, J Brooks, J Renée, J Roden, and M Saurer, editors. Tree Physiology 8:291-305. https://researchonline.jcu.edu.au/74837/7/74837.pdf

Cho, N, C Agossou, E Kim, J-H Lim, T Hwang, and S Kang. 2022. Recent field findings and modeling on non-structural carbohydrates (NSCs): How to synthesize? Ecological Informatics 70:101695. https://doi.org/10.1016/j.ecoinf.2022.101695

Felicetti, LA, CC Schwartz, RO Rye, MA Haroldson, KA Gunther, DL Phillips, and CT Robbins. 2003. Use of sulfur and nitrogen stable isotopes to determine the importance of whitebark pine nuts to Yellowstone grizzly bears. Canadian Journal of Zoology 81(5):763-770. https://doi.org/10.1139/z03-054

Hansen, AJ, A East, RE Keane, M Lavin, K Legg, Z Holden, C Toney, and F Alongi. 2021. Is whitebark pine less sensitive to climate warming when climate tolerances of juveniles are considered? Forest Ecology and Management 493:119221. https://doi.org/10.1016/j.foreco.2021.119221

Keen, RM., SL Voelker, BJ Bentz, S-YS Wang, and R Ferrell. 2020. Stronger influence of growth rate than severity of drought stress on mortality of large ponderosa pines during the 2012–2015 California drought. Oecologia 194(3):359-370. https://doi.org/10.1007/s00442-020-04771-0

Letts, MG, KN Nakonechny, KE Van Gaalen, and CM Smith. 2009. Physiological acclimation of *Pinus flexilis* to drought stress on contrasting slope aspects in Waterton Lakes National Park, Alberta, Canada. Canadian Journal of Forest Research 39(3):629- 641. https://doi.org/10.1139/X08-206

Macfarlane, WW, JA Logan, and WR Kern. 2013. An innovative aerial assessment of Greater Yellowstone Ecosystem mountain pine beetle-caused whitebark pine mortality. Ecological Applications 23(2):421-437. https://doi.org/10.1890/11-1982.1

Rinne, KT, T Boettger, NJ Loader, I Robertson, VR Switsur, and JS Waterhouse. 2005. On the purification of α-cellulose from resinous wood for stable isotope (H, C and O) analysis. Chemical Geology 222(1):75-82. https://doi.org/10.1016/j.chemgeo.2005.06.010

Sevanto, S, NG Mcdowell, LT Dickman, R Pangle, and WT Pockman. 2014. How do trees die? A test of the hydraulic failure and carbon starvation hypotheses. Plant, Cell & Environment 37(1):153-161. https://doi.org/10.1111/pce.12141

Siegwolf, R, M Lehmann, G Goldsmith, OC Churakova (Sidorova), C Mirande-Ney, G Timofeeva, R Weigt, and M Saurer. 2022. Updating the dual C and O isotope – gas exchange model: A concept to understand plant responses to the environment and its implications for tree rings [Preprint]. https://doi.org/10.22541/au.166862167.73927217/v1

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

Webster, KL, and EA Johnson. 2000. The importance of regional dynamics in local populations of limber pine (*Pinus flexilis*). Écoscience 7(2):175-182. https://doi.org/10.1080/11956860.2