

AN ABSTRACT OF THE THESIS OF

Jamie E. Mosel for the degree of Master of Science in Forest Ecosystems and Society presented on December 12, 2016.

Title: Physiological Responses of Loblolly pine and Douglas-fir Seedlings from Various Provenances to Timing and Frequency of Drought Stress.

Abstract approved:

Barbara Lachenbruch

Drought is expected to increase in many parts of the world and has been shown to affect tree physiology and growth, with seedlings being particularly vulnerable. Seedling drought responses are often species dependent, and even within species, different populations may demonstrate a spectrum of responses to drought, from susceptibility to resistance. As both loblolly pine (*Pinus taeda*) and Douglas-fir (*Pseudotsuga menziesii*) have broad geographic ranges, they provide an opportunity to assess drought resistances of provenances associated with a variety of site climate conditions across their ranges.

Furthermore, while studies of singular drought events have revealed important information about seedling stress responses, it has been shown that drought may impart effects even following the release from drought. In some cases, effects of drought exposure can be detrimental or lethal; however, exposure to non-lethal drought may also lead to drought acclimation, which could potentially enhance seedling functioning under subsequent droughts. This potential phenotypic plasticity, i.e. the extent to which a seedling can acclimate to drought conditions, may vary across species and across provenances within a species.

This study used physiological (electron transport rate, fluorescence, and water potentials) and growth (biomass accumulation and height increment) responses for two goals: 1) to assess the drought resistances of three provenances of loblolly pine and three provenances of Douglas-fir associated with varying site climate conditions, and 2) to investigate whether previous exposure to a drought results in acclimation to a second drought. We hypothesized that: 1) drought responses would differ among the provenances in accordance with associated site climate

conditions (i.e., provenances associated with mesic site climates as characterized by low climatic moisture deficit and high mean annual precipitation would be drought susceptible, and more xeric site climates as characterized by high climatic moisture deficit and low mean annual precipitation would be more drought resistant) as evidenced by physiological measures of electron transport rate, fluorescence, water potentials and growth; and 2) previous exposure to drought would result in acclimation to drought as evidenced by maintenance of physiological function (i.e., higher levels of electron transport rate and fluorescence) in previously drought exposed seedlings compared to previously unexposed seedlings. We also hypothesized that levels of acclimation would vary among provenances.

The study yielded some evidence to support the first hypothesis regarding provenance differences in drought resistances in both species. Provenance drought resistances conformed largely to expectations, though differences were less than expected. In loblolly pine, although not statistically significant, during the second drought there was a pattern of lower maximum electron transport rates, which appeared sooner in the more mesic provenance than in the other two provenances. There were also provenance differences in seedling heights, with the most xeric of the three provenances being shorter at the beginning and the end of the study. In Douglas-fir, there were significant differences in provenance and in the interaction of treatment and provenance for maximum electron rates and fluorescence. Dark-adapted fluorescence was lower in the Coos Bay (mesic site climate) provenance during drought than in the Cascades (mesic site climate) and New Mexico (xeric site climate) provenances. The New Mexico provenance showed the least differences in fluorescence between droughted and watered treatments.

The study also yielded some evidence to support the second hypothesis regarding drought acclimation in both species and among provenances. During the second drought, there was a pattern of higher maximum electron transport rates and fluorescence in previously drought exposed seedlings as compared to seedlings previously unexposed to drought. This difference was significant in the mesic provenance (North Carolina) of loblolly pine, with maximum electron transport rates significantly higher in the previously drought exposed treatment compared to the newly exposed treatment during and following the second drought. Patterns of lower electron transport rates in seedlings previously unexposed to drought compared to

seedlings previously exposed to drought also appeared in Douglas-fir during the second drought, though not with statistical significance. However, the most xeric provenance showed the reverse pattern during and following the second drought, with lower maximum electron transport rates in the previously drought exposed treatment compared to the treatment previously unexposed to drought. Chlorophyll fluorescence values were significantly higher during the second drought in the previously droughted treatments compared to newly drought-exposed treatments in some provenances of loblolly pine and Douglas-fir. Lastly, seedlings exposed to an early drought had significantly lower final heights than seedlings unexposed to an early drought in both species, although the differences were greater in loblolly pine than Douglas-fir. It may be that growth acclimation, especially in the form of height reductions, influenced physiological responses during a second drought. Further studies are necessary to provide more conclusive evidence in support or against the two hypotheses. Nonetheless, this study provides valuable information on the drought responses of young, greenhouse-grown seedlings of two species that are widespread in North America and that are economically important throughout the world. Further studies in a wider range of age classes, incorporating field studies or more natural settings, may help better predict plant responses in the face of changing climate.

Copyright by Jamie E. Mosel
December 12, 2016
All Rights Reserved

Physiological Responses of Loblolly Pine and Douglas-fir Seedlings from Various Provenances
to Timing and Frequency of Drought Stress

by
Jamie E. Mosel

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Presented December 12, 2016
Commencement June 2017

Master of Science thesis of Jamie E. Mosel presented on December 12, 2016

APPROVED:

Major Professor, representing Forest Ecosystems and Society

Head of the Department of Forest Ecosystems and Society

Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Jamie E. Mosel, Author

ACKNOWLEDGEMENTS

I would like to sincerely thank my major professor, Barb Lachenbruch, as well as my committee members Rick Meinzer, Chris Still, Dave Woodruff, and Andy Jones for their help, time, patience, and support. I would also like to thank Weyerhaeuser Co, who made this research possible through their generosity. In particular, I would like to thank Edgar Fuchs for always providing encouragement, kind words, mentorship, and immense help. I would also like to thank Bob Purnell, Pam Nielsen, Dave Marshall, and Greg Johnson, all of whom provided assistance and support. I would like to thank Mike Lopez, Shannon Dunfee, and Kyler Jacobo for their help in the greenhouse, and Shane Wilson for help with microscopy. I would like to thank Lisa Ganio for her continual support and kindness. I would also like to thank Ariel Muldoon for her continual guidance in statistics and for all of her patience. I would also like to thank Jessica Bagley for all of her help and advice.

I would like to profoundly thank Gail Woodside for her friendship, dedication, and wisdom. Gail has instilled and nurtured courage in me, and her commitment to justice and goodness continues to inspire me. I cherish the gift of her love and guidance. I would like to thank Dean Thomas Maness, Troy Hall, Randy Rosenberger and the College of Forestry for their willingness to pursue important issues of cultural inclusion. In particular, I thank Thomas for the support he has shown in the inclusion of Indigenous voices, a commitment that is of utmost importance. There are many friends who I would like to thank, and who have helped to support me along the way. I would especially like to thank Randi Shaw for her depth of strength, character, and love. I would also like to thank my fellow labmates and good friends Sarah Greenleaf – who has always been kind and loving in encouraging me forward – as well as Elise Heffernan. I would like to thank the many individuals and leaders of the Diverse Perspectives in Forestry Group, who have formed a foundation of community, commitment, and positive energy. Such work is vital in addressing issues of diversity, equity, and inclusion. I would also like to thank my family: my mom Laurie, my dad Jim, my sister Maddie, and my little brother Paul. I especially thank my father and my sister, for their love and patience despite the number of times I have persuaded them to help me with measurements. I would also like to thank my aunt Diane without whom I could not have made it to Oregon, and my aunt Lisa, without whom I would not have made it to the Pacific Northwest. I would like to thank the Richardson family, the Waring

family, and the Gruetter family for their generous support. Thank you to everyone who has helped me along the way.

Finally, I would like to acknowledge and deeply thank the Nine Tribes of Oregon, and especially the Kalapuya peoples, on whose land this university sits. With love and gratitude, I dedicate this work to the land, to its long history and to its future, and to all the many relations who live upon it.

CONTRIBUTION OF AUTHORS

Chapter 2: Jamie Mosel designed, carried out, analyzed, and wrote the thesis. Barbara Lachenbruch, Frederick C. Meinzer, Christopher Still, and David Woodruff provided guidance on design, data collection, analysis, interpretation, writing, and editing. Edgar Fuchs provided guidance on design, data collection, and analysis, as well as assistance in seedling transplanting and transport.

TABLE OF CONTENTS

	<u>Page</u>
1. Introduction.....	2
Introduction to Drought in Forests at the Stand Level.....	3
Overview of Water Stress Responses.....	4
Effects of Drought on Carbon Economy and Plant Temperature.....	8
Effects of Drought on Growth, Morphology, and Anatomy.....	10
Drought Effects Differ for Seedlings and Mature Trees.....	12
Drought Resistance.....	14
Variation, Acclimation and Plasticity.....	15
Experimental Approach and Limitations.....	18
Overview of Research Questions.....	21
References.....	22
 2. Physiological responses of loblolly pine and Douglas-fir seedlings from varying provenances to timing and frequency of drought stress.....	 32
Introduction	33
Methods	38
Plant Material	38
Study Design	42
Environmental Monitoring	43
Drought Treatments.....	44
Physiological Measurements	45
Predawn and Midday Water Potentials	45
Fluorescence (F_v/F_m) and Electron Transport Rate.....	45
Growth Measurements.....	47
Statistical Analysis	48
Results	48
General Observations on Foliar Coloration and Wilt.....	49
Water Potentials.....	49

TABLE OF CONTENTS (Continued)

	<u>Page</u>
Electron Transport Rates	51
Fluorescence (F_v/F_m)	54
Biomass	56
Heights	59
Discussion	60
Conclusions.....	71
References.....	74
Tables.....	82
Figures.....	89
3. Conclusions	100
References.....	107
Appendices.....	109
Appendix A.....	110
Appendix B.....	143
Appendix C.....	153

LIST OF TABLES

<u>Table</u>	<u>Page</u>
1. Loblolly pine (<i>Pinus taeda</i>) and Douglas-fir (<i>Pseudotsuga menziesii</i>) provenances and site climate characteristics.....	82
2. Loblolly pine average predawn and midday leaf water potentials (Ψ), summarized for all droughts and treatments by provenance.....	83
3. Douglas-fir average predawn and midday leaf water potentials (Ψ), summarized for all droughts and treatments by provenance.	84
4. Statistical analyses (ANOVA F-Tests) for loblolly pine (<i>Pinus taeda</i>) maximum electron transport rates (ETR_{max}).....	85
5. Statistical analyses (ANOVA F-tests) for Douglas-fir (<i>Pseudotsuga menziesii</i>) maximum electron transport rates (ETR_{max}).....	86
6. Pairwise comparisons for loblolly pine (<i>Pinus taeda</i>) maximum electron transport rates (ETR_{max}).....	87
7. Pairwise comparisons for Douglas-fir (<i>Pseudotsuga menziesii</i>) maximum electron transport rates (ETR_{max}).....	88

LIST OF FIGURES

<u>Figure</u>	<u>Page</u>
1. Timeline of dates of drought treatments for loblolly pine and Douglas-fir during the summer of 2015.....	89
2. Seedling needle water potentials (Ψ) at the end of the first drought (D1) and the second drought (D2).....	90
3. Rapid light curves showing electron transport rates (ETR) vs light level (PAR) for loblolly pine seedlings of the three provenances.....	91
4. Rapid light curves showing electron transport rates (ETR) vs light level (PAR) for Douglas-fir seedlings of the three provenances.....	92
5. Dark-adapted fluorescence (F_v/F_m) of loblolly pine seedlings (A-D) by treatment for each provenance measured mid-drought (27 Sept) during the second drought (n = 7) and of Douglas-fir seedlings (E-H) by treatment for each provenance at the end (8 Oct) of the second drought (n = 5).....	93
6. Loblolly pine total overall biomass (A-D) and root/shoot ratio (E-H) at the end of the second drought for each of the provenances and provenances pooled.....	94
7. Loblolly pine needle (top), root (middle), and stem (bottom) biomass at the end of the second drought (n = 7).....	95
8. Relative growth rates of loblolly pine (A-D) and Douglas-fir (E-G).....	96
9. Douglas-fir total overall biomass (A-D) and root/shoot ratio (E-H) at the end of the second drought for each of the provenances and provenances pooled.....	97
10. Douglas-fir needle (top), root (middle), and stem (bottom) biomass at the end of the second drought (n = 5).....	98
11. Heights of loblolly pine heights (A) and Douglas-fir heights (B) of the three provenances measured through the summer of 2015.....	99

Physiological responses of loblolly pine and Douglas-fir seedlings from various provenances to timing and frequency of drought stress

1. INTRODUCTION

Introduction to Drought in Forests at the Stand Level

Climate change is predicted to influence forests in a variety of ways, with anticipated increases in heat waves, fire, drought, insect or pathogen outbreaks, and extreme weather (Dale et al. 2001). Drought, which is typically a combination of heat and water stress caused by a prolonged period of moisture deficiency relative to local normal conditions (Palmer 1965, Dai et al. 2004, Dai 2011), can have many effects on trees. Seasonal and even multi-year droughts and aridity are characteristic of some regions, and often a determining factor in tree species distributions (Engelbrecht et al. 2007). However, drought associated with climate change is anticipated to increase in frequency and intensity in many parts of the world (IPCC 2007, Dai 2011, 2013). Drought effects associated with climate change, such as forest dieback, reductions in growth, reductions in tree vigor, and increases in tree mortality, have already been observed in trees and forests across the globe (Breshears et al. 2005, Allen et al. 2010). In the United States, consequences of climate change-associated drought for tree and forest health are likely to vary by region (Hanson and Weltzin 2000). In the western United States, increases in background rates of tree mortality in coniferous species and across multiple age classes, sites, and life histories are associated with water deficits connected to regional warming (van Mantgem and Stephenson 2007, van Mantgem et al. 2009). In the same studies by van Mantgem et al., modeled rates of recruitment were lower than mortality rates. Even in the generally wetter southeastern U.S., *Pinus* species have shown decreases in growth rates and increases in mean relative mortality rates with increasing drought severity (Klos et al. 2009).

Understanding how drought stress affects trees and forests is of great importance in the face of climate change. Tree mortality and reductions in tree growth, vigor, or health may alter forest compositions or ranges, alter local microclimates, and may also have consequences for the sustainability of natural resources. Seedlings may be particularly vulnerable to abiotic stresses

such as drought (Grossnickle 2012), and are vital for forest regeneration. Moreover, disturbances and abiotic and biotic stressors often have interactive effects (Anderegg et al. 2015). Although the focus of this study is on the water-stress component of drought, water-stress is often aggravated by heat stress, and can predispose trees to disease or other stressors (Adams et al. 2009, Niinemets 2010).

Overview of Water Stress Responses

Water stress, often driven by drought, can pose a major abiotic stress for terrestrial plants, and has often resulted in morphological and physiological adaptations. Woody plants such as trees have evolved to stand freely and often grow to great heights and must therefore be able to transport large amounts of water long distances. Rates of water use in trees can range from 10 kg day⁻¹ in trees in a *Quercus petraea* plantation to as much as 1,180 kg day⁻¹ in a *Eperua purpurea* tree in the Amazon, with typical maximum rates ranging between 10 and 200 kg day⁻¹ for trees averaging 21 m in height (Wullschleger et al. 1998).

Water transport through the xylem is driven by transpiration, and in particular by the difference in water potential between the atmosphere and the substomatal cavity (Tyree and Ewers 1991, Manzoni et al. 2013). Stomata, located on the leaf, are the primary sites of gas exchange between the plant and the atmosphere, and are key in the regulation of water loss and water transport (Sperry et al. 2002). When there is high vapor pressure deficit (VPD), transpiration is increased if stomatal conductance is not proportionately reduced. When water potentials are near zero at the plant-atmosphere interface, the driving force for water movement through the soil-plant-air continuum and consequently through the xylem is also low. At very negative water potentials (an indication of the amount of tension of the water column) such as occur under dry soil conditions, there is increasing risk of the formation of air bubbles known as

embolisms in the xylem conduits (Tyree and Sperry 1988, Manzoni et al. 2013). These embolisms can lead to breaks in the water column in the xylem, sometimes resulting in hydraulic failure: the accumulation of embolisms and hydraulic failure can lead to shedding of foliage or tissue, and more severely to death or dieback (Urli et al. 2013).

Transpiration is closely linked to CO₂ uptake. When stomata are open, water is lost as CO₂ enters the leaf, with water loss generally increasing with widening stomatal aperture. Stomatal opening and closing results from changes in the turgor pressure of the guard cells and subsidiary cells surrounding the stomata. Under plant water deficits, guard cells become increasingly flaccid, leading to stomatal closure. Closure may result through desiccation, and also thorough regulation of osmotic solutes and the accumulation of abscisic acid (Hsiao 1973, Kozlowski and Pallardy 2002). Plants must balance the need to supply water to maintain functions such as photosynthesis with the need to conserve water to minimize risk of embolism (Brodribb 2009). The concepts of anisohydry and isohydry are helpful to describe the stomatal responses to water availability and the suite of other associated traits. Most tree species fall along a spectrum of anisohydry and isohydry rather than strictly within one category (Klein 2014). Species towards the anisohydric end typically continue to transpire under relatively negative water potentials. Isohydric species, on the other hand, close stomata at relatively moderate water potentials (Brodribb et al. 2014). Another concept to characterize the regulation of water transport is related to the relationship between midday and predawn leaf water potentials. With this approach it is possible to consider the plant's sensitivity to soil moisture in the context of the vulnerability of the hydraulic system of the plant (Martínez-Vilalta et al. 2014, Meinzer et al. 2016). Metrics such as P50, the water potential at which 50% of the xylem's conductivity has been lost, are related to the safety margin at which a plant is operating, and can be represented by a vulnerability curve (Johnson et al. 2012, Manzoni et al. 2013, Meinzer and McCulloh 2013). In

fact, many species operate near their hydraulic safety margins, such that increasing drought and temperature may have particularly extensive effects across arid and even wet forest types (Choat et al. 2012). More recently, the use of a ‘hydroscape’ has also been proposed as a means to quantify stomatal control of plant water status in drying soil conditions, which incorporates the water potentials where stomata maintain control of water potential and slope of the daily range of water potential with declining predawn water potential (Martínez-Vilalta et al. 2014, Meinzer et al. 2016).

Water stress can affect the overall ability of the xylem to deliver water to the leaves in many ways, including by triggering changes in stomatal behavior, and by inducing formation of embolisms in the xylem. If an individual of a species is relatively anisohydric, as water potentials decrease, it may continue to photosynthesize, thus losing water through transpiration, while risking embolism or hydraulic failure. In contrast, species towards the isohydric end of the spectrum may close stomata in conditions that threaten water stress before reaching water potentials that would cause significant decreases in water transport through xylem embolism. Other responses to water stress include drought avoiding strategies, such as induction of dormancy, dropping of leaves, or behavior that avoids transpiration in the driest or hottest part of the day or season. While dormancy may help protect the tree against water stress, it also generally results in slower growth which may pose a competitive disadvantage. Similarly, extreme drought adaptations may come at the expense of growth (Jansen et al. 2013). Drought stress can also affect turgor. Loss of turgor during drought can be countered in some species through osmotic adjustment, in which solutes such as sorbitol, glucose, fructose, malic acid, or potassium are transported in greater concentration into living turgor-maintained cells. Interestingly, osmotic adjustment is often lessened in the sudden onset of drought as opposed to a gradual onset of drought (Kozlowski and Pallardy 2002).

These strategies reduce risk of embolism, although embolisms still occur. In most coniferous species, tracheid bordered pits serve as valves that trap embolisms within individual tracheids, within a range of pressure differentials across these pits, preventing or slowing the spread of embolisms in the first place (Hacke and Jansen 2009). When an air-seeded bubble expands to fill a tracheid, the internal pressure approaches 0 MPa, whereas the water in adjacent tracheids has a value < 0 MPa, causing the pit membrane to be pushed from the gas-filled tracheid. The impermeable center of the membrane, the torus, blocks the aperture of the pit border. This same action occurs in all the bordered pits within that tracheid, thus isolating the embolism (Petty 1972).

These strategies for decreased xylem stress or for trapping embolisms decrease embolism, but most trees operate with a certain degree of embolism without decreasing performance (McCully et al. 1998, Johnson et al. 2012, Meinzer and McCulloh 2013). When embolism does occur, there is also evidence that some species can re-fill embolisms on a nightly or seasonal basis. To re-fill embolisms, it is thought that trees transport sugars from xylem parenchyma or phloem to the locations of embolism, allowing water to passively follow due to the gradient in osmotic potential (Secchi et al. 2012). Xylem parenchyma cells are therefore involved in transport between phloem and xylem, as well as osmotic storage capacity and water storage capacity, and are likely involved in xylem refilling and embolism removal (Secchi et al. 2016).

There are hypothesized to be two primary causes of mortality in situations of water stress such as caused by drought: hydraulic failure and carbon starvation (McDowell et al. 2008, Urli et al. 2013, Sevanto et al. 2014). In many cases, it is likely that mortality is driven by both causes. Carbon starvation is closely related to water transport, but also to photosynthesis and to the

storage of non-structural carbohydrates such as sugars and starch. Hydraulic failure and carbon starvation can also interact with biotic stresses such as insects and pathogens.

Effects of Drought on Carbon Economy and Plant Temperature

Because transpiration is closely tied to carbon assimilation due to the shared pathway of water and CO₂ through the stomata, if water stress results in stomatal closure, it will affect photosynthetic activity. Stomata in many species are highly sensitive to soil water potentials. Water stress is also connected to decreases in photophosphorylation and thereby decreasing regeneration of Ribulose-1,5-bisphosphate (RuBP) (Tezara et al. 1999). As drought continues and diffusion of CO₂ into the stomata remains limited due to stomatal closure, over time the photosynthetic apparatus becomes down-regulated due to low carbon availability, with reductions in enzymes of the Calvin cycle, Rubisco maximum carboxylation capacity, and RuBP regeneration (Chaves et al. 2002, Flexas et al. 2006). In addition, during periods of drought, cells can sustain oxidative damage: reactive oxygen species are produced as a result of aerobic metabolic processes, and can accumulate under drought stress (Mittler 2002).

Water limitations can also be antagonistic to heat dissipation. Evaporative cooling via transpiration can dissipate as much as 25% of heat absorbed by leaves (Sage and Kubien 2007). The rapid flow of water through the seedling stem can reduce stem temperature by as much as 30 °C under modeled conditions, and typically as much as 15 °C in extreme observed conditions (Kolb and Robberecht 1996). However, the effectiveness of cooling via transpiration and rapid water flow depends largely on water availability. If stomata close due to stress or low soil moisture, leaves may not be able to regulate temperatures, even in non-heat wave periods, which can cause increases in leaf temperature with water stress (Ehrlir 1973, Sepulcre-Cantó et al. 2006). Increases of leaf temperature, thus, can be indicative of stomatal closure and decreases in

transpiration, and can indicate the onset of water stress (Tanner 1963, Gates 1964, Erhler 1973, Jackson et al. 1981). Even under moderate air temperatures, leaves exposed to direct sunlight may experience increased temperatures of many degrees C (Wiegand and Namken 1966, Jackson et al. 1981). In drought scenarios, which often (though not always) involve high temperatures, water stress may place trees at further risk of heat damage or contribute to mortality (Adams et al. 2009).

Water stress may also affect, or be potentially mitigated by amounts of non-structural carbohydrates reserves. Hoch et al. (2003) found deciduous tree species on average to have higher concentrations of non-structural carbohydrates (NSC) in their stem sapwood than in evergreen conifers. As discussed above, water stress typically decreases photosynthetic activity, which in turn reduces the ability of the plant to produce sugars. In that way, water stress may reduce inputs of available sugars via a reduction of photosynthesis. It may also reduce available sugars by inhibiting the ability of the plant to transport sugars in the phloem (Sala et al. 2010, Sevanto et al. 2014, Woodruff 2014).

During the early stages of stress, plants may increase allocation to storage carbohydrates, especially if growth declines faster than photosynthesis. NSCs accumulate or decline based on the carbon source-sink balance represented by photosynthesis versus respiration and growth (Hoch et al. 2003, Sala et al. 2010). However, when drought persists for long time periods, NSC reserves are gradually depleted because of continued respiration for cell maintenance and repair in the face of decreased photosynthesis. In a drought study of piñon pine (*Pinus edulis*), seedlings were shaded to promote carbon starvation, while other seedlings were provided full light but subjected to severe drought to promote hydraulic failure (Sevanto et al. 2014). The NSCs of the shaded seedlings declined, with no change in water potential until mortality. The

fast-droughted seedlings likely died from hydraulic failure, but had large NSC reserves remaining at mortality. The slow-droughted seedlings maintained conservative water potentials until the days prior to death at which point water potentials dropped indicating hydraulic failure, but also showed relatively depleted NSC. Seedlings that survived longest had lowest levels of NSCs at death. O'Brien et al. (2011) also found NSCs to contribute to drought survival in tree seedlings. Initial NSC content did not correlate with survival, but needle starch content did correlate with survival. The study by Sevanto et al. (2014) also suggests that, in the case of the slow-drought, seedlings likely utilized NSCs for osmoregulation to maintain relatively constant water potentials up until just before death. Phloem sap under water stress has also been shown to have higher viscosity when glucose and fructose concentrations are considered, which might further reduce phloem conductivity (Woodruff 2014).

Effects of Drought on Growth, Morphology, and Anatomy

Drought conditions generally result in growth reductions in many tree species (Myers and Landsberg 1989, Klos et al. 2009), with observations of reductions in gross primary productivity also associated with drought (Grier and Running 1977, Le Dantec et al. 2000, Law et al. 2002, Ciais et al. 2005, Bréda et al. 2006). Reductions in leaf area index (LAI), for example, have been observed inter-annually as a result of repeated drought incidents in temperate forests. That is, drought decreased LAI in the year following the drought. Drought has often been shown to lead to reductions in height and radial growth in seedlings (Bauweraerts et al. 2014). Reduced bud production in response to drought (Power 1994, Bréda et al. 2006) affects leaf number, surface area, and stem and branch extension in following years. In species that exhibit multiple flushes during the growing seasons, drought can limit flushes and further suppress growth relative to years with optimal conditions. In red pine (*Pinus resinosa*), shoot elongation in main stem terminals was found to be 40% greater in irrigated trees than trees experiencing drought, with

decreasing differences towards the base of the crown; average needle elongation was also around 40% greater in irrigated trees compared to drought trees, with an observed extension of needle elongation into September for irrigated trees as opposed to mid-August for droughted trees, and with buds smaller in droughted trees at the end of the season than in irrigated trees (Lotan and Zahner 1963). In loblolly pine (*Pinus taeda*), mid and late-summer droughts reduced intermodal flushes and stopped terminal growth as much as four weeks earlier than in watered trees (Zahner 1962). In loblolly pine seedlings, water availability had larger effects on stem height and diameter growth than the combined effects of high temperature and high CO₂ concentration (Bauweraerts et al. 2014). Additionally, drought can trigger increases in root biomass as a result of increased carbon allocation to roots compared to stem wood production (Teskey et al. 1987). However, depending on drought severity and factors such as species, site and soil-type, drought can also result in die-back of fine root tips, or even affect mycorrhizal associations (Valdés et al. 2005, Olesinki et al. 2011).

Wood formation and wood characteristics are also affected by drought. In a comparison of droughted vs. irrigated Scots pine (*Pinus sylvestris* L.) trees, drought stress shortened the period of wood formation by about one month, resulted in smaller radial increments, and a two to four week earlier transition from early- to latewood (Eilmann et al. 2011). The same study reported wider, rather than narrower earlywood lumen diameters in the droughted Scots tree, but decreases in tracheid diameters have been widely observed in response to water deficits or xeric growing conditions, often along thicker cell walls (Bréda et al. 2006, Sheriff and Whitehead 2006). Narrower tracheids generally lead to low hydraulic conductivity and are generally associated with higher resistance to embolism (Hacke and Sperry 2001). For example, earlywood radial widths in Scots pine have also been found to be significantly narrower in trees from xeric sites (Gruber et al. 2010). As such, there is often a positive relationship between wood density

and drought resistance, though this relationship does not always hold (Bréda et al. 2006, Lachenbruch and McCulloh 2014). There is also some evidence that drought may disrupt the lignification process of cells that are developing at the time of drought in conifers. This may lead to zones of a growth ring that lack or have very low lignin content (Donaldson 2002, Piermattei et al. 2015).

Drought Effects Differ for Seedlings and Mature Trees

Throughout growth and development, stresses can affect trees in different ways (Niinemets et al. 2010, Hinckley et al. 2011). Seedlings are especially vulnerable to abiotic stresses such as drought compared to mature trees, and often show high mortality rates (Minore 1986, Cui and Smith 1991, Shevtsova et al. 2009, Grossnickle 2012). Moreover, seedlings also often occupy different microclimates than older trees. These microclimate differences can include increased temperatures with relatively rapid fluctuation at the soil surface, with surface and sun-exposed temperatures sometimes reaching lethal and extreme levels (Kolb and Robberecht 1996), in addition to larger fluctuations in available soil moisture .

Compared to seedlings, mature trees have larger, deeper root systems that can access sources of water that are less prone to drying and rapid heating (Dawson 1996). Because of their size, mature trees also have higher internal water storage capacity than seedlings. This stored water can help support daily water demands (Waring and Running 1978, Tyree and Yang 1990, Phillips et al. 2003), and can be especially important during periods of drought, allowing stomata to open and photosynthesis to occur. Seedlings, in contrast, rely more on soil moisture and external water sources, and may be prone to higher stress and lower water potentials than neighboring mature trees. Juvenile age trees often exhibit greater mortality during drought than trees of reproductive age within the same site (Donovan and Ehleringer 1991). Leaf mass

fractions may be greater during the seedling stage than in mature trees, with their extensive root systems, accumulated woody biomass, and increased in stem mass fraction. Leaf mass per area can decrease in drought conditions (Bansal et al. 2015), suggestive that the higher leaf mass per area in mature trees than in seedlings and saplings (Thomas and Winner 2001, Ishida et al. 2005) may reflect a greater drought experienced by the younger plants. The strategy of leaf shedding during drought could therefore be proportionally more costly for seedlings than mature trees.

Mature trees typically have greater reserves of non-structural carbohydrates than seedlings, which can be utilized to sustain cell functions and maintenance during periods of drought. For example, *Pinus ponderosa* exhibits increasing non-structural carbohydrates with tree height (Sala and Hoch 2009, Woodruff and Meinzer 2011). Seedlings, in contrast, often rely more on immediate photosynthesis than non-structural carbohydrate reserves. Moreover, seedling responses to water stress often include reduced net photosynthesis and reduced carbon gain (Wertin et al. 2009). As a result, if stomata close due to water deficit, limitations on photosynthesis and carbon gain may have more immediate effects on growth and cell functions in seedlings; low levels of carbohydrates can also prevent osmoregulation and hydraulic function. In *Pinus flexilis* seedlings, just prior to mortality, hydraulic conductivity decreased by 90%, with significant increases in hydraulic resistance, leading to accumulations of NSCs in aboveground tissues, likely caused by damage in phloem-transport which even further restricted water uptake by limiting root function (Reinhardt et al. 2015). Whereas a mature tree may be able to shed leaves or branches, seedlings risk losing a greater proportion of their overall biomass in doing so. Similarly, if damage is incurred during drought, smaller NSC reserves in seedlings may limit their capacity to produce new foliage or tissues in that season, or to survive in the next season. Furthermore, low levels of NSC reserves can restrict a seedling's ability to respond to other abiotic stresses, such as temperature, and to biotic stresses, such as pathogens. For

example, without sufficient carbohydrate reserves, a seedling may not be able to allocate resources towards defense.

The vulnerability of seedlings to drought stress has consequences for forest regeneration and species assemblages. If seedlings are unable to mature, forest compositions may be altered or, in extreme cases, forests may shift or be replaced by other ecosystems. Those seedlings that are capable of surviving must demonstrate drought resistance if they are to grow and compete, and certain provenances may be more or less adapted to oncoming stressful conditions (St. Clair and Howe 2007). This variation in drought resistance and survival may influence the course of forest regeneration (Grubb 1977, Suarez and Kitzberger 2008).

Drought Resistance

With increasing concerns related to drought, there is considerable interest in the drought resistances of trees. Drought resistance encompasses a number of strategies for coping with drought (Lo Gullo and Salleo 1988, Kozlowski and Pallardy 2002). These strategies include tolerance, avoidance, escape, and recovery (Levitt 1980, Fang and Xiong 2015). Drought tolerance refers to the ability of a tree to maintain physiological activity even at relatively negative water potentials. Drought tolerance may include the use of osmoregulation and osmotic adjustment to main cell turgor, gene regulation to reduce or repair damage from stress, and antioxidant defense (Farooq et al. 2012, Fang and Xiong 2015). Species with behavior that is more isohydric – characterized by sensitivity to decreasing soil moisture and closing of stomata at early signs of possible water stress – typically utilize the strategy of drought avoidance. Drought avoidance refers to minimizing water loss and minimizing water stress. This can occur via the shutting of stomata and high sensitivity to changes in soil moisture in order to minimize

water loss, in order to avoid water stress and associated damage. When soil moisture increases, stomata will typically re-open.

Plants that exhibit drought avoidance may also have waxy or small leaves, or other morphological adaptations that minimize water loss, such as enhanced water storage organs, or the development of extensive root systems to access deeper soil moisture (Fang and Xiong 2015). Drought escape involves behaviors such as dormancy during times characterized by seasonal or periodic drought, or adjusting growth periods or key life cycles to likewise avoid seasonal drought. For example, in arid environments, many species will remain dormant until a precipitation event. Seeds might remain dormant for many years and germinate only after an increase in soil moisture. Species that utilize the strategy of drought escape minimize the risk of drought stress by reducing or completely avoiding activity during dry seasons. Finally, drought recovery is the ability of a tree to recover from drought. For example, after surviving drought (either through tolerance or avoidance, for example), a species might be effective at repairing damaged tissue, re-growing tissue that has died back, re-sprouting, recovering growth, or otherwise effectively recovering function following drought.

Variation, Acclimation, and Plasticity

Across species and within species, there is considerable variation in drought resistance, with species distributions often determined in large part by drought resistance and site climate conditions. To become established plants must pass the regeneration phase. Likewise, tree species with broad geographic ranges may contain local populations with considerable genetic and adaptive variability (Zhang and Marshall 1995, Aranda et al. 2012) conferring varying extents of drought resistance (Ducrey et al. 2008), and suggesting that the seedling phases were successful in some climates and sites. Moreover, traits of species or populations have been

shown in some cases to be associated with site climate conditions (Dudley 1996). For example, Douglas-fir (*Pseudotsuga menziesii*) seedlings from xeric provenances have been shown in some studies to be more resistant to embolism than those from mesic provenances, thus allowing stomata to remain open at more negative xylem water potentials (Kavanagh et al. 1999). Provenance and thereby site conditions appear to exert selection for drought-resistance in Douglas-fir to varying degrees, with sites typified by low-summer precipitation or dry conditions associated with drought-resistance compared to more moist sites, and with considerable genetic variability for drought resistance across its range (Ferrell and Woodard 1966, White 1987, Joly et al. 1989, Kavanagh et al. 1999, Eilmann et al. 2012, Jansen et al. 2013, Bansal et al. 2015). Water stress responses can have high inter-species genetic variability, especially in phenotypic traits such as water use efficiency (Aranda et al. 2012).

Being long-lived organisms, trees experience many environmental conditions and stresses throughout their lives (Hinckley et al. 2011). While long-term genetic adaptations over multiple generations may help to insure that a species or individual tree will be able to persist in the specific overall site climate conditions of its range, nonetheless phenotypic plasticity can be an important trait to cope with stresses that may vary throughout one lifetime. Given increasing risk of intense and frequent drought stress as a result of climate change, especially in regions historically not characterized by droughts, it may be necessary for trees to adapt, particularly if migration proves to be too slow or otherwise limited (Aitken et al. 2008). However, in the face of rapid changes such as those associated with climate change, adaptation over multiple generations may not be sufficient for some species. Douglas-fir, for example, may not be able to adapt swiftly enough to projected future optima for its range, due in part to slow generation turnover, leading to maladaptation to future climates (St. Clair and Howe 2007). Given that even adaptation may require multiple generations, the ability of trees to acclimate to drought is of

particular interest. Acclimation can in some cases be triggered as a result of exposure to a given stress, and could enhance survival during drought. Abiotic and biotic stresses may result in epigenetic changes, conferring enhancements in drought resistance following exposure. For example, Raj et al. (2011) found evidence of DNA methylation and differing transcript patterns in response to water stress in *Populus* planted in contrasting locations. In loblolly pine seedlings exposed to cycles of drought, transcript profiles changed, with increases in genes expression connected with acclimation responses (Watkinson et al. 2003), including genes associated with heat shock proteins and aquaporins, dehydration-responsive element-binding proteins, and abscisic acid-responsive element-binding proteins. Provenances of Douglas-fir seedlings exposed to drought (both heat and water stress) have demonstrated shifts in preferences of metabolic pathways towards metabolites conferring protection against high temperature, osmotic stress, and reactive oxygen species, which is suggested to support maintenance of physiological activity under heat waves (Jansen et al. 2014). Kozłowski and Pallardy (2002) note that seedlings with previous exposure to water stress have been observed to have smaller growth inhibitions following transplanting compared to unexposed seedlings. Enhancements in drought tolerance following drought stress exposure may be linked to osmotic adjustment, as well as higher root to shoot ratios which may assist in accessing water (Kozłowski and Pallardy 2002). Provenances of Scots pine (*Pinus sylvestris* L) seedlings exposed to previous drought treatments exhibited lower drought mortality (Seidel and Menzel 2016) than non-droughted controls. Decreased height and aboveground dry weights were observed which may have further contributed to avoidance of lethally negative water potentials. There is evidence for plasticity of anatomical responses to variable water ability between years in *Larix decidua* and *Picea abies*, with increases in hydraulic safety associated with drought (Bryukhanova and Fonti 2013). The extent to which a species or population is able to acclimate following stress exposure may be an important determining factor in persistence on the landscape in the face of climate change. Acclimation

could be particularly important for survival in seedlings, with their small stature, low reserves, and low ability to buffer environmental conditions.

To summarize, possible forms of drought acclimation that can follow drought exposure include adjustments of photosynthesis, biochemistry, morphology, phenology, growth, metabolism, levels of osmotic adjustment, stomatal behavior, allocation of above and belowground biomass, sapwood area, epicuticular waxes, and gene expression as some examples. Common forms of acclimation include conservative growth, with more conservative growth often a response to xeric conditions or stress. Growth reductions can reduce resource requirements and water demands, and can also be a consequence of shut down and reduced photosynthesis during drought. Increased root biomass as a form of drought acclimation can increase access to soil moisture and expand rooting area. Morphological changes such as decreased leaf to sapwood area and anatomical changes such as smaller tracheid dimensions can reduce vulnerability to embolism, while changes such as increases of epicuticular waxes can further reduce water loss even when stomata are closed. Biochemical changes such as increases in osmotic adjustment, involving the use of solutes to drive pressure gradients, can help to maintain water transport under negative water potentials. Acclimation can often be a combination of the strategies listed here. However, acclimation towards greater drought resistance can come with trade-offs, such as the trade-off of reduced growth, and so there can be limits on the extent and on which traits can be expressed while meeting other needs.

Experimental Approach and Limitations

This study used loblolly pine (*Pinus taeda*) and Douglas-fir (*Pseudotsuga menziesii*) seedlings to investigate the variation of drought resistances (as measured by physiological responses and growth) among provenances from diverse site climates, and to investigate the

effects of drought acclimation. Seedlings were assigned randomly to drought treatments, with one treatment exposing seedlings to drought early in the summer, another treatment exposing seedlings to drought late in the summer, another treatment exposing seedlings to both early and late droughts, and a control treatment that was watered throughout the summer. This experimental approach was used to allow for general comparisons of drought resistance among the respective provenances, and also to allow for comparisons of timing and repeated exposure to drought with respect to possible acclimation. Seedlings in treatments that had experienced previous drought (two drought treatment) could be compared to seedlings that experienced drought for the first time (late drought) with the aim of detecting acclimation to drought.

Because seedlings represent a vulnerable life stage, studying drought responses in loblolly pine and Douglas-fir seedlings is important in understanding forest regeneration in natural settings as well as in industrial or managed settings. There is great interest by the forest industry in assuring survival and quality growth when out-planting. In the face of climate change, many forest industry companies will be affected by increasing frequency and severity of drought across lands under their management. Understanding variation in drought resistance is therefore of great concern to planting decisions. In natural settings, understanding seedling regeneration is important for predicting future species assemblages, forest composition, and succession, especially as there is considerable mortality during the first year of growth. The specific ages of this study represent two highly vulnerable stage: germinated seedling facing a first growing season for loblolly pine, and nursery grown one-year-old seedlings prepared to be out-planted for Douglas-fir.

The study was carried out in a greenhouse setting, with loblolly pine seedlings that were germinated from seed four months prior to the start of the study and with Douglas-fir seedlings

that were around one and a half years old at the start of the study. The provenances were selected on the basis of their site climate conditions, with the aim of including varying conditions for comparison, although ultimately some provenances were found to be similar in mean annual precipitation. Because plant material was provided by an industrial partner, we had limited control of material selected. The provenances were color-coded to reduce bias; their identities were only revealed after data had been collected. Seedling ages were used in part as a result of availability from the industrial partner that had access to seed, seedlings, and facilities to germinate and stratify the seedlings. In physiological studies, it is often helpful to know the life history that a seedling has experienced, and thus using germinated loblolly pine seedlings and transplanted Douglas-fir seedlings from an industrial partner can have the advantage of more full information of past conditions. Because the Douglas-fir seedlings differed in age from the loblolly pine seedlings, we did not attempt to compare across species. Douglas-fir plant material represented half-siblings, as did two of the three loblolly pine provenances; the Lost Pines provenance represented open-pollinated seed collected from Lost Pines loblolly pine stands in Texas.

The experiment was carried out over the course of one summer, representing one growing season. Although studies of drought responses in seedlings and trees are ideally carried out over multiple seasons, one summer nonetheless provides insight into early strategies and responses, which may be useful in developing multi-year studies or guiding subsequent investigations. Moreover, there existed limitations on resources and time. Further limitations include the fact that the experiment was conducted in greenhouse conditions. While greenhouse studies provide advantages of controlled conditions, they are not necessarily representative of field conditions that seedlings might normally experience, and thus place some additional limitations on inferences.

Overview of Research Questions

This study sought to address multiple questions. 1) The study aimed to compare the drought resistances of loblolly pine and Douglas-fir seedlings from various provenances that are associated with differing site climate conditions. We hypothesized that seedlings of both species from provenances associated with xeric site climate conditions (e.g., low annual and summer precipitation, and high climatic moisture deficit) would exhibit higher drought resistance than seedlings from provenances associated with more mesic site climate conditions (e.g., high annual and summer precipitation and low climatic moisture deficits). 2) The study aimed to investigate the extent of acclimation following exposure to drought. We hypothesized that previous drought exposure would result in enhanced performance in subsequent drought. Performance was inferred from stem water potentials, foliar physiology, and biomass accumulation. In connection to drought acclimation, the study investigated the extent of plasticity among provenances of each species. We hypothesized that provenances associated with mesic site climate conditions would demonstrate a greater degree of plasticity in terms of acclimation to drought compared to provenances from more xeric site climate conditions. Drought resistance, degree of acclimation, and plasticity were inferred through periodic measurements of stem water potential, foliar physiology, and biomass accumulation.

References

- Adams, H., Guardiola-Claramonte, M., Barron-Gafford, G., Camilo Villegas, J., Breshears, D., Zou, C., Troch, P., Huxman, T. 2009. Temperature sensitivity of drought-induced tree mortality portends increased regional die-off under global-change-type drought. *Proceedings of the National Academy of Sciences (PNAS)* 106(17): 7063-7066.
- Aitken, S., Yeaman, S., Holliday, J., Wang, T., Curtis-McLane S. 2008. Adaptation, migration or extirpation: climate change outcomes for tree populations. *Evolution Applications* 1: 95-111.
- Allen, C., Macalady, A., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M., Kitzberger, T., Rigling, A., Breshears, D., Hogg, E., Gonzalez, P., Fensham, R., Xhang, Z., Castro, J., Demidova, N., Lim, J., Allard, G., Running, S., Semerci, A. Cobb, N. 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.
- Anderegg, W., Hicke, J., Fisher, R., Allen, C., Aukema, J., Bentz, B., Hood, S., Lichstein, J., Macalady, A., McDowell, N., Pan, Y., Raff, K., Sala, A., Shaw, J., Stephenson, N., Tague, C., Zeppel, M. 2015. Tree mortality from drought, insects, and their interactions in a changing climate. *New Phytologist* 208(3): 674-683.
- Aranda, I., Gil-Pelegrín, E., Gascó, A., Guevara, M., Cano, J., De Miguel, M., Ramírez-Valiente, A., Peguero-Pina, J., Perdigüero, P., Soto, A., Cervera, M., Collada, C. 2012. Drought response in forest trees: from the species to the gene. In: *Plant Responses to Drought*. R. Aroca (ed.). Springer-Verlag Berlin Heidelberg pp 293-333.
- Bansal, S., Harrington, C., Gould, P., St. Clair, B. 2015. Climate-related genetic variation in drought-resistance of Douglas-fir (*Pseudotsuga menziesii*). *Global Change Biology* 21(2): 947-958.
- Bauweraerts, I., Ameye, M., Wertin, T., McGuire, M., Teskey, R., Steppe, K. 2014. Water availability is the decisive factor for the growth of two tree species in the occurrence of consecutive heat waves. *Agricultural and Forest Meteorology* 189-190: 19-29.
- Bréda, N., Huc, R., Granier, A., Dreyer, E. 2006. Temperate forest trees and stands under severe drought: a review of ecophysiological responses, adaptation processes and long-term consequences. *Annals of Forest Science* 63(6): 625-644.
- Breshears, D., Cobb, N., Rich, P., Price, K., Allen, C., Balice, R., Romme, W., Kastens, J., Floyd, L., Belnap, J., Anderson, J., Myers, O., Meyer, C. 2005. Regional vegetation die-off in response to global-change-type drought. *Proceedings of the National Academy of Sciences (PNAS)* 102 (42): 15144-15148.
- Brodribb, T.J. 2009. Xylem hydraulic physiology: the functional backbone of terrestrial plant

productivity. *Plant Science* 177: 245–251.

Brodrigg, T.J., McAdam, Scott A.M., Jordan, Gregory J., and Martins, Samuel C.V. 2014. Conifer species adapt to low rainfall climates by following one of two divergent pathways. *Proceedings of the National Academy of Sciences (PNAS)* 111 (40): 14489-14493.

Bryukhanova, M., Fonti, P. 2013. Xylem plasticity allows rapid hydraulic adjustment to annual climatic variability. *Trees* 27(3): 485-496.

Chaves, M., Flexas, J., Pinheiro, C. 2009. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. *Annals of Botany* 103: 551-560.

Chaves, M., Pereira, J. Maroco, J., Rodrigues, M., Ricardo, C. Osorio, M., Carvalho, I., Faria, T., Pinheiro C. 2002. How plants cope with water stress in the field. Photosynthesis and growth. *Annals of Botany* 89: 907-916.

Choat, B., Jansen, S., Brodrigg, T., Cochard, H., Delzon, S., Radika, B., Bucci, S., Field, T., Gleason, S., Hacke, U., Jacobsen, A., Lens, F., Maherali, H., Martinez-Vilalta, J., Mayr, S., Mencuccini, M., Mitchell, P., Nardini, A., Pittermann, J., Pratt, R.B., Sperry, J., Westoby, M., Wright, I., Zanne, A. 2012. Global convergence in the vulnerability of forests to drought. *Nature* 491: 752-755.

Ciais, P., Reichstein, M., Viovy, N., Granier, A., Ogee, J., Allard, V., Aubinet, M., Buchmann, N., Bernhofer, C., Carrara, A., Chevallier, F., De Noblet, A., Friend, P., Friedlingstein, P., Grunwald, T., Heinesch, B., Keronen, P., Knohl, A., Krinner, G., Loustau, D., Manca, G., Matteucci, G., Miglietta, F., Ourcival, J., Papale, D., Pilegaard, K., Rambal, S., Seufert, G., Soussana, J., Sanz, M., Schulze, E., Vesala, T., Valentini, R. 2005. Europe-wide reduction in primary productivity caused by the heat and drought in 2003. *Nature* 437: 529-533.

Cui, M., Smith, W.K. 1991. Photosynthesis, water relations and mortality in *Abies lasiocarpa* seedlings during natural establishment. *Tree Physiology* 8: 37–46.

Dai, A., Trenberth, K., Qian, T. 2004. A global dataset of Palmer Drought Severity Index for 1870-2002: relationship with soil moisture and effects of surface warming. *Hydrometeorology* 5: 1117-1130.

Dai, A. 2011. Drought under global warming: a review. *Wiley Interdisciplinary Reviews: Climate Change* 2(1): 45-65.

Dai, A. 2013. Increasing drought under global warming in observations and models. *Nature Climate Change Letters* 3: 52-58.

- Dale, V., Joyce, L., McNulty, S., Neilson, R., Ayres, M., Flannigan, M., Hanson, P., Irland, L., Lugo, A., Peterson, C., Simberloff, D., Swanson, F., Stocks, B., Wotton, M. 2001. Climate change and forest disturbances. *Biosciences* 51(9): 723-734.
- Dawson, T. 1996. Determining water use by trees and forests from isotopic, energy balance and transpiration analyses: the roles of tree size and hydraulic lift. *Tree Physiology* 16: 263-272.
- Donaldson, L. 2002. Abnormal lignin distribution in wood from severely stressed *Pinus radiata* trees. *International Association of Wood Anatomists (IAWA) Journal* 23: 161-178.
- Donovan, L. Ehleringer, J. 1991. Ecophysiological differences among juvenile and reproductive plants of several woody species. *Oecologia* 86(4): 594-597.
- Ducrey, M., Huc, R., Ladjal, M., Guehl, J. 2008. Variability in growth, carbon isotope composition, leaf gas exchange and hydraulic traits in the eastern Mediterranean cedars *Cedrus libani* and *C. brevifolia*. *Tree Physiology* 28: 689-701.
- Dudley, S. 1996. Differing selection on plant physiological traits in response to environmental water availability: a test of adaptive hypotheses. *Evolution* 50(1): 92-102.
- Eilmann, B., Zweifel, R., Buchmann, N., Graf Pannatier, E., Rigling, A. 2011. Drought alters timing, quantity, and quality of wood formation in Scots pine. *Journal of Experimental Botany* 6 (8): 2763-2771.
- Eilmann, B., de Vries, S.M., den Ouden, J., Mohren, G., Sauren, P., Sass-Klaassen, U. 2012. Origin matters! Difference in drought tolerance and productivity of coastal Douglas-fir (*Pseudotsuga menziesii* (Mirb.)) provenances. *Forest Ecology and Management* 302: 133-143.
- Ehrler, W. 1973. Cotton leaf temperatures as related to soil water depletion and meteorological factors. *Agronomy Journal* 65: 404-409.
- Engelbrecht, B., Comita, L., Condit, R., Kursar, T., Tyree, M., Turner, B., Hubbell, S. 2007. Drought sensitivity shapes species distribution patterns in tropical forests. *Nature* 447: 80-82.
- Fang, Y., Xiong, L. 2015. General mechanisms of drought response and their application in drought resistance improvement in plants. *Cellular and Molecular Life Sciences* 72: 673-689.
- Farooq, M., Hussain, M., Wahid, A., Siddique, K. 2012. Drought stress in plants: an overview. In: *Plant Responses to Drought Stress* (Ed. R Aroca), Springer Press, Berlin-Heidelberg. 1-33.
- Ferrell, W.K., Woodard, E.S. 1966. Effects of seed origin on drought resistance of Douglas-fir (*Pseudotsuga menziesii*) (Mirb.) Franco. *Ecology* 47: 499-503.

- Flexas, J., Ribas-Carbo, M., Bota, J., Galmes, J., Henkle, M., Martinez-Canellas, S., Medrano, H. 2006. Decreased Rubisco activity during water stress is not induced by decreased relative water content but related to conditions of low stomatal conductance and chloroplast CO₂ concentration. *New Phytologist* 172: 73-82.
- Gates, D. 1964. Leaf temperature and transpiration. *Agronomy Journal* 56: 273-277.
- Grier, C., Running, S. 1977. Leaf area of mature Northwestern coniferous forests: relation to site water balance. *Ecology* 58: 893-899.
- Grossnickle, S.C. 2012. Why seedlings survive: influence of plant attributes. *New Forests* 43:711–738.
- Grubb, P.J. 1977. The maintenance of species richness in plant communities: the importance of the regeneration niche. *Biological Review* 52: 107-145
- Gruber, A., Strobl, S., Veit, B., Oberhuber, W. 2010. Impact of drought on the temporal dynamics of wood formation in *Pinus sylvestris*. *Tree Physiology* 30(4): 490-501.
- Hacke, U.G., Sperry, J. 2001. Functional and ecological xylem anatomy. *Perspectives in Plant Ecology, Evolution, and Systematics* 4: 97-115.
- Hacke, U.G., Jansen, S. 2009. Embolism resistance of three boreal conifer species varies with pit structure. *New Phytologist* 182(3): 675-86.
- Hanson, P., Weltzin, J. 2000. Drought disturbance from climate change: response of United States forests. *Science of the Total Environment*. 262: 205-220.
- Hsiao, T. 1973. Plant responses to water stress. *Annual Review of Plant Physiology*. 24: 519-70.
- Hinckley, T.M., Lachenbruch, B., Meinzer, F.C., Dawson, T.E. 2011. A lifespan perspective on integrating structure and function in trees in Size-and Age-Related Changes in Tree Structure and Function pp 3-30.
- IPCC, 2007. Climate change 2007: the physical science basis. In: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M., Miller, H.L. (Eds.), Contribution of Working Group I to the Fourth Assessment. Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom/New York, NY, USA, 996pp.
- Ishida, A., Yazaki, K., Lai Hoe, A. 2005. Ontogenetic transition of leaf physiology and anatomy from seedlings to mature trees of a rain forest pioneer tree, *Macaranga gigantea*. *Tree Physiology* 25: 513-522.
- Jackson, R., Idso, S., Reginato, R., Pinter, P. 1981. Canopy temperature as a crop water stress

indicator. *Water Resources Research* 17(4): 1133-1138.

Jansen, K., Baoguo, D., Kayler, Z., Siegwolf, R., Ensminger, I., Rennenberg, H., Kammerer, B., Jaeger, C., Schaub, M., Kreuzwieser, J., Gessler, A. 2014. Douglas-fir exhibit metabolic responses to increased temperature and atmospheric drought. *PLoS One* 9(12): e114165.

Jansen, K., Sohr, J., Kohnle, U., Ensminger, I., Gessler, A. 2013. Tree ring isotopic composition, radial increment and height growth reveal provenance-specific reactions of Douglas-fir towards environmental parameters. *Trees* 27 (1): 37-52.

Johnson, D.M., McCulloh, K.A., Woodruff, D.R., Meinzer, F.C. 2012. Hydraulic safety margins and embolism reversal in stems and leaves: why are conifers and angiosperms so different? *Plant Science* 195: 48-53.

Joly, R.J., W.T. Adams, S.G. Stafford. 1989. Phenological and morphological responses of mesic and dry site sources of coastal Douglas-fir to water deficit. *Forest Science* 35: 987-1005.

Kavanagh, K.L., Bond, B.J., Aitken, S.N., Gartner, B.L., Knowe, S., 1999. Shoot and root vulnerability to xylem cavitation in four populations of Douglas-fir seedlings. *Tree Physiology* 19: 31-37.

Klein, T. 2014. The variability of stomatal sensitivity to leaf water potential across tree species indicates a continuum between isohydric and anisohydric behaviors. *Functional Ecology* 28(6): 1313-1320.

Klos, R., Wang, G., Bauerle, W., Rieck, J. 2009. Drought impact on forest growth and mortality in the southeast USA: an analysis using Forest Health and Monitoring data. *Ecological Applications* 19(3): 699-708.

Kolb, P., Robberecht, R. 1996. High temperature and drought stress effects on survival of *Pinus ponderosa* seedlings. *Tree Physiology* 16(8): 665-72.

Kozlowski T., and Pallardy, S. 2002. Acclimation and adaptive Responses of woody plants to environmental stresses. *Botanical Review* 68(2): 270-334.

Lachenbruch, B., McCulloh, K.A. 2014. Traits, properties, and performance: how woody plants combine hydraulic and mechanical functions in a cell, tissue, or whole plant. *New Phytologist* 204:747-764.

Law, B., Falge, E., Gu, L., Baldocchi, D., Bawkin, P., Berbigier, P., David, K., Dolman, A., Falk, M., Fuentes, J., Goldstein, A., Granier, A., Grelle, A., Hollinger, D., Janssens, I., Jarvis, P., Jensen, N., Katul, G., Mahli, Y., Matteucci, G., Meyers, T., Monson, R., Munger, J., Oechel, W., Olson, R., Pilegaard, K., Paw, U., Thorgeirsson, H., Valentini, R., Verma, S., Vesala, T., Wilson, K., Wofsy, S. 2002. Environmental controls over carbon dioxide and water vapor exchange of

terrestrial vegetation. *Agricultural and Forest Meteorology* 113: 97-120.

Le Dantec, V., Dufrene, E., Saugier, B. 2000. Interannual and spatial variation in maximum leaf area index of temperate deciduous stands. *Forest Ecology and Management* 134: 71-81.

Levitt, J. 1980. Responses of plants to environmental stresses. In: Kozlowski TT Responses of plants to environmental stresses. Volume II. Water, radiation, salt, and other stresses. 1980. Ed. 2 pp. 607

Lo Gullo, M., Salleo, S. 1988. Different strategies of drought resistance in three Mediterranean sclerophyllous trees growing in the same environmental conditions. *New Phytologist* 108: 267-276.

Lotan, J., Zahner, R. 1963. Shoot and needle responses of 20-year-old red pine to current soil moisture regimes. *Forest Science* 9(4): 497-506.

Martinez-Vilalta, J., Poyatos, R., Aguade, D. 2014. A new look at water transport regulation in plants. *New Phytologist* 204(1): 105-115.

Manzoni, S., Vico, G., Katul, G., Palmroth, S., Jackson, R.B., Porporato, A. 2013. Hydraulic limits on maximum plant transpiration and the emergence of the safety-efficiency trade-off. *New Phytologist* 198: 169-178.

McCully, M.E, Huang, C.X., Ling, L.E. 1998. Daily embolism and refilling of xylem vessels in the roots of field-grown maize. *New Phytologist* 138:327–342.

McDowell, N, Pockman W.T., Allen, C.D., Breshears, D.D., Cobb N, Kolb T, Plaut, J., Sperry J., West A, Williams, D.G. 2008. Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytologist* 178: 719–739.

Meinzer, F.C., McCulloh, K. 2013. Xylem recovery from drought-induced embolism: where is the hydraulic point of no return? *Tree Physiology* 33(4): 331-334.

Meinzer, F.C., Woodruff, D., Marias, D., Smith, D., McCulloh, K., Howard, A., Magedman, A. 2016. Mapping ‘hydroscares’ along the iso- to anisohydric continuum of stomatal regulation of plant water status. *Ecology Letters* 19(11): 1343-1352.

Mittler, R. 2002. Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science* 7: 405-410.

Minore, D. 1986. Germination, survival and early growth of conifer seedlings in two habitat types. Res. Pap. PNW-RP-347. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Forest and Range Experiment Station. 63 pp.

- Myers, B. Landsberg, J. 1989. Water stress and seedling growth of two eucalypt species from contrasting habitats. *Tree Physiology* 5: 207-218.
- Niinemets, U. 2010. Responses of forest trees to single and multiple environmental stresses from seedlings to mature plants: Past stress history, stress interactions, tolerance and acclimation. *Forest Ecology and Management* 260: 1623-1639.
- O'Brien, M., Leuzinger, S., Philipson, C. Tay, J., Hector, A. 2011. Drought survival of tropical tree seedlings enhanced by non-structural carbohydrate levels. *Agricultural and Forest Meteorology* 151: 1632-1640.
- Olesinski, J., Lavigne, M., Krasowski, M. 2011. Effects of soil moisture manipulations on fine root dynamics in a mature balsam fir (*Abies balsamea* L. Mill.) forest. *Tree Physiology* 31: 339-348.
- Palmer, W. 1965. Meteorological drought. Research Paper No. 45. U.S. Department of Commerce, 1965, 58 pp.
- Piermattei, A., Crivellaro, A., Carrer, M., Urbinati, C. 2015. The “blue ring”: anatomy and formation hypothesis of a new tree-ring anomaly in conifers. *Trees Structure and Function* 29: 612-620.
- Phillips, N., Ryan, M., Bond, B., McDowell, N., Hinckley, T., Cermak, J. 2003. Reliance on stored water increases with tree size in three species in the Pacific Northwest. *Tree Physiology* 23: 237-245.
- Power, S. 1994. Temporal trends in twig growth of *Fagus sylvatica* L. and their relationships with environmental factors. *Forestry*. 67: 13-30.
- Raj, S., Brautigama, K., Hamanishi, E., Wilkins, O., Thomas, B., Schroederf, W., Mansfield, S., Planth, A., Campbell, M. 2011. Clone history shapes *Populus* drought responses. *Proceedings of the National Academy of Sciences (PNAS)* 106: 12521-12526.
- Reinhardt, K., Germino, M., Kueppers, L., Domec, J., Mitton, J. 2015. Linking carbon and water relations to drought-induced mortality in *Pinus flexilis* seedlings. *Tree Physiology* 35: 771-782.
- Sage, R., Kubien, D. 2007. The temperature response of C3 and C4 photosynthesis. *Plant, Cell and Environment* 30: 1086-1106.
- Sala, A., Hoch, G. 2009. Height-related growth declines in ponderosa pine are not due to carbon limitation. *Plant Cell Environment* 32: 22-30.
- Sala., A, Piper, F., Hoch, G. 2010. Physiological mechanisms of drought-induced tree mortality are far from being resolved. *New Phytologist* 186: 274–281.

- Secchi, F., Zwieniecki, M.A. 2012. Analysis of xylem sap from functional (nonembolized) and nonfunctional (embolized) vessels of *Populus nigra*: chemistry of refilling. *Plant Physiology* 160:955–964.
- Secchi, F., Pagliarani, C., Zwieniecki, M. 2016. The functional role of xylem parenchymacells and aquaporins during recovery from severe water stress. *Plant, Cell and Environment* Epub 2016 Dec 15
- Seidel, H., Menzel, A. 2016. Above-ground dimensions and acclimation explain variation in drought mortality of Scots pine seedlings from various provenances. *Fronts in Plant Science* 7 (1014): 1-14.
- Sepulcre-Cantó, G., Zarco-Tejada, P., Jiménez-Muñoz, J., Sobrino, J., Miguel E., Villalobos, F. 2006. Detection of water stress in an olive orchard with thermal remote sensing imagery. *Agricultural and Forest Meteorology* 136: 31-44.
- Sevanto, S., McDowell, N., Dickman, L.T., Pangle, R., Pockman, W. 2014. How do trees die? A test of the hydraulic failure and carbon starvation hypotheses. *Plant, Cell and Environment* 37: 153-161.
- Shevtsova A, Graae, B.J., Jochum, T., Milbau, A., Kockelbergh, F., Beyens, L., Nijs, I. 2009. Critical periods for impact of climate warming on early seedling establishment in subarctic tundra. *Global Change Biology* 15: 2662–2680.
- Sheriff, D. and Whitehead, D. 2006. Photosynthesis and wood structure in *Pinus radiata* D. Don during dehydration and immediately after rewatering. *Plant Cell and Environment* 7(1): 53-62.
- Sperry, J., Hacke, U., Oren, R., Comstock, J. 2002. Water deficits and hydraulic limits to leaf water supply. *Plant, Cell and Environment* 25: 251-263.
- St. Clair, B., Howe, G. 2007. Genetic maladaptation of coastal Douglas-fir seedlings to future climates. *Global Change Biology* 13: 1441-1454.
- Suarez, M.L., Kitzberger, T. 2008. Recruitment patterns following a severe drought: long-term compositional shifts in Patagonian forests. *Canadian Journal of Forest Research* 38: 3002-3010.
- Tanner, C. 1963. Plant temperatures. *Agronomy Journal* 55: 210-211.
- Teskey, R., Bongarten, B., Cregg, B., Dougherty, P., Hennessey, T. 1987. Physiology and genetics of tree growth responses to moisture and temperature stress: an examination of the characteristics of loblolly pine. *Tree Physiology* 3: 41-61.
- Thomas, S., Winner, W. 2001. Photosynthetic differences between saplings and adult trees: an integration of field results by meta-analysis. *Tree Physiology* 22: 117-127.

- Tyree, M., Yang, S. 1990. Water-storage capacity of *Thuja*, *Tsuga* and *Acer* stems measured by dehydration isotherms: The contribution of capillary water and cavitation. *Planta* 182: 420-426.
- Tezara, W., Mitchell, V.J., Driscoll, S.D., Lawlor, D.W. 1999. Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. *Nature* 401: 914–917.
- Tyree, M., Sperry, J. 1988. Do plants operate near the point of catastrophic xylem dysfunction caused by dynamic water stress? *Plant Physiology* 88: 574-580.
- Tyree, M., Ewers, F. 1991. The hydraulic architecture of trees and other woody plants. *New Phytologist* 119: 345-360.
- Urli, M., Porte, A., Cochard, H., Guengant, Y., Burlett, R., Delzon, S. 2013. Xylem embolism threshold for catastrophic hydraulic failure in angiosperm trees. *Tree Physiology*. 33: 672-683.
- Valdés, M., Asbjornsen, H., Gómez Cárdenas, M., Vogt, K. 2005. Drought effects on fire-root and ectomycorrhizal-root biomass in managed *Pinus oaxacana* Mirov stands in Oaxaca, Mexico. *Mycorrhiza* 16(2):.117-24.
- van Mantgem, P., Stephenson, N., Byrne, J., Daniels, L., Franklin, J., Fule, P., Harmon, M., Larson, A., Smith, J., Taylor, A., Veblen, T. 2009 Widespread increase of tree mortality rates in the western United States. *Science* 323: 521-524.
- van Mantgem, P., Stephenson, N. 2007. Apparent climatically induced increase of tree mortality rates in a temperate forest. *Ecology Letters* 10: 909-916.
- Waring, R., Running, S. 1978. Sapwood water storage: its contribution to transpiration and effect upon water conductance through the stems of old growth Douglas-fir. *Plant Cell and Environment* 1:131-140.
- Watkinson, J., Sioson, A., Vasquez-Robinet, Shukla, M, Deept, K., Ellis, M., Heath, L., Ramakrishnan, N., Chevone, B., Watson, L., van Zyl, L., Egertsdotter, U., Sederoff, R., Grene, R. 2003. Photosynthetic acclimation is reflected in specific patterns of gene expression in drought-stressed loblolly pine. *Plant Physiology* 133(4): 1702-1716.
- White, T. 1987. Drought tolerance of southwestern Oregon Douglas-fir. *Forest Science* 33(2): 283-293.
- Wertin, T., McGuire, M., Teskey, R. 2009. The influence of elevated temperature, elevated atmosphere CO₂ concentration and water stress on net photosynthesis of loblolly pine (*Pinus taeda* L.) at northern, central and southern sites in its native range. *Global Change Biology* 16(7): 2089-2103.

- Wiegand, C. and Namken, L. 1966. Influences of plant moisture stress, solar radiation and air temperature on cotton leaf temperature. *Agronomy Journal* 58: 552-556.
- Woodruff, D.R., Meinzer, F.C. 2011. Water stress, shoot growth and storage of non-structural carbohydrates along a tree height gradient in a tall conifer. *Plant, Cell and Environment* 34(11): 1920-1930.
- Woodruff, D.R. 2014. The impacts of water stress on phloem transport in Douglas-fir trees. *Tree Physiology* 34: 5–14.
- Wullschleger, S.D., Meinzer, F.C., Vertessy, R.A. 1998. A review of whole-plant water use studies in trees. *Tree Physiology* 18: 499-512.
- Zahner, R. 1962. Terminal growth and wood formation by juvenile loblolly pine under two soil moisture regimes. *Forest Science* 8: 345-352.
- Zhang, J., Marshall, J. 1995. Variation in carbon isotope discrimination and photosynthetic gas exchange among populations of *Pseudotsuga menziesii* and *Pinus ponderosa* in different environments. *Functional Ecology* 9: 402-412.

2. PHYSIOLOGICAL RESPONSES OF LOBLOLLY PINE AND DOUGLAS-FIR
SEEDLINGS FROM VARIOUS PROVENANCES TO TIMING AND FREQUENCY
OF DROUGHT STRESS

INTRODUCTION

Plants, and trees in particular due to their longevity, cope with an array of abiotic and biotic stresses throughout their lives in order to survive. Abiotic stresses such as drought and high temperature are predicted to increase in many parts of the world (IPCC 2007a, IPCC 2007b, Christensen et al. 2007, Dai 2011, 2013). Tree mortality and range shifts associated with climate change have already been observed across multiple continents (Parmesan and Yohe 2003, Allen et al. 2010, Anderegg et al. 2013) and are predicted to continue, likely in interactive ways (McDowell et al. 2011, Anderegg et al. 2015). Seedlings, furthermore, are more vulnerable to environmental stresses than mature trees, with mortality rates at young life-stages often high (Minore 1986, Cui and Smith 1991, Shevtsova et al. 2009, Grossnickle 2012). However, seedling growth and survival is vital to forest regeneration (Kobe 1996). Therefore, a greater understanding of the physiological responses of seedlings to abiotic stresses such as drought is important in a changing world, from both an ecological and management point of view.

Considerable work has been done to investigate the physiological responses of trees to drought (Hsiao 1973, Hinckley et al. 1979, Morgan 1984, Hanson and Weltzin 2000, Breshears et al. 2009). Drought can result in decreases in photosynthesis, damage to leaf tissues, and embolism of vascular tissue, which can lead to hydraulic failure, and other physiological consequences, along with reductions in growth. Effects of drought can be temporary, or long-lasting. For example, under mild water stress, many plant species have been shown to recover pre-drought photosynthetic rates within one to two days (Flexas 2004). However, after severe water stress, *Eucalyptus pauciflora* seedlings recovered only 40-60% of maximum photosynthetic rates the day after re-watering, and some individuals never returned to pre-stress rates (Kirschbaum 1988). In the same study, photosynthetic recovery after severe water stress was described as a two-stage process during which leaves rehydrated and stomata re-opened,

after which some photosynthetic enzymes such as ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) were re-synthesized. Moreover, photosynthesis, stomatal opening and closing, mesophyll conductance, and biochemical responses under water deficit are often species-dependent (Chaves et al. 2009).

Even within species, different populations and provenances may demonstrate a range of responses to drought. For example, both loblolly pine (*Pinus taeda*) and Douglas-fir (*Pseudotsuga menziesii*) show variation in drought resistance. The Lost Pines population, a disjunct population of loblolly pine in Bastrop County Texas at the farthest western edge of its range, have been reported to have higher drought resistance than other sources, likely as a result of natural selection and high selection pressure (Zobel and Goddard 1955, Bilan et al. 1977). Lost Pines seedlings close stomata sooner than other eastern Texas sources under drought conditions (Bilan et al. 1977), have fewer stomata per unit surface area and needle volume, and thicker epidermal cells compared to other sources (Thames 1963, Bilan and Knauf 1974). These traits are consistent with selection to reduce water loss.

Ecotypic variation refers to trait differences between populations reflective of adaptation to selection pressures, and can result in genetically distinct populations or ‘ecotypes’ (Turesson 1922). Yet, species can also show phenotypic variation and plasticity in acclimating to environmental conditions (Lande 2009, Richter et al. 2012). Douglas-fir also demonstrates considerable adaptation to varying environmental conditions (Rehfeldt 1978), some degree of plasticity (Koehn et al. 2010), as well as variation in drought sensitivity by site, population, and provenance (Heiner and Lavender 1972, White 1987, Joly et al. 1989, Jansen et al. 2013, Eilmann et al. 2012, Bansal et al. 2015). Douglas-fir seedlings from xeric environments maintain higher needle turgidity under drying conditions than seedlings from mesic environments

(Zavitkovski and Ferrell 1968), and dry site interior sources have shown greater drought resistance than coastal sources (Ferrell and Woodard 1966). Furthermore, coastal and interior Douglas-fir from xeric sites have shown greater resistance to embolism than coastal and interior Douglas-fir from mesic sites. These differences were observed even when grown in common gardens, suggesting ecotypic variation (Kavanagh et al. 1999).

Beyond immediate effects, drought can also have persistent effects on physiology, morphology, growth, and performance. Long periods of drought, for example, may lead to shifts in allocation of carbohydrates available for necessary functions such as cell maintenance, defense, and growth (McDowell 2011). A study investigating widespread aspen forest die-off reported that hydraulic damage persisted in the years following drought, and in some cases trees accumulated xylem damage, leading to increased vulnerability to embolism and increased vulnerability to drought (Anderegg et al. 2013). Interestingly, in the short-term, because drought can initially result in greater reductions in growth than photosynthesis (Hsiao 1973), drought may even cause accumulations of non-structural carbohydrates (NSC) (McDowell 2011). However, NSCs become depleted over long periods of drought. After exposing loblolly pine (*Pinus taeda*) seedlings to repeated drought cycles, Teskey et al. (1987) also reported a loss of aboveground productivity on droughty sites because of increased carbon allocation to roots and at the expense of stem wood production.

In response to drought conditions in previous years or over the course of a season, some tree species may adjust photosynthetic rates, growth rates, and xylem characteristics, showing potential for drought acclimation. In response to eight weeks of moisture-stress conditioning, for example, loblolly pine seedlings subjected to subsequent drought maintained higher photosynthetic rates at lower needle water potentials compared to un-conditioned controls, and

increased water-use efficiency by 67% (Seiler and Johnson 1985). Other studies have shown increased drought resistance with stress conditioning in nursery practices in the short-term (Arnott et al. 1993), although this increased resistance has not always translated to increased survival after field planting (Royo et al. 2001). Similarly, alterations in growth due to drought exposure, with reductions in aboveground biomass, can affect drought resistance (Seidel and Menzel 2016). Gene expression may also be influenced by environment (Bartholome et al. 2015). In response to abiotic stresses such as drought, genes may be turned on or off, leading to enhanced expression of traits that may be beneficial to performance under drought (Shinozaki and Yamaguchi-Shinozaki 1997, Shinozaki and Yamaguchi-Shinozaki 2007, Harb et al. 2010). A study that subjected loblolly pine seedlings to mild and more severe drought cycles (leaf water potentials around -1 MPa and -1.5 MPa, respectively) showed patterns of gene expression correlated with acclimation to drought stress, with increases in transcript abundances of stress-associated genes under mild stress. The study also suggested differences in response pathways depending on stress level (Watkinson et al. 2003).

The degree of phenotypic plasticity in response to drought, i.e. the degree to which an individual is able to acclimate to drought conditions following exposure, is often species dependent. This plasticity may provide important advantages in future scenarios where drought may become more frequent throughout a season, or over multiple years (Nicotra et al. 2010). For example, changes in xylem anatomy that reduce vulnerability to embolism, increases in sensitivity of stomata to dry soil, higher levels of osmotic adjustment, increased allocation to root biomass, more conservative growth or increases in water use efficiency as a result of drought acclimation could all potentially reduce damage incurred from drought. These changes could promote maintenance of cell functioning and possibly assist seedling survival to less vulnerable ages. Therefore, along with overarching questions of interspecific drought responses, or of

intraspecific responses of populations, provenances, or ecotypes, it is also important to consider effects of *repeated* stress given that drought-exposure may potentially alter growth, alter morphological and physiological characteristics, or impart lasting effects even following release from drought. The ability of seedlings to acclimate to drought as a result of previous exposure may thus play an important role in survival and growth of species in increasingly drought-prone regions (Nicotra et al. 2010, Seidel and Menzel 2016).

The goals of this study were first, to investigate the drought resistances of seedlings from various provenances and second, to investigate the acclimation response of seedlings from the respective provenances after drought exposure during a second drought. Specifically, this study investigated the physiological responses of loblolly pine seedlings from three provenances (North Carolina, Alabama, and Lost Pines) and Douglas-fir seedlings from three provenances (Coos Bay, Cascades, and New Mexico) to an early and late drought in the same growing season to better understand drought resistances and drought acclimation. The provenances represent mean site climate conditions at sites of origin that range from generally mesic to somewhat more xeric as shown by mean annual precipitation, mean summer precipitation, and climatic moisture deficit. Connected to questions of drought resistances among the provenances and to questions of potential for acclimation in response to drought exposure, the study also investigated acclimation and plasticity among the provenances. The study used physiological measures (chlorophyll fluorescence, photosynthetic electron transport rate, plant water potential) to assess these questions in a greenhouse setting, and tracked growth (through repeated height measurements and biomass harvests).

We hypothesized that 1) provenances from mesic site climate conditions (North Carolina for loblolly pine, and Coos Bay and Cascades for Douglas-fir) are more susceptible to drought

stress than provenances from more xeric site climates, assessed by measurements of electron transport rate, variation in chlorophyll fluorescence, water potential, and growth. Provenances associated with more xeric site climate conditions (Lost Pines and Alabama for loblolly pine and New Mexico for Douglas-fir) will demonstrate moderate to high drought resistance in the same measures. We hypothesized that 2a) previous drought exposure would lead to acclimation, and the expression of more conservative traits in seedlings, helping to maintain performance or mitigating its decline. These patterns were studied by comparing electron transport rate, fluorescence, water potentials and growth during a second drought in seedlings that had been previously exposed to drought and seedlings that were not previously exposed to drought. We predicted that 2b) during the second drought, the treatments that received two droughts as compared to treatments that only received the second drought would maintain higher electron transport rates and higher levels of variable chlorophyll fluorescence, less negative water potentials, and less growth decline during the second drought. We predicted that 2c) plasticity (in terms of ability to acclimate to drought stress) would be greatest in seedlings from mesic provenances, such as North Carolina for loblolly pine and Coos Bay for Douglas-fir. There are limits to plasticity in plants (Givnish 2002, van Kleunen and Fischer 2005, Valladares 2007) and provenances associated with more consistently xeric site climates may possibly already be expressing traits conducive to drought resistance to the limits of their adaptive abilities, and may not be able to afford further trade-offs. More mesic provenances may have more room to acclimate if they have not yet been pushed to their adaptive limits in their provenance.

METHODS

Plant Material

Loblolly pine (*Pinus taeda*) and Douglas-fir (*Pseudotsuga menziesii*) are among the dominant timber species in North America. Both species have broad natural geographic

distributions (Franklin and Dyrness 1973). Loblolly pine is native to the southeast United States, ranging from Texas, east to Florida and north to Delaware (Burns and Honkala 1990). To the west, this range is primarily limited by precipitation and to the north by winter temperatures. Of particular interest to this study are the Lost Pines of eastern Texas, a disjunct population from Bastrop County, Texas, about 160 km west of the continuous population. The Lost Pines receive on average 200-500 mm less annual precipitation than the rest of the range loblolly pine (Baker and Langdon 1990, Al-Rabab'ah and Williams 2004). Douglas-fir grows throughout the western United States. The coastal variety (*Pseudotsuga menziesii* var. *menziesii*) ranges from British Columbia and south into Santa Barbara County, California, and the interior variety (*Pseudotsuga menziesii* var. *glauca*) ranges inland to the Sierra Nevada and southern Rocky Mountains, and becomes increasingly disjunct as far south as northern Mexico (Lavender and Hermann 2014). Plant material studied was provided by Weyerhaeuser Company (Seattle, Washington) from three provenances for each of the two species, loblolly pine and Douglas-fir, on the basis of varying geographic location, and what were intended to be varying provenance site climate conditions.

Site climates associated with the respective provenances varied in mean annual or summer precipitation, climatic moisture deficit, and geographic location, though some provenances experienced similarities in annual precipitation (Table 1). The loblolly pine provenances are North Carolina (mesic site climate), Alabama (less mesic site climate), and an eastern Texas source Lost Pines provenance (disjunct source at the limit of the loblolly pine range with xeric site climate). These loblolly pine provenances will be referred to hereafter as North Carolina, Alabama, and Lost Pines provenances. The North Carolina and Alabama provenances represent half siblings, each derived from one open-pollinated mother tree. The North Carolina seed orchard is in Onslow County, NC including pollen sources from trees

selected for good growth properties on the Atlantic coastal plain. The Alabama provenance came from a seed orchard near Tuscaloosa, AL that included pollen sources from trees selected for good growth properties from the upland coastal plain. The Lost Pines provenance is self-seeded material from many possible open-pollinated mother trees in non-industrial woodland in eastern Texas. We estimated climate for the provenances by using ClimateWNA.com (Wang et al. 2012), a database that includes temperature and precipitation data from 1961-1999 (Table 1). The North Carolina and Alabama provenances have similar rainfall and mean annual temperature, but the North Carolina provenance has more summer precipitation and a lower computed climatic moisture deficit (CMD), which is the difference between reference evapotranspiration and measured precipitation (Wang et al. 2012). The Lost Pines provenance is from the highest elevation, has the lowest precipitation (both annual and summer) and the highest temperature, and so the climate moisture deficit is the highest of the three provenances. North Carolina is therefore considered to have the most moisture available (moist), followed by Alabama (mesic) and Lost Pines (xeric). The Lost Pines provenance have slightly more growing days above 5 °C, with the other two provenances similar in growing days. Loblolly pine were grown from seed in greenhouses in Centralia WA until the start of the experiment. Seedlings were, planted in 18 cm long x 5 cm diameter Cone-tainers (Stuewe and Sons, Tangent OR), in 80:20 peat perlite potting mixture, and sown on 8 April 2015.

For Douglas-fir, the provenances within this study are Coos Bay (coastal OR, mesic site climate), high-elevation Cascades (high elevation western Cascades, WA, mesic site climate) and Lincoln, New Mexico (xeric site climate). These Douglas-fir provenances will be referred to hereafter as Coos Bay, Cascades, and New Mexico provenances. Site climate conditions for Douglas-fir provenances were also estimated using ClimateWNA.com. The Coos Bay and Cascades provenances both have high precipitation, with the Cascades provenance receiving

more mean annual precipitation but slightly less summer precipitation than the Coos Bay provenance. Climatic moisture deficit is highest for the New Mexico provenance, followed by the Coos Bay provenance and then the Cascades provenance, although site climate conditions are more similar between the Coos Bay and Cascades provenances. The Coos Bay provenance ranges from lower elevations, and the Cascades and New Mexico provenances range from higher elevation. The New Mexico provenance receives lowest mean annual precipitation. The Coos Bay and New Mexico provenances have similar degree growing days above 5 °C, and the Cascades provenance has slightly fewer growing days (Table 1). Douglas-fir seedlings were provided by Weyerhaeuser Company, started from seed then grown in a Weyerhaeuser nursery (Mima, Washington, USA) for one year before being lifted and supplied as bare root transplants in April 2015. Seedlings in the Coos Bay and Cascades provenances represent half siblings, each derived from one open-pollinated mother tree with many potential father trees. The New Mexico provenance, however, is self-seeded material collected from the Lincoln National Forest from many possible open-pollinated mother trees. The Douglas-fir seedlings were kept in frozen storage, thawed, and transplanted into 25 x 7.5 x 7.5 cm containers in an 80:20 peat/perlite potting mixture on 24 April 2015.

Seedlings were regularly watered and fertilized with Jack's Professional (NPK 20:10:20), and allowed to grow under optimal conditions in Weyerhaeuser Company greenhouses (Centralia, Washington, USA) for approximately three months, until transport to the Oregon State University greenhouses (Corvallis, Oregon, USA 123.28 W, 44.57 N) on 26 June, 2015. Seedlings of each provenance were marked blindly with colored flagging at the time of planting, to reduce potential bias. Provenance color-codes were revealed only after completion of the experiment. Seedlings were allowed several weeks to acclimate to the Oregon State greenhouses,

with regular watering and fertilizing. Greenhouse temperatures were set to cool when air temperature exceeded approximately 25 °C, and received no supplemental light.

Study Design

In mid-July, rows of loblolly pine seedlings and individual Douglas-fir seedlings were assigned randomly to one of four treatments: no drought (ND), early drought (ED), late drought (LD), and two drought (2D). The no drought treatment received regular watering throughout the experiment. The ED treatment received only the first of two droughts, LD received only the second of two droughts, and 2D received both the first and the second drought. There were about 60 seedlings of each provenance in each treatment (about 720 to 800 seedlings of each species total).

Rows of loblolly pine seedlings and individual Douglas-fir seedlings of each of the three provenances and four treatments were marked with colored flagging to indicate treatment/provenances and were distributed randomly throughout one greenhouse bench to minimize edge effects and other variations in greenhouse environment. We randomized and sampled loblolly pine seedlings differently due to concerns about their smaller seedling size making them potentially more sensitive to sampling damage, and because the Cone-tainers were not freestanding. As a result, for loblolly pine, combinations of provenance and drought treatment were assigned randomly to rows on greenhouse benches with seven rows of 10 seedlings for each combination of treatment and provenance. In this way, seedlings could be destructively subsampled when necessary, while retaining sufficient plant material. Rows were consolidated with extra seedlings to maintain the same spacing and buffering throughout the experiment. As such, measurements represent averages of seedlings for rows for loblolly pine, with the row being the experimental unit, and represent individual seedlings for Douglas-fir, with the seedling

being the experimental unit. For both species, extra seedlings were placed at the edges of benches to form buffer rows.

Environmental Monitoring

Environmental conditions of the greenhouse were monitored with a Decagon (Pullman, Washington, USA) Weather Station (VP-3 sensor, PAR sensor, EM-50 datalogger) and logged every 5 minutes daily between 18 July and 18 October 2015. Greenhouse temperatures averaged 24.9 °C during July, 22.0 °C during August, 21.2 °C during September, and 23 °C during October. Light levels were relatively low, averaging about 300 $\mu\text{moles m}^{-2} \text{s}^{-1}$ of PAR and reaching a maximum of no more than about 450 $\mu\text{moles m}^{-2} \text{s}^{-1}$ on clear days (Fig A1).

We installed Decagon EC-5 soil moisture probes were installed in one pot for each of the four treatments for each provenance (12 total sensors) of Douglas-fir, and logged data to a Campbell Scientific (Logan, Utah, USA) CR-10X datalogger every 10 minutes from 22 July to 9 October 2015 (Fig A2). Because soil moisture values were only used for general monitoring, they are reported only in the appendix (Fig A2). Loblolly pine pots were too small to accommodate soil moisture probes. Reference weights were taken daily of subsamples for both species to monitor moisture loss; this weighing approach was driven by logistical consideration given the large sample sizes. Douglas-fir were weighed in trays containing 12 to 13 well-watered or 12 to 13 droughted seedlings to approximate weights by treatment, and loblolly pine seedlings were weighed in racks containing all watered or all droughted rows of seedlings. Seedlings were weighed individually as time permitted. Saturated (field capacity) pot weights and rack/tray weights were determined for both species at field capacity, to insure that weights were maintained above 75% of saturated weights for controls and in the well-watered conditions for the respective treatments in periods and treatments for which drought regimes were not being

imposed. Fertilizer was included in general every third watering (usually bi-weekly) with a watering frequency of about three times per week. We used Jacks Professional, and applied it diluted in water to 125 ppm N.

Drought Treatments

In this study, drought stress refers to conditions where soil moisture is limiting. The experimental design showing drought treatment timeline is shown in Figure 1. In the first drought (applied to ED and 2D) for the Douglas-fir seedlings, seedlings were saturated to field capacity on 5 August after which water applied was progressively reduced to avoid shock from rapid dry down and to simulate increasingly droughted conditions. Water content was tracked by tray weights (as described above), and held at about 75% of saturated weight through minimal watering and occasional light misting for 10 days. Weights were then allowed to decrease to 60% of saturated weight for five days and then 50% of saturated weights for 10 days by completely withholding water. Douglas-fir seedlings were re-watered to field capacity on 30 August, 2015. Seedlings that were not being droughted during the first drought (ND and LD treatments) were watered normally, about every two to three days, to insure that tray weights did not drop below 75% of saturated weight. After 30 August, droughted seedlings were allowed to recover and watered normally and all treatments were fertilized. Periods following drought were termed the recovery. The second drought treatment (applied to LD and 2D) was started on 10 September following the same procedure as the first drought, and ended on 8 October. Seedlings were again allowed to recover for about 10 days, at which point the experiment was ended (Fig 1). General qualitative observations of foliage and wilt were made throughout the experiment.

The droughts for the two species were staggered for logistical reasons. Drought treatments, however, followed the same protocol for both species except that loblolly pine

required more gradual reductions in watering due to smaller pot sizes that were more prone to drying quickly. The first drought treatment (given to ED and 2D) was started for loblolly pine on 10 August and ended on 3 September. Seedlings were allowed to recover, and then the second drought treatment (given to LD and 2D) was started on 13 September and ended on 1 October. For loblolly pine, the second drought was slightly more severe than the first drought as judged by more negative water potentials. General qualitative observations of foliage and wilt were made throughout the experiment.

Physiological Measurements

Water Potentials: Predawn and midday water potentials were measured at the end of both droughts in both species using the Scholander pressure chamber method (PMS Instrument Company, Albany, OR). Predawn water potentials were measured between 4:00 AM and 7:00 AM (PST), prior to sunrise. Midday water potentials were measured between 11:30 AM and 2:00 PM. Seedlings measured for predawn and midday water potentials were selected randomly from each treatment/provenance combination. For loblolly pine, because seedlings were young and most had not yet branched, water potentials were measured on the youngest, fully mature fascicles, generally in the upper two thirds of the stem. For Douglas-fir, water potentials were measured on small branch segments (~1.5 cm long) taken from the main stem, approximately midway from the base of the seedling. Target sample sizes were seven seedlings (each from a different row representing each treatment/provenance combination) for loblolly pine and five seedlings from each treatment/provenance combination for Douglas-fir.

Fluorescence and Electron Transport Rate (ETR): Ambient and dark-adapted fluorescence (calculated as both F_v/F_m and F_v/F_o) were estimated using an integrated fluorometer and gas exchange system (ADC BioScientific/Opti-Sciences iFL, England, UK) throughout the

drought treatments and recovery periods. Fluorescence is an indicator of abiotic stresses, such as heat stress and water stress. Energy from absorbed photons can be dissipated by three pathways: heat, photochemistry, and fluorescence. An increase in efficiency of one of these pathways results in a decrease in the efficiency of the other pathways. Thereby, measurements of chlorophyll fluorescence can reveal information about photochemistry and heat dissipation. Measurements of dark-adapted fluorescence calculated as F_v/F_m provides the ratio of variable (F_v defined as $F_m - F_o$ where F_m is maximum fluorescence and F_o is minimum fluorescence) to maximum (F_m) fluorescence and by extension the quantum efficiency of open photosystem II centers, which in many cases can provide information about relative changes in the status of photosystem II which can be related to reductions in photosynthetic rate from optimal conditions. A sustained decrease in dark-adapted F_v/F_m and increase in F_o has been used to detect water stress (Ogren 1990, Maxwell and Johnson 2000).

Electron transport rates (ETR) were estimated during the recovery period following the first drought and during the second drought and recovery period (about six to eight days following re-watering). ETR was measured using rapid light curves stepwise by PAR at intervals of $100 \mu\text{moles m}^{-2} \text{s}^{-1}$, from PAR 100 up to PAR $1600 \mu\text{moles m}^{-2} \text{s}^{-1}$ with the iFL, with target sample sizes of five to seven seedlings. Preliminary light curves showed that seedlings reached light saturation by approximately PAR 1200 for loblolly pine and PAR 1000 for Douglas-fir. ETR is directly related to photosystem II efficiency ($Y(\text{II})$), which is a light-adapted measure of plant stress representing energy use for photochemistry of photosystem II under steady-state light, and is correlated to carbon assimilation linearly for most C4 plants and curve-linearly for most C3 plants under stress (Genty et al. 1989, Genty et al. 1990, Krall and Edwards 1992). ETR, shown to be sensitive to drought stress, is then calculated from $Y(\text{II})$ via the equation of $Y(\text{II})$ quantum efficiency (calculated as fluorescence yield) \times PAR \times 0.84 (a , average ratio of

light absorbed by leaf, termed the leaf absorption coefficient, for C3 plants) x 0.5 (average ratio of photosystem II to photosystem I reaction centers, with transport of one electron requiring absorption of two quanta via two photosystems) outlined as: $ETR \text{ (sometimes called } J_T) = \Delta F/F_m' * PFFD * a * 0.5$ (Flexas et al. 1999, Iacono and Sommer 2000). Maximum electron transport rates (ETR_{max}) were calculated using the average of the three highest electron transport rates for each curve, and subsequently averaged for treatment and provenance.

Dark-adapted fluorescence and ETR were measured on the youngest, fully mature needles for both species, generally mid-drought during the second drought, at the end of the second drought, and during the recovery from the second drought. Exact dates are provided in all figures and tables for both fluorescence and ETR. For dark-adapted fluorescence measurements, needles were collected (excised), placed in envelopes and kept in the dark for approximately two hours before making measurements ($n = 5$ to 9 for each combination of treatment and provenance). For ETR, needles were measured attached to the seedlings ($n = 5$ to 9 for each combination of treatment and provenance).

Growth Measurements

Height was measured weekly by ruler, from the base of the stem to the base of the terminal bud on all seedlings. Basal stem diameter was measured with digital calipers in mid-July and at the end of each drought. Biomass was collected prior to the start of the first drought, at the end of the first drought, at the end of the recovery period (before the second drought) and at the end of the second drought. Target sample sizes were seven seedlings per provenance per treatment for loblolly (one seedling from each row), and 5 seedlings per provenance per treatment for Douglas-fir. Roots were washed to remove soil, then separated from the tops at the root collar with clippers. Shoots and roots were placed in paper bags, and oven-dried at 60°C for

at least five days. Dry shoots were then separated into needles and stems. Biomass was weighed separately for roots, stems, and needles. Total biomass was calculated as roots plus stems plus needles. Root/shoot biomass was calculated as root biomass divided by needle and shoot biomass combined. Relative growth rate was calculated as total biomass at the end of the second drought subtracted from total biomass at the end of the first drought, all divided by the total biomass at the end of the first drought; this quotient was not compared statistically.

Statistical Analysis

A linear mixed model was used to analyze ETR, F_v/F_m , water potential, heights, and biomass data from the study, always separately for the two species, with four levels of the factor “Treatment” (ND, ED, LD, and 2D) and three levels of the factor “Provenance” (North Carolina, Alabama, and Lost Pines for loblolly pine and Coos Bay, Cascades, and New Mexico for Douglas-fir). Assumptions of independence, equal variance, and normality were checked for the various physiological datasets and found to be acceptably met. All statistics were run using R 3.2.3 (R Core Team 2016).

Although the interaction between treatment and provenance was not always statistically significant, we investigated both the main effects level (treatment) and at the interaction level (treatment by provenance) for two reasons. First, patterns appeared to differ subtly by provenance, sometimes in unique or opposing ways which would be lost by pooling to the main effect (or treatment) level. Second, the study was designed to seek the effects of treatments on the provenances, not only on the species.

Further measurements and results not included in the results section are provided in the Appendix A (data and analyses for which methods are described in this chapter) and Appendix B

(methods, data, and analyses for which methods are not described in this chapter); tables and figures are listed, for example, as Table A1 (in Appendix A) or Figure B1 (in Appendix B). Appendix C catalogues how and where data, programs, and samples are archived.

RESULTS

Observations on Foliage Coloration and Wilt:

In loblolly pine, we observed wilt towards the later-half of the first drought (D1), and particularly during the second drought (D2). Wilt was especially evident in the late drought (LD) treatment, along with needle speckling. Speckling appeared as small, discolored spots along needles. Foliar damage and wilt was most apparent in the North Carolina coast provenance, though we observed it in all provenances. During the second drought, top dieback occurred in loblolly pine and was especially evident in the LD treatment, although it appeared in both LD and two drought (2D) treatments. In Douglas-fir, minimal wilt was observed during the first drought, generally towards the last days of drought, and was most common in the Coos Bay provenance followed by the Cascades provenance. Wilt was rare in the New Mexico provenance. There was no noticeable branch die-back. There was slight needle speckling, usually in the Coos Bay provenance, with some occurring in the Cascade provenances, especially during the second drought. Needles of New Mexico seedlings were in general bluer in hue, and somewhat shorter and thicker than the Coos Bay and Cascade seedlings.

Water Potentials

Water potentials were generally more negative at midday (Ψ_m) than predawn (Ψ_p) for both species and all provenances (Tables 2, 3). For loblolly pine, differences between Ψ_p and Ψ_m were about 0.40 MPa, and for Douglas-fir between 0.60 and 0.85 during D1. During D2, for loblolly pine differences in Ψ_p and Ψ_m ranged from 0.04 to 0.48 MPa, usually more negative in

Ψ_m , although there were several instances where Ψ_p was more negative than Ψ_m (Table 2). For Douglas-fir during D2, differences in Ψ_p and Ψ_m ranged from zero to 0.85 MPa, usually with more negative values in Ψ_m than Ψ_p , but also with some instances of the reverse. For loblolly pine, Ψ_p and Ψ_m during D1 were similar for a given treatment for the three provenances (Table 2). Average Ψ_p in the D1 drought treatments (experienced by both ED and 2D) ranged from provenance means of -1.64 to -1.75 MPa at the end of approximately three weeks of drought (Table 2). At that same period for Douglas-fir, the Coos Bay and Cascade provenances demonstrated similar Ψ_p , -1.75 and -1.65 MPa respectively, but the New Mexico provenance had less negative values (-1.05 MPa, Table 3).

At the end of D1, the only statistically significant differences in Ψ_p for either species were for treatment ($p < 0.00001$). At the end of D2, only treatment was significant for loblolly pine only treatment ($p < 0.00001$). However, for Douglas-fir at the end of D2, treatment ($p < 0.00001$), provenance ($p < 0.005$), and the interaction between treatment x provenance ($p < 0.05$) were all statistically significant (Table A1, A2).

Loblolly pine 2D vs LD: In loblolly pine, at the end of D2, there was a tendency for the LD treatment to exhibit more negative Ψ_p than the 2D treatment (Table 2, Table A3). Average Ψ_p of the provenances during D2 ranged between -2.40 and -2.66 MPa in the LD treatment, and between -2.14 and -2.30 MPa in the 2D treatment (Table 2). However, estimates of the differences between the 2D and LD treatment for Ψ_p at the end of D2 for loblolly pine were not statistically significant for any of the provenances, and are shown at the treatment level (Fig 2B, Table A3).

Douglas-fir 2D vs LD: In Douglas-fir, at the end of D2, there was also some tendency for the LD treatment to exhibit more negative Ψ_p than the 2D treatment (Table 3, Table A4). Average Ψ_p of the provenances at the end of D2 ranged from -1.75 to -3.43 MPa in the LD treatment, and from -2.48 to -2.92 MPa in the 2D treatment (Table 3). Estimates of differences in Ψ_p between the 2D and LD treatments at the end of the D2 were not statistically significant for the Coos Bay or Cascades provenances, but were statistically significant for the New Mexico provenance ($p < 0.05$). However, unlike the pattern of more negative Ψ_p in the LD treatment than the 2D treatment in other provenances, it is estimated that the LD treatment was 0.73 MPa (95% C.I. = -0.050 to 1.40) *less* negative for the New Mexico provenance (Fig 2F, Table A4).

Electron Transport Rates (ETR Rapid Light Curves)

Provenances: Drought-stressed seedlings of both species demonstrated lower electron transport rates (ETR) and lower maximum electron transport rates (ETR_{max}) than watered controls, as measured by rapid light curves of ETR with increasing PAR.

In loblolly pine, the watered controls (ND) in the North Carolina provenance often reached slightly higher ETR_{max} than did the seedlings of the same treatment in the Alabama provenance, whereas the Lost Pines provenance often demonstrated the lowest ETR_{max} (Fig 3). In measurements taken mid-drought during D2 for loblolly pine, the North Carolina provenance was the first to show a noticeably lower ETR_{max} in the droughted treatments relative to well-watered control levels, most noticeably in the LD treatment (Fig 3A). The Lost Pines provenance was also affected by drought as evidenced by low ETR_{max} as drought progressed, and the Alabama provenance tended to maintain highest ETR_{max} during drought. Loblolly pine seedlings from all provenances eventually demonstrated lowered ETR_{max} in response to drought (2D and LD), and a gradual though not always immediate recovery to watered control (ND) levels of

ETR following release from drought. At the end of the experiment, there was also no evidence for lingering differences in ETR_{\max} between early drought (ED) and no drought (ND) controls, and comparisons of ED and ND treatments not significant.

Overall, however, there was not strong statistical evidence for an interaction between treatment and provenance during or after drought for ETR_{\max} in loblolly pine, and differences were sometimes small. In ETR_{\max} measured mid-drought (22 Sept) during D2, for example, there was no statistical evidence for an interactive effect of drought treatment and provenance, and no evidence for an effect of provenance, but moderate evidence for an effect of drought treatment ($p < 0.05$, Table 4). Interactive effects and provenance effects were similar to the results displayed above when measured mid-drought as when measured at the end of drought and during recovery following drought, though there was evidence for an effect of provenance during the recovery from drought ($p < 0.05$, Table 4).

For Douglas-fir, treatment, provenance and the interaction of treatment x provenance all significantly affected ETR_{\max} as measured mid-drought during D2 (2 Oct, $p < 0.001$), and at the end of D2 (5 Oct, $p < 0.0001$, Table 5). During the recovery from D2 (15 Oct), only treatment and provenance significantly affected ETR_{\max} ($p < 0.05$). The New Mexico provenance generally reached highest levels of ETR_{\max} among the ND controls, with the Cascades and Coos Bay provenances performing at similar levels of ETR_{\max} . In droughted treatments (2D and LD), the New Mexico provenance maintained highest rates of ETR_{\max} , followed by the Cascades provenance and then by the Coos Bay provenance.

Loblolly pine 2D vs LD: In loblolly pine, seedlings exposed to previous drought (2D) maintained slightly higher ETR_{\max} (4 to 18 $\mu\text{mol electron m}^{-2} \text{s}^{-1}$) during and after subsequent

drought compared to seedlings that experienced only a late drought (LD). For example, in measurements of ETR using rapid light curves taken mid-drought during D2 (22 Sept), the LD treatment in the North Carolina provenance exhibited lower ETR_{max} compared to watered control (ND) levels and to the 2D treatment. The LD treatment in the North Carolina provenance exhibited low ETR_{max} relative to the watered control (ND) as measured during the second drought before the 2D treatment similarly showed low ETR_{max} relative to the watered control (Fig 3). The difference in ETR_{max} between the 2D and LD treatments in the North Carolina provenance, measured mid-drought (22 Sept) during D2, was statistically significant ($p < 0.05$), though not in the Alabama provenance or the Lost Pines provenance (Table 6). When all provenances were pooled overall by treatment at the main effects level, the difference in ETR_{max} between LD and 2D treatments as measured mid-drought (Sept 22) during the second drought was also significant ($p < 0.05$, Table 6). Near the end of D2 (measured 28 Sept) there was little difference between LD and 2D treatments in any of the provenances, with all droughted treatments (2D and LD) having declined to comparable levels of ETR (around $40 \mu\text{mol electrons m}^{-2} \text{ s}^{-1}$) relative to watered controls (ND) in the final days, and with 2D treatments maintaining rates only slightly higher than LD treatments. However, estimates of the difference in ETR_{max} between the 2D and LD treatments measured one week after the end of D2 (10 Oct) were statistically significant for the North Carolina ($p < 0.05$) and for Lost Pines provenance ($p < 0.05$), but not for the Alabama provenance. During the recovery period, measured one week after the end of D2 (10 Oct), drought treatment overall had a significant effect on ETR_{max} ($p < 0.0000005$), with the difference in ETR_{max} between the LD and 2D treatments overall also statistically significant ($p < 0.005$, Table 6).

Douglas-fir 2D vs LD: For Douglas-fir, as with loblolly pine, differences in ETR_{max} between 2D and LD treatments were not always significant in all provenances, although a similar

and relatively consistent pattern was observed in the Coos Bay provenance: the 2D treatment typically maintained slightly higher ETR_{max} than the LD treatment during and following drought. There was no evidence for a difference between LD and 2D treatments in the Cascades provenance. However, the New Mexico provenance showed the *opposite* pattern to that of the Coos Bay provenance, with a lower ETR_{max} in the 2D treatment than in the LD treatment near the end of D2 (Fig 4). This difference between 2D and LD treatments for the New Mexico provenance had borderline statistical significance ($p = 0.073$) as measured during the recovery from D2 (Table 7).

Fluorescence (F_v/F_m)

Dark-adapted fluorescence (F_v/F_m) was not particularly sensitive to drought stress, and often remained around a “healthy” level 0.80 with small deviations below the healthy level during drought. Ratios calculated as F_v/F_o (variable/minimum fluorescence) were somewhat more revealing of differences (Fig A6, A7). We chose to show F_v/F_m because this is the most commonly used ratio for dark-adapted fluorescence, and because ratios of F_v/F_m and F_v/F_o both represent the same data.

For loblolly pine, treatment had a significant effect on F_v/F_m as measured mid-drought on 23 Sept ($p < 0.00005$) and near the end of D2 (27 Sept, $p < 0.0000005$) but there were no significant effects of provenance or the interaction of treatment x provenance. For Douglas-fir, treatment had a significant effect on F_v/F_m on both 3 Oct and 8 Oct ($p < 0.005$). Provenance had a borderline significant effect on F_v/F_m mid-drought on 3 Oct ($p = 0.085$) but not on 8 Oct. The interaction of treatment x provenance significantly affected F_v/F_m in Douglas-fir on 3 Oct ($p < 0.05$) and with borderline significance on 8 Oct ($p = 0.079$). The New Mexico provenance demonstrated the smallest decreases in F_v/F_m in droughted treatments relative to watered

treatments, followed by the Cascades, with largest differences between Coos Bay droughted and watered treatments (Fig 5, Table A6).

Loblolly pine 2D vs LD: F_v/F_m followed a similar pattern to ETR, with LD often showing lower values than 2D treatments during D2. At the overall treatment level, F_v/F_m was significantly lower in the LD treatment than in the 2D treatment on both dates measured during D2 ($p < 0.05$, Fig 5, Table A5). Estimates of differences between LD and 2D treatments during D2 were statistically significant for F_v/F_m both mid-drought and at the end of D2 in the Alabama provenance ($p < 0.05$) and had borderline significance mid-drought ($p = 0.064$), and were significant near the end of D2 for the Lost Pines ($p < 0.05$).

Douglas-fir 2D vs LD: Patterns in fluorescence measurements in Douglas-fir were less apparent. Overall, for all treatments pooled as measured during D2 on 3 Oct, F_v/F_m was not significantly lower in the LD treatment than 2D treatment. The LD treatment was significantly lower than the 2D treatment for Coos Bay ($p < 0.005$), though not for the Cascades or the New Mexico provenances on 3 Oct. By the final days of D2 (8 Oct), the LD treatment in the Coos Bay provenance showed significantly lower values than the 2D treatment ($p < 0.0005$). It is estimated that on 8 Oct, F_v/F_m was 0.124 lower (95% C.I. = 0.064 to 0.184) in the LD treatment of the Coos Bay provenance than in the 2D treatment. The Cascades provenance also began to show decreased values in the droughted treatments (LD and 2D) as compared to watered treatments (ND and ED) during the course of D2, but there was no statistically significant difference between LD and 2D treatments by the end of D2. The New Mexico droughted treatments (LD and 2D) during D2 exhibited F_v/F_m values similar to watered levels (ND and ED) throughout D2, and with no significant differences in LD and 2D treatments by the end of D2 (Fig 5G, Table A6).

Biomass

Loblolly pine: Biomass accumulation at the end of the second drought (D2) showed some effects of drought in loblolly pine. Total overall biomass at the end of D2 was significantly affected by treatment ($p < 0.0000005$) and provenance ($p < 0.05$), but there was no significant interaction (Fig 6, Table A7). Needle, stem, and root biomass in all three provenances at the end of D2 were greatest in the ND treatments. Needle, stem, and root biomass were consistently lowest in the 2D treatment at the end of D2, and among provenances these values were highest in the ND treatment of the Alabama provenance. Values of stem and root biomass were lower in the Lost Pines provenance than the other provenances, and needle biomass values were lower in the North Carolina provenance than in the Lost Pines provenance (Fig 7). At the end of D2, needle biomass was significantly affected by treatment ($p < 0.00000005$), but not significantly affected by provenance or the interaction of treatment and provenance. Root biomass was also significantly affected by treatment ($p < 0.005$), but not by provenance or the interaction of treatment x provenance. For stem biomass, there was a significant effect of treatment ($p < 0.0000005$) and of provenance ($p < 0.000005$), but not of the interaction of treatment x provenance (Table A9).

There was no evidence for effects of treatment, provenance, or an interactive effect on root/shoot biomass, nor were there large differences among treatments or provenances, except in the Lost Pines provenance where there was a significantly higher root/shoot biomass for the 2D treatment relative to the watered (ND) treatment ($p < 0.05$, Fig 6G). Relative growth rates were greatest in the ND treatment, with droughted treatments (ED, LD, and 2D) all showing lower rates in the North Carolina and Lost Pines provenances (Fig 8A-D). In the Alabama provenance, relative growth rates were greatest in the ED treatment, and other treatments (ND, LD, and 2D) were similar to each other.

Douglas-fir: There was a borderline significant effect of provenance ($p = 0.056$) on total overall biomass at the end of D2, but no evidence for an effect of treatment or treatment x provenance (Table A8). In the New Mexico provenance, total overall biomass was lower in the ED and LD treatments, and similar in ND and 2D treatments (Fig 9). The 2D treatment showed largest values of needle biomass across the three provenances (Fig 10). Root biomass at the end of D2 was largest in the ED treatment among treatments in the Coos Bay and Cascade provenances, but lowest in the New Mexico provenance; root biomass was borderline significantly lower in the New Mexico ED treatment compared to the ND treatments ($p = 0.057$, Table A10). The ND, LD, and 2D treatments showed similar root biomasses among provenances. There was no evidence for a statistically significant effect of treatment, provenance, or the interaction of treatment x provenance on Douglas-fir stem biomass harvested at the end of D2 (Table A10). Stem biomass was similar among treatments within the Coos Bay and Cascade provenances, but were largest in the 2D treatment for the New Mexico provenance. Needle biomass was significantly affected by treatment ($p < 0.05$), but not by provenance or the interaction of treatment by provenance. There was no evidence for a statistically significant effect of treatment or the interaction of treatment x provenance on root biomass as harvested at the end of D2, but there was evidence for a significant effect of provenance ($p < 0.005$). There was a significant effect of provenance on root to shoot biomass ($p < 0.05$), but no evidence for a significant effect of treatment or the interaction of treatment x provenance. Relative growth rates were greatest in the ED treatment for the Coos Bay and Cascades provenances, though relative growth rate was negative and low in the New Mexico provenances for ED as well as LD treatments (Fig 8 E-H).

Loblolly pine 2D vs LD: For loblolly pine, there was not strong evidence for significant differences between LD and 2D treatments in total overall biomass among any of the

provenances at the end of D2 (Fig 6), though there was a statistically significant difference between 2D and LD total overall biomass at the treatment level with all provenances pooled ($p < 0.05$, Table A11). There was not strong evidence for statistically significant differences between 2D and LD treatments in any of the provenances for needle, root, or stem biomass harvested at the end of D2 (Table A9). There was also not strong evidence for significant differences between LD and 2D treatments in root/shoot biomass among any of the provenances at the end of D2 (Table A12). Relative growth rate was slightly lower in the 2D treatment than in the LD treatment (Fig 8A-D).

Douglas-fir 2D vs LD: For Douglas-fir, there was no evidence for differences in total biomass between LD and 2D treatments for the Coos Bay or Cascades provenances, but there was significantly lower total biomass in the LD treatment than the 2D treatment for the New Mexico provenance ($p < 0.05$, Table A13). There was no evidence for statistically significant differences between 2D and LD treatments in any of the provenances for needle, root, or stem biomass harvested at the end of D2, though there was borderline significance for differences between 2D and LD needle ($p = 0.071$) and stem ($p = 0.069$) biomass in the New Mexico provenance (Table A10). There was also no evidence for statistically significant differences between ND and 2D treatments or between ND and LD treatments for needle, root, or stem biomass harvested at the end of D2 in the Douglas-fir provenances, with the exception of a significant difference between ND and 2D and between ND and ED needle biomass in the Cascades provenance ($p < 0.05$, Table A10). There was not strong evidence for differences in root/shoot biomass between LD and 2D treatments in any of the provenances, although there was a borderline significantly lower ($p = 0.072$, Table A14) root/shoot biomass in the Cascades provenance. Relative growth rates were similar between LD and 2D treatments in the Coos Bay provenance, and somewhat higher in the 2D treatment for the Cascades provenance (Fig 8E-F).

In the New Mexico provenance, relative growth rate was slightly negative in the LD treatment, but positive in the 2D treatment.

Heights

Loblolly pine: Provenances were initially significantly different in height prior to any drought treatments, with initial and final heights lowest in the Lost Pines provenance in all treatments ($p < 0.0000001$, Table A15, Fig 11A). Final heights for the North Carolina and Alabama provenances were similar in both watered and droughted treatments. There was a significant effect of treatment and provenance on final heights ($p < 0.00005$), but not a significant interaction (Table A15). For all provenances, final heights were lower in the ED and 2D treatments than in ND and LD, which were similar to each other (Fig 11A). ED was significantly smaller than LD in final heights in all provenances ($p < 0.05$), and 2D was significantly lower than LD in the Alabama and Lost Pines provenances ($p < 0.05$, Table A18).

Douglas-fir: Provenances were also significantly different in initial height, with initial and final heights lower in the New Mexico provenance ($p < 0.00005$, Table A16, Fig 11B). Although treatments were assigned randomly, in the Cascades provenance the 2D treatment was also significantly shorter initially than other treatments ($p < 0.005$, Table A19). There was no evidence for an effect of treatment on final heights, but there was a significant effect of provenance ($p < 0.005$, Table A19). Final heights were similar between the Coos Bay and Cascade provenances, though slightly larger in the Coos Bay provenances in all treatments. For the Coos Bay and Cascades provenances, final heights were lower in 2D compared to other treatments. However, differences in final heights among treatments were mostly similar among treatments in the Coos Bay provenance. In the New Mexico provenance, final heights were similar among ND, ED, and 2D, with slightly lower values in LD (Fig 11B).

DISCUSSION

Provenance drought resistances:

Results show some support for the hypothesis that the respective provenances of loblolly pine and Douglas-fir seedlings exhibit differences in drought resistance in association with site climate conditions, based on responses in a greenhouse scenario. However, there were not strong provenance differences for loblolly pine in this study, though there was more evidence for provenance differences in Douglas-fir. For both species, general patterns suggest that provenances associated with more mesic site climates (high mean annual precipitation and low climatic moisture deficit among the respective provenances) exhibited somewhat lower drought resistances as compared to provenances associated with more xeric site climates (low mean annual precipitation and high climatic moisture deficit among the respective provenances). For example, the Alabama provenance (slightly less mesic) for loblolly pine and the New Mexico provenance (xeric) for Douglas-fir both appeared to perform somewhat higher under drought, based on some of the physiological measures (electron transport rate and dark-adapted fluorescence) in this study. The North Carolina provenance (mesic) for loblolly pine and the Coos Bay and Cascades provenances (mesic) for Douglas-fir exhibited lower resistances as characterized by the physiological measures used in this study.

There were not, however, significant differences in predawn water potentials (Ψ_p) between loblolly pine provenances, though there were significant provenance differences in Ψ_p for Douglas-fir during the second drought (D2). For example, differences in Ψ_p between provenances in droughted treatments of loblolly pine during D2 ranged from 0.10 to -0.36 MPa. For Douglas-fir, differences between provenances in Ψ_p in droughted treatments ranged from 0.14 to 1.68 MPa, with the most negative Ψ_p in the Coos Bay provenance, followed by the Cascades provenance, and least negative in the New Mexico provenance. It might be expected

that more drought resistant provenances would show less negative water potentials (especially if employing, for example, a drought avoidance strategy that might reduce water use by shutting down). Alternatively, drought tolerant provenances could in theory show more negative Ψ_p by continuing to operate as soil moisture decreases but maintaining function and water transport. The New Mexico provenance showed less negative Ψ_p among Douglas-fir provenances and less effect of drought on ETR and F_v/F_m during D2 than other provenances, perhaps suggesting avoidance of negative water potentials.

To put water potentials (Ψ) observed in this study into context, turgor loss points for loblolly pine have been observed around osmotic potentials of -1.5 MPa (Bongarten and Teskey 1986). In Douglas-fir, turgor loss can occur between Ψ_p of about -2 and -2.5 MPa and considerable embolism has been observed between -1.3 and -3.4 MPa (Kubiske and Abrams 1991). Roots can be more vulnerable to embolism than stems, embolizing at less negative water potentials, around -2.09 for small roots of Douglas-fir trees (Sperry and Ikeda 1997) and likely at even less negative values for seedlings (between -1 and -1.4 MPa). For loblolly pine, 50 to 75% losses of conductivity have been observed in roots at -1.5 MPa (Hacke et al. 2000). Thus, it is likely that all provenances in both species did experience some level of stress during D2. Although differences in Ψ_p were not always as large as expected between provenances, observed differences could still possibly impart physiological or biological effects, especially when approaching turgor loss points or Ψ_p values beyond which might increase occurrence of considerable embolism. Overall, however, drought resistances across provenances did not differ as drastically as anticipated and likely require additional measures to more fully characterize possible differences in resistances.

For loblolly pine, the Lost Pines provenance has often been considered a high drought performer and drought resistant due to its persistence at a disjunct dry site. Past studies of Lost Pines seedlings have shown high resistance under drought, and in some studies have shown higher values of transpiration, stomatal conductance, and plant hydraulic conductivity under watered conditions, and lower values of water conduction under dry conditions, suggesting that maintenance of high water conduction when water was available also contributed to their survival (Bongarten and Teskey 1986, Wakamiya-Noborio et al. 1999). However, the Lost Pines were found to be only moderately drought resistant in regards to electron transport rate (ETR), water potentials, and growth (height and biomass) in this study. For example, the Lost Pines did not show particularly high maximum ETR (ETR_{max}) in the watered control (ND), at least in comparison to other provenances and did not maintain significantly less negative Ψ_p than the other provenances. The Alabama provenance maintained somewhat larger height growth, relative growth, and biomass production than the Lost Pines. The North Carolina provenance for loblolly pine, in contrast, showed lower ETR_{max} earlier than the other two provenances, which may suggest greater drought susceptibility. Nonetheless, overall, differences in resistance between provenances were not as great as expected in terms of physiological responses to drought in loblolly pine, and evidence presented herein remains tentative. In the case of drought scenarios, however, it is possible that even small differences in performance may potentially influence seedling survival or in the outlasting of a drought period, and a greater suite of measurements may better characterize differences.

Moderate drought resistance combined with slightly higher relative growth rates and ETR (suggestive of photosynthetic activity) in the Alabama provenance compared to the Lost Pines may be of interest with regard to planting selection decisions or with regard to seedling survival and regeneration. Higher relative growth rates in the Alabama provenance droughted treatments

(ED, LD, and 2D) than in droughted treatments of the other two provenances also suggest that the Alabama provenance was more drought resistant, although these measures were not compared statistically. Disjunct “drought-adapted” provenances may appear appealing choices in environments with projected increases in the frequency and severity of drought, as well as for crossing with other populations to enhance drought performance. This drought resistance may possibly come at the expense of growth rates and carbon gain, both of which seem to be more conservative in the Lost Pines for loblolly pine and also in the New Mexico Douglas-fir provenance in this study. For example, loblolly pine from mesic sites such as Louisiana typically exhibit faster growth under moderate drought stress compared to Texas sources and despite greater reported drought susceptibility in mesic sources (Newton et al. 1985, Teskey et al. 1987). The more conservative nature of the Lost Pines, as demonstrated by slower growth and water conserving characteristics, could be a strategy that has aided their persistence in such a droughty environment, west of the rest of its geographic range. Similarly, in long-term studies Douglas-fir provenances from more xeric southern Oregon have shown slower growth than more mesic provenances (White and Ching 1985). Kerr et al. (2015) discussed similar findings in a study of Ponderosa pine (*Pinus ponderosa*) from mesic and dry sites, noting that dry site seedlings demonstrated more conservative behavior compared to mesic site seedlings.

For Douglas-fir, results were similar to loblolly pine with respect to provenance and observed drought resistance patterns, with more mesic provenances appearing less resistant and the more xeric provenance more resistant. Whereas differences were sometimes subtle in loblolly pine, the New Mexico provenance (xeric site climate) exhibited higher apparent drought resistance than the other two provenances. For example, in greenhouse conditions especially during the first drought (D1), drought did not induce as negative Ψ in the New Mexico provenance as compared to the other two provenances (Coos Bay and Cascades) despite

receiving the same limited watering regime and dry-down process. This reaction suggests that the New Mexico provenance was perhaps more conservative in water use and able to prevent as negative water potentials. However, the New Mexico provenance grew more slowly than the other two provenances. Additionally, droughted seedlings of the New Mexico provenance showed some signs of decreased performance during the second drought compared to during the first drought, as shown by water potentials and ETR. Whereas during D1, Ψ_p in the New Mexico droughted treatment did not become as negative as in other provenances relative to the watered control (ND) and remained around -1.05 MPa, during D2 decreases in Ψ_p did show sensitivity to drought and somewhat greater decreases than in D1, though still less negative than the other two provenances. Moreover, the New Mexico provenance was the only provenance to show lower ETR_{max} in the two drought (2D) treatment than in the late drought (LD) treatment during the second drought and after in the week following re-watering. These trends in the New Mexico provenance are intriguing; especially when considering the site climate conditions of the New Mexico provenance, which typically experience a dry early summer followed by a monsoon season leading into the fall. Perhaps the New Mexico provenance demonstrates a strategy reflective of anticipating rain late in the season and focusing its drought resistance strategy early in the season. This could include employing the drought avoidance aspect of drought resistance early in the summer when moisture is most limiting. During the late summer and early fall, then, it may perhaps exhibit more drought susceptible behavior in anticipation of late summer precipitation as compared to more drought resistant behavior earlier in the summer.

The Cascades and Coos Bay provenances were largely similar in their observed drought resistances, though in fluorescence (F_v/F_m) there were somewhat lower values in the Coos Bay provenance drought treatments (LD and 2D) compared to the other two provenances during the second drought. Furthermore, both the Coos Bay and Cascades provenances are associated with

similar site climate conditions, with some overlap in range of conditions. The range of site climate conditions for the Cascades provenance does include slightly greater mean annual precipitation (MAP) and lower climatic moisture deficit (CMD) than the Coos Bay provenance. Because the Coos Bay and Cascades provenances were associated with a range of site climate conditions rather than a specific site, there is also some uncertainty as to the differences between the site climates for the two provenances. There are also fewer degree growing days above 5 °C for the Cascades provenance than the Coos Bay provenance. However, given higher CMD in the Coos Bay provenance, it may be somewhat unexpected to observe lower drought resistance relative to the Cascades provenance. For the Coos Bay and Cascades provenances, MAP and CMD may also not be the strongest or only factors associated with drought resistance, or may have resulted in similar drought resistances through different environment factors. Temperature and elevation in particular, for example, likely play a role in drought resistance. White (1987) found higher elevation Douglas-fir seedlings to better survive drought. Such results would align with the results of this study, as the Cascades provenance was associated with a higher elevation range, lower mean annual temperatures, and fewer degree growing days above 5 °C, and possibly showed higher drought resistance in chlorophyll fluorescence than the Coos Bay provenance. Furthermore, if higher elevation provenances like the Cascades provenance are closely adapted to colder growing conditions and greater cold hardiness, which are adaptations that overlap with drought resistance (Bansal et al. 2016), then higher elevation provenances like the Cascades provenance may be unable to balance further trade-offs thus demonstrating less plasticity. This could be one explanation for provenance differences and for why there was less evidence for acclimation in ETR in the Cascades provenance, and more evidence in the Coos Bay provenance.

Regarding the drought resistances, this study considered young seedlings at only one age (half a year for loblolly pine and one and a half years old for Douglas-fir), and therefore provides

only one specific window on drought responses. Survival, growth, and physiological performance under drought may all be different in younger seedlings or change in older-aged seedlings with ontogeny, and many factors ultimately contribute to regeneration, including the ability to germinate at all. Moreover, roots have a greater ability to grow rapidly to acquire water in a field situation, where volume is less restricted than in containers.

Effects of previous drought on possible acclimation:

Results also show some support that repeated drought influences seedling physiology in loblolly pine and Douglas-fir seedlings, varying somewhat in effect by provenance and for the two species. This influence of drought may be acclimation. In both species, in some provenances, effects of repeated drought on drought performance as measured via ETR_{max} and F_v/F_m showed a pattern of higher values in seedlings that experienced the two drought (2D) treatment compared to seedlings that experienced only a late drought (LD) when both treatments were subjected to drought simultaneously. This pattern was most distinct in loblolly pine, in some cases resulting in statistical significance. For example, in loblolly pine, rapid light curves demonstrating ETR following D2 as well as measurements of F_v/F_m following D2 often showed lower values in the LD treatment, primarily for the North Carolina, occasionally for the Lost Pines, and less so for the Alabama provenance as compared to the 2D treatment. While F_v/F_m was not especially sensitive to drought, as has been seen in other studies (Ogren 1990, Baker and Rosenqvist 2004, Razavi et al. 2008), nonetheless there were still significantly lower values in some of the provenances in the LD treatment compared to the 2D treatment. In Douglas-fir, F_v/F_m was somewhat more sensitive, and showed the same pattern as with loblolly pine.

It is possible that lower values of ETR_{max} and F_v/F_m in LD treatments compared to 2D treatments are a reflection of slight soil moisture differences between LD and 2D treatments

during the second drought rather than necessarily the result of physiological adjustment for example in photosynthetic apparatus or biochemistry. Loss of turgor and closing of stomata can limit photosynthesis, and both ETR and F_v/F_m are related to photosynthesis. As seen in Ψ_p by treatment across provenances, in both species there were less negative values in the 2D treatments than the LD treatments at the end of the second drought, with the exception of the New Mexico provenance where this was the reverse (Table 2, Table 3). Differences in Ψ_p between 2D and LD treatments were not statistically significant; however, confidence intervals of estimates of differences between the LD and 2D treatments in loblolly, for example, overlap both zero and also more negative water potentials that could possibly impart biologically meaningful differences in stress severity. That is, although *statistically* LD and 2D treatment Ψ_p are not different, the confidence intervals include possible differences of as much as 1 MPa along with differences of 0 MPa. As such, there may still be some uncertainty as to the degree to which treatments experienced comparable Ψ_p during the second drought, and more negative Ψ_p could have contributed to lowering ETR and F_v/F_m and limiting photosynthesis. Measuring water potential and ETR simultaneously and more frequently throughout drought treatments could help clarify whether ETR is directly related to water potential differences between the treatments. Variation in water potentials among pots, and small sample sizes, also likely influenced the ability to detect statistical differences and effects on measures like ETR.

Previous drought exposure also had at least some effect on seedling size and growth, and these effects may have in turn translated to differences in soil moisture via reduction in water demand by smaller seedlings, with 2D seedlings somewhat smaller in size (though not generally statistically smaller) compared to LD seedlings. Although there were not strong statistical differences in biomass, there were greater differences in heights. For loblolly pine, seedlings in the 2D treatment were generally shorter going into the second drought than seedlings in the LD

treatment, as can be seen in lower final heights in the ED and 2D treatments. There were also height differences between 2D and LD treatments for Douglas-fir. However, the two xeric provenances, the Lost Pines and New Mexico provenances, were generally shorter compared to other provenances throughout the experiment. Differences in size, even slight, could influence water use and demand and thereby affect drought severity experienced by seedlings. In a study of Scots pine seedlings, for example, exposure to previous drought reduced mortality in subsequent drought. Reduced mortality was attributed to acclimation, but also particularly to greater aboveground biomass and height in seedlings un-exposed to drought compared to seedlings previously exposed to drought, which may have influenced previously drought-exposed seedlings in avoiding critically negative or lethal water potentials (Seidel and Menzel 2016). Changes in allocation and aboveground morphology, such as decreases in leaf to sapwood areas, have also shown plastic responses to increases in VPD in *Pinus* species, though not necessarily in non-*Pinus* conifer species (DeLucia et al. 2000). Besides heights, total biomass in loblolly pine was slightly lower in provenances that experienced the first drought (ED and 2D) compared to the watered control (ND) and LD treatment. These biomass differences may have further contributed to differences in ETR and F_v/F_m in the 2D and LD treatment for loblolly pine. However, for Douglas-fir, biomass differences among treatments are not as clear, and smaller differences between provenances could have contributed to less distinct patterns of acclimation. Allocation to root biomass is often a strategy in drought, but there were not large differences in root biomass in this study. Inferences from root biomass in this study are likely limited due to the influence of pots on root growth as opposed to in field conditions.

It is also possible that the drought treatments resulted in changes in seedling physiology more directly and that there were effects of drought exposure via forms of acclimation besides or in addition to growth. Acclimation in seedling physiology could include effects on biochemistry.

For example, accumulation of ABA causes stomatal closure (Roberts and Dumbroff 1986, Brodribb et al. 2014). It is possible that previous drought exposure resulted in increased stomatal sensitivity to ABA which could have assisted previously drought exposed seedlings during subsequent drought. Increased stomatal sensitivity could have aided 2D seedlings to marginally delay the onset of more severe stress as compared to LD seedlings, thus assisting 2D seedlings in maintaining slightly higher ETR_{max} and fluorescence during D2. If so, such influences on seedling behavior may lend interesting implications for possible benefits of prior drought exposure, at least in a greenhouse scenario, that may be worth exploring in future studies. In a study of Norway spruce (*Picea abies* (L.) H. Karst) families, Chmura et al. (2016) found reductions in stomatal conductance and light-saturated maximum photosynthetic rates in saplings following drought, and that these measures remained lower in droughted saplings as compared to control saplings even following re-watering, implying that drought imparted a lasting effect on sapling physiology, which could in theory translate to differences in water use. In studies of moisture stress conditioning with loblolly pine seedlings, Seiler and Johnson (1985) found possible evidence of osmotic adjustment in conditioned seedlings. Conditioned loblolly pine seedlings also maintained higher photosynthesis at negative water potentials compared to un-conditioned seedlings during drought. Mesophyll resistance to CO_2 diffusion was also calculated to have decreased in conditioned seedlings compared to control seedlings and was lower during drought compared to un-conditioned seedlings (Seiler and Johnson 1988). Less resistance to CO_2 diffusion could allow for continued carbon fixation at smaller stomatal apertures. Continued carbon fixation with smaller stomatal aperture could in turn further reduce water loss, and increase water-use efficiency.

Finally, if the results of the current study do demonstrate acclimation to drought, then it is interesting to observe that the more mesic and possibly more drought-susceptible provenances

(North Carolina for loblolly pine and Coos Bay for Douglas-fir) tended to have higher ETR and F_v/F_m in the 2D treatment than in the LD treatment during D2. The Alabama provenance in loblolly pine and the Cascades provenance in Douglas-fir demonstrated less evidence (possibly less plasticity) for differences in ETR and F_v/F_m between 2D and LD treatments. The New Mexico provenance, which seemed to have high drought resistance, even showed the reverse effect compared to the Coos Bay provenance for ETR_{max} . These patterns suggest possible differences in plasticity among the provenances. It may be that provenances from more mesic site climates, with species ranges covering heterogeneous conditions rather than persistent aridity, are associated with greater plasticity or capacity to acclimate to drought if exposed to (and if seedlings survive) drought compared to provenances from xeric sites. One explanation is that seedlings growing at more xeric sites are possibly already adapted to perform at the peak of their abilities to cope with drought and to manage various tradeoffs (Givnish 2002). More mesic provenances might utilize fast growth, given that water is typically less limiting at such sites and slow growth could be disadvantageous when resources are commonly available, while xeric provenances may have faced strong adaptive pressure towards conservative growth that mitigates risks such as embolism or tissue loss. Corcuera et al. (2011) also reported higher plasticity in xeric-grown mesic provenances of *Pinus pinaster* compared to more xeric provenances in a study of P_{50} (xylem water potential at 50% loss of conductivity) and stem specific conductivity. In the same species, seedlings from more mesic sites showed more plasticity in changes of carbon isotope discrimination when exposed to drought than seedlings from more xeric sites (Aranda et al. 2010). Curiously, however, the Lost Pines also showed some evidence for acclimation in response to drought in the current study, despite associations with high drought resistance in other literature and a xeric site climate for its range. These results are in contrast to low acclimation found by Seiler (1988) in east Texas loblolly pine sources in response to

moisture stress conditioning. Differences in dry-down process, drought length, greenhouse conditions, or other artifacts of the experiment could all have influenced results.

Overall, results suggest intraspecific variation in plasticity among the provenances studied, in both species. Differences in plasticity can influence plant and ultimately forest dynamics (Sultan 2003), perhaps especially in species with broad geographic ranges like loblolly pine and Douglas-fir, that are associated with various site climate conditions across those ranges, and which will be increasingly subject to climate change across those ranges. Field studies in combination with greenhouse studies of seedlings over multiple seasons may be especially revealing, both in quantifying general drought responses and clarifying whether drought resistances and acclimation carries forward into subsequent years since the results of this study are limited to greenhouse scenarios over one summer.

CONCLUSIONS

The first goal of this study was to investigate the drought resistances of seedlings from the selected provenances of loblolly pine and Douglas-fir associated with varying site climates and to investigate the physiological responses of the provenances to repeated drought stress with respect to potential acclimation. The drought resistances of provenances of both species largely conformed to expectations of the respective provenances, with the mesic-associated provenances in both loblolly pine (North Carolina) and Douglas-fir (Coos Bay) exhibiting drought susceptibility, and the more xeric-associated provenances exhibiting greatest drought resistance. However, differences in drought resistance were not as strong as expected and primarily showed patterns as opposed to statistical significance and thus would require further study to elucidate if these patterns are robust. In loblolly pine, it was somewhat unexpected that the Alabama provenance would seemingly demonstrate higher drought resistance than the Lost Pines

provenance. In Douglas-fir, in contrast, the provenance associated with the most xeric site climate (New Mexico) exhibited highest drought resistance, to the extent that it avoided more negative water potentials (Ψ) during the first drought (D1) and second drought (D2) than other provenances. The Cascades and Coos Bay provenances were relatively similar in drought resistances, although there is some suggestion in F_v/F_m that the Cascades provenance may have slightly higher resistance and is associated with slightly higher mean annual precipitation and lower climatic moisture deficit.

The second goal of this study was to investigate evidence for drought acclimation following drought exposure. We observed a pattern of lower electron transport rate (ETR) and fluorescence (F_v/F_m) in seedlings newly exposed to drought compared to seedlings previously exposed to drought during the second drought. This difference would appear to be acclimation. These results were found to be significant as measured by ETR_{max} mid-drought and in the recovery following release from D2, primarily in some of the mesic-associated provenances of both species (North Carolina for loblolly and showing a pattern but not significance for the Coos Bay provenance for Douglas-fir). These results are suggestive that seedlings from provenances associated with more xeric site climates and higher drought resistance are less plastic in their ability to acclimate to drought as compared to more drought susceptible seedlings from more mesic sites, although for this possible acclimation to manifest, seedlings must be able to survive initial drought exposure.

The inferences of this study are limited to greenhouse scenarios and the young seedlings from the provenances of loblolly pine and Douglas-fir used within this study. This study was also somewhat limited in measures and sample size. More in-depth research is necessary to better characterize and investigate the effects of differences in drought resistance, and effects of

drought exposure on seedlings in relation to acclimation. In that regard, field studies in combination with greenhouse studies may be especially illuminating. The ability to correlate measures such as Ψ with measures like ETR and F_v/F_m with simultaneous measurements of gas exchange would provide greater clarity. Nonetheless, the results of this study suggest differences in drought resistances and potential for acclimation, both of which could be influential in seedling responses to projected increases in frequency and severity of drought.

References

- Allen, C.D., Macalady, A.K., Chenchouni H, Bachelet, D., McDowell, N., Vennetier, M., Kitzberger, T., Rigling, A., Breshears, D.D., Hogg, E.H. 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and Management* 259: 660–684.
- Al-Rabab'ah, M., Williams, C. 2004. An ancient bottleneck in the Lost Pines of central Texas. *Molecular Ecology* 13(5): 1075-1084.
- Anderegg, W.R., Plavcova, L., Anderegg, L.D., Hacke, U.G., Berry, J.A., Field, C.B. 2013. Drought's legacy: multiyear hydraulic deterioration underlies widespread aspen forest die-off and portends increased future risk. *Global Change Biology* 19(4): 1188-96.
- Anderegg, W., Kane, J., Anderegg, L. 2013. Consequences of widespread tree mortality triggered by drought and temperature stress. *Nature Climate Change* 3: 30-36.
- Anderegg, W., Hicke, J., Fisher, R., Allen, C., Aukema, J., Bentz, B., Hood, S., Lichstein, J., Macalady, A., McDowell, N., Pan, Y., Raffa, K., Sala, A., Shaw, J., Stephenson, L., Tague, C., Zeppel, M. 2015. Tree mortality from drought, insects, and their interactions in a changing climate. *New Phytologist* 208 (3): 674-683.
- Aranda, I., Alia, R., Ortega, U., Dantas, A., Majada, J. 2010. Intra-specific variability in biomass partitioning and carbon isotopic discrimination under moderate drought stress in seedlings from four *Pinus pinaster* populations. *Tree Genetics and Genomes* 6: 169-178.
- Arnott, J., Grossnickle, S., Puttonen, P., Mitchell, A., Folk, R. 1993. Influence of nursery culture on growth, cold hardiness, and drought resistance of yellow cypress. *Canadian Journal of Forest Research* 23(12): 2537-2547.
- Baker, J., Langdon, O. 1990. *Pinus taeda* L., Loblolly Pine. *Silvics of North America*, Vol. 1. *Conifers Agriculture Handbook* 1(654): 497–512. Forest Service, United States Department of Agriculture, Washington DC.
- Baker, N., Rosenqvist, E. 2004. Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities. *Journal of Experimental Botany* 55(403):1607-1621.
- Bansal, S., Harrington, C., Gould, P., St. Clair, B. 2015. Climate-related genetic variation in drought-resistance of Douglas-fir (*Pseudotsuga menziesii*). *Global Change Biology* 21(2): 947-958.

- Bansal, S., Harrington, C., St. Clair, B. 2016. Tolerance to multiple climate stressors: a case study of Douglas-fir drought and cold hardiness. *Ecology and Evolution* 6 (7): 2074-2083.
- Bartholome, J., Mabiala, A., Savelli, B., Bert, D., Brendel, O., Plomion, C., and Gion, J.M. 2015. Genetic architecture of carbon isotope composition and growth in *Eucalyptus* across multiple environments. *New Phytologist* 206:1437-1449.
- Bilan, V., Knauf, T. 1974. Needle variation in Loblolly pine from mesic and xeric seed sources. *Forest Science* 20(1): 88-90.
- Bilan, V., Hogan, C., Carter, B. 1977. Stomatal opening, transpiration, and needle moisture in Loblolly pine seedlings from two Texas seed sources. *Forest Science* 23 (4): 457-462.
- Bongarten, B., Teskey, R. 1986. Water relations of loblolly pine seedlings from diverse geographic origin. *Tree Physiology* 1:265-276.
- Breshears, D.D., Myers, O.B., Meyer, C.W., Barnes, F.J., Zou, C.B., Allen, C.D., McDowell, N.G., Pockman, W.T. 2009. Tree die-off in response to global change-type drought: mortality insights from a decade of plant water potential measurements. *Frontiers in Ecology and the Environment* 7(4):185-189.
- Brodribb, T. McAdam, S., Jordan, G., Martins, S. 2014. Conifer species adapt to low-rainfall climates by following one of two divergent pathways. *Proceedings of the National Academy of Science (PNAS)* 111(40): 14489-14493.
- Burns, R. M., B. H. Honkala (eds.). 1990. *Silvics of North America, Volume 1, Conifers*. U.S. Department of Agriculture, Forest Service, Agriculture Handbook 654, Washington, D.C. Pp. 675
- Chaves, M., Pereira, J. Maroco, J., Rodrigues, M., Ricardo, C. Osorio, M., Carvalho, I., Faria, T., Pinheiro C. 2002. How plants cope with water stress in the field. *Photosynthesis and growth*. *Annals of Botany* 89: 907-916.
- Chmura, D.J., Guzicka, M., McCulloh, K., Zytowskiak, R. 2016. Limited variation found among Norway spruce half-sib families in physiological response to drought and resistance to embolism. *Tree Physiology* 36 (2): 252-66.
- Christensen, J.H., Hewitson, B., Busuioc, A., Chen, A., Gao, X., Held, I., Jones, R., Kolli, R.K., Kwon, W.-T., Laprise, R., Magana Rueda, V., Mearns, L., Menendez, C.G., Raisanen, J., Rinke, A., Sarr, A., Whetton, P. 2007. Regional climate projections. In: Solomon, S., et al. (Eds.), *Climate Change. 2007: The Physical Science Basis. Contributions of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, United Kingdom/New York, NY.

- Corcuera, L., Cochard, H., Gil-Pelegrin, E., Noivol, E. 2011. Phenotypic plasticity in mesic populations of *Pinus pinaster* improves resistance to xylem embolism (P_{50}) under severe drought. *Trees* 25: 1033-1042.
- Cui, M., Smith, W.K. 1991. Photosynthesis, water relations and mortality in *Abies lasiocarpa* seedlings during natural establishment. *Tree Physiology* 8:37–46
- Dai, A. 2011. Drought under global warming: a review. *Wiley Interdisciplinary Reviews: Climate Change* 2 (1): 45-65.
- Dai, A. 2013. Increasing drought under global warming in observations and models. *Nature Climate Change Letters* 3: 52-58.
- DeLucia, E., Maherali, H., Carey, E. 2000. Climate-driven changes in biomass allocation in pines. *Global Change Biology* 6: 587-593.
- Eilmann, B., de Vries, S.M., den Ouden, J., Mohren, G., Sauren, P., Sass-Klaassen, U. 2012. Origin matters! Difference in drought tolerance and productivity of coastal Douglas-fir (*Pseudotsuga menziesii* (Mirb.)) provenances. *Forest Ecology and Management* 302: 133-143
- Ferrell, W., Woodard, S. 1966. Effects of seed origin on drought resistance of Douglas-fir (*Pseudotsuga Menziesii*) (Mirb) Franco. *Ecology* 47 (3): 499-503.
- Flexas, J., Escalona, J.M., Medrano, H. 1999. Water stress induces different levels of photosynthesis and electron transport rate regulation in grapevines. *Plant, Cell and Environment* 22: 39-48.
- Flexas, J., Bota, J., Loreto, F., Cornic, G., Sharkey, T. 2004. Diffusive and metabolic limitations to photosynthesis under drought and salinity in C3 plants. *Plant Biology* 6: 269-279.
- Franklin, J.F., and C.T. Dyrness. 1973. Natural vegetation of Oregon and Washington. USDA Forest Service. General Technical Report. PNW-8.
- Genty B., Briantais J. M. & Baker N. R. 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence, *Biochimica et Biophysica Acta* 990: 87-92
- Genty B., Harbinson J., Baker N.R. 1990. Relative quantum efficiencies of the two photosystems of leaves in photo respiratory and non-photo respiratory conditions. *Plant Physiology and Biochemistry* 28: 1-10

- Givnish, T. 2002. Ecological constraints on the evolution of plasticity in plants. *Evolutionary Ecology* 16: 213-242.
- Grossnickle, S.C. 2012. Why seedlings survive: influence of plant attributes. *New Forests* 43:711–738.
- Hacke, U., Sperry, J., Ewers, B., Ellsworth, D., Schafer, K., Oren, R. 2000. Influence of soil porosity on water use in *Pinus taeda*. *Oecologia* 124:495-505.
- Hanson, P., Weltzin J., 2000. Drought Disturbance from Climate Change: Response of United States Forests. *Science of the Total Environment* 262: 205-220.
- Harb, A., Krishnan, A., Ambavaram, M., Pereira, A. 2010. Molecular and Physiological Analysis of Drought Stress in *Arabidopsis* Reveals Early Responses Leading to Acclimation in Plant Growth. *Plant Physiology* 154 (3): 1254-1271.
- Heiner, T.C., Lavender, D.P. 1972. Early growth and drought avoidance in Douglas-fir seedlings. Res. Pap. 14. Corvallis, Or.: Forest Research Laboratory, School of Forestry, Oregon State University. 9 p.
- Hinckley, T., Dougherty, P. Lassoie, J., Roberts, J. Teskey, R. 1979. A severe drought: impact on tree growth, phenology, net photosynthetic rate, and water relations. *American Midland Naturalist* 102 (2): 307-316.
- Hsiao, T. 1973. Plant Responses to Water Stress. *Annual Review of Plant Physiology* 24: 519-70.
- Iacono, F., Sommer, K. 2000. Response of electron transport rate of water stress-affected grapevines: Influence of leaf age. *Vitis* 39(4): 137-144.
- IPCC, 2007a. Climate change 2007: the physical science basis. In: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M., Miller, H.L. (Eds.), Contribution of Working Group I to the Fourth Assessment. Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom/New York, NY, USA, 996pp.
- IPCC, 2007b. Climate change 2007: impacts, adaptation and vulnerability. Parry, M.L., Canziani, O.F., Palutikof, J.P., van der Linden, P.J., Hanson, C.E. (eds.), Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK, 976 pp.

- Jansen, K., Sohr, J., Kohnle, U., Ensminger, I., Gessler, A. 2013. Tree ring isotopic composition, radial increment and height growth reveal provenance-specific reactions of Douglas-fir towards environmental parameters. *Trees* 27 (1): 37-52.
- Jansen, K. Du, B., Kayler, Z., Seigwolf, R., Ensminger, I., Rennenberg, H., Kammerer, B., Jaeger, C., Schaub, M., Kreuzwieser, J., Gessler, A. 2014. Douglas-fir seedlings exhibit metabolic responses to increased temperature and atmospheric drought. *PLoS ONE* 9 (12): 1-21.
- Joly, R.J., W.T. Adams and S.G. Stafford. 1989. Phenological and morphological responses of mesic and dry site sources of coastal Douglas-fir to water deficit. *Forest Science* 35:987-1005.
- Kerr, K., Meinzer, F., McCulloh, K., Woodruff, D., Marias, D. 2015. Expression of functional traits during seedling establishment in two populations of *Pinus ponderosa* from contrasting climates. *Tree Physiology* 35: 535-548.
- Kirschbaum, M. 1988. Recovery of photosynthesis from water stress in *Eucalyptus pauciflora* – a process in two stages. *Plant, Cell & Environment* 11: 685–694.
- Kobe, R. 1996. Intraspecific variation in sapling mortality and growth predicts geographic variation in forest composition. *Ecological Monographs* 66 (2): 181-201.
- Koehn, A. C., McDonald, G. I., Turner, D. L., Adams, D. L. 2010. Dynamic phenotypic plasticity in photosynthesis and biomass patterns in Douglas-fir seedlings. Res. Pap. RMRS-RP-79. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. 14 p.
- Kubiske, M., and Abrams, M. 1991. Seasonal, diurnal and rehydration-induced variation of pressure-volume relationships in *Pseudotsuga menziesii*. *Physiologia Plantarum* 83: 107-116.
- Krall, J., Edwards, G. 1992. Relationship between photosystem II activity and CO₂ fixation in leaves. *Physiologia Plantarum* 86: 180-187.
- Lande, R. 2009. Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. *Journal of Evolutionary Biology* 22, 1435–1446
- Lavender, D., Hermann, R.K. 2014. 2. Natural Range in Douglas-fir: The Genus *Pseudotsuga*. Oregon Forest Research Laboratory, Oregon State University, Corvallis. Pp 15-24
- Maxwell, K., Johnson, G. 2000. Chlorophyll fluorescence – a practical guide. *Journal of Experimental Botany* 51 (345): 659-668.
- McDowell, N. 2011. Mechanisms linking drought, hydraulics, carbon metabolism, and vegetation mortality. *Plant Physiology* 155:1051-1059.

- McDowell, N., Beerling, D., Breshears, D., Fisher, R., Raffa, K., Stitt, M. 2011. The interdependence of mechanisms underlying climate-driven vegetation mortality. *Trends in Ecology and Evolution* 26(10):523-532.
- Minore, Don. 1986. Germination, survival and early growth of conifer seedlings in two habitat types. Res. Pap. PNW-RP-347. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Forest and Range Experiment Station. 63 p
- Morgan, J. 1984. Osmoregulation and water stress in higher plants. *Annual Reviews of Plant Physiology* 35: 299-319.
- Newton, R.J., Sen, S., and van Buijtenen, J.P. 1985. Growth changes in loblolly pine (*Pinus taeda* L.) cell cultures in response to drought-stress. pp. 64-73 in *Proceedings of the 18th Forest Tree Improvement Conference*. University of So. Mississippi, Long Beach, May 21-23.
- Ogren, E. 1990. Evaluation of Chlorophyll Fluorescence as a probe for Drought Stress in Willow Leaves. *Plant Physiology* 93: 1280-1285.
- Nicotra, A., Atkin, O., Bonser, S., Davidson, A., Finnegan, E., Mathesius, U., Poot, P., Purugganan, M., Richards, C., Valladares, F., van Kleunen, M. 2010. Plant phenotypic plasticity in a changing climate. *Trends in Plant Science* 15 (12): 684-692.
- R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>
- Razavi, F., Pollet, B., Steppe, K., Van Labeke, M.C. 2008. Chlorophyll fluorescence as a tool for evaluation of drought stress in strawberry. *Photosynthetica* 46(4): 631-633.
- Rehfeldt, G.E. 1978. Genetic differentiation of Douglas-fir populations from the northern Rocky Mountains. *Ecology* 59: 1264–1270
- Richter, S., Kipfer, T., Wohlgemuth, T., Guerrero, C.C., Ghazoul, J., Moser, B. 2012. Phenotypic plasticity facilitates resistance to climate change in a highly variable environment. *Oecologia* 169:269–279.
- Roberts, D.R., Dumbroff, E.B. 1986. Relationships among drought resistance, transpiration rates, and abscisic acid levels in three northern conifers. *Tree Physiology* 1: 161-167.
- Royo, A., Gil, L., Pardos, J. 2001. Effect of water stress conditioning on morphology, physiology and field performance of *Pinus halepensis* Mill. Seedlings. *New Forests* 21:127-140.
- Seidel, H., and Menzel, A. 2016. Above-ground dimensions and acclimation explain variation in drought mortality of Scots pine seedlings from various provenances. *Fronts in Plant Science* 7 (1014): 1-14.

- Seiler, J., Johnson, J. 1985. Photosynthesis and transpiration of loblolly pine seedlings as influenced by moisture-stress conditioning. *Forest Science* 31 (3):742-749.
- Seiler, J., Johnson, J. 1988. Physiological and morphological responses of three half-sib families of loblolly pine to water-stress conditioning. *Forest Science* 34(2): 487-495.
- Shevtsova, A., Graae, B.J., Jochum, T., Milbau, A., Kockelbergh, F., Beyens, L., Nijs, I. 2009. Critical periods for impact of climate warming on early seedling establishment in subarctic tundra. *Glob Change Biology* 15:2662–2680.
- Shinozaki, K., Yamaguchi-Shinozaki, K. 1997. Gene Expression and Signal Transduction in Water-Stress Response. *Plant Physiology* 115(2):327-334.
- Shinozaki K, Yamaguchi-Shinozaki, K. 2007. Gene networks involved in drought stress response and tolerance. *Journal of Experimental Botany* 58 (2): 221-227.
- Sperry, J., Ikeda, T. 1997. Xylem cavitation in roots and stems of Douglas-fir and white fir. *Tree Physiology* 17: 275-280.
- Sultan, S. 2003. Phenotypic plasticity in plants: a case study in ecological development *Evolution and Development*. 5 (1): 25-33.
- Teskey, R., Bongarten, B., Cregg, B., Dougherty, P., Hennessey, T. 1987. Physiology and genetics of tree growth responses to moisture and temperature stress: an examination of the characteristics of loblolly pine. *Tree Physiology* 3: 41-61.
- Turesson, G. 1922. The species and the variety as ecological units. *Hereditas* 3:100–113.
- van Kleunen, M., Fischer, M. 2005. Constraints on the evolution of adaptive phenotypic plasticity in plants. *New Phytologist* 166:49-60.
- Wakamiya-Noborio, I., Heilman, J., Newton, R., Messina, M. 1999. Diurnal changes in water conduction in loblolly pine (*Pinus taeda*) and Virginia pine (*P. virginiana*) during soil dehydration. *Tree Physiology* 19:575-581.
- Wang, T., Hamann, A., Spittlehouse, D., Murdock TQ. 2012. ClimateWNA – High resolution spatial climate data for wester north America. *Journal of Applied Meteorology and Climatology* 51:16-29
- Watkinson, J., Sioson, A., Vasquez-Robinet C., Shukla, M., Kumar, D., Ellis, M., Heath, L., Ramakrishnan, N., Chevone, B., Watson, L., van Zyl, L., Egertsdotter, U., Sederoff, R., Grene,

R. 2003. Photosynthetic acclimation is reflected in specific patterns of gene expression in drought-stressed loblolly pine. *Plant Physiology* 133 (4): 1702-1716.

White, T., Ching, K. 1985. Provenance study of Douglas-fir in the Pacific Northwest region IV. Field Performance at age 25 years. *Silvae Genetica* 34: 84-90.

White, T. 1987. Drought tolerance of southwestern Oregon Douglas-fir. *Forest Science* 33(2):283-293.

Zavitkovski, J., Ferrell, W. 1968. Effect upon rates of photosynthesis, respiration, and transpiration of seedlings of two ecotypes of Douglas-fir. *Botanical Gazette* 129 (4):346-350.

Zobel, B. J., R. E. Goddard. 1955. Preliminary results on tests of drought hardy strains of loblolly pine (*Pinus taeda* L.). Texas Forest Service Research Note 14, 22 p.

Tables

Table 1. Loblolly pine (*Pinus taeda*) and Douglas-fir (*Pseudotsuga menziesii*) provenances and site climate characteristics. For Douglas-fir, the provenance data include a range representing sites of origin of all trees in the seed orchards, not just the mother tree. Temperature and precipitation data from 1961-1999 Climate Normals from ClimateWNA (ClimateWNA.com) database of North America. (DD>5 represents degree-days above 5° C, CMD represents climatic moisture deficit, which is the sum of monthly difference between reference atmospheric evaporative demand and precipitation)

Provenance	Site type	Elevation (m)	Mean Annual Precipitation (mm)	May-Sept Precipitation (mm)	CMD (mm)	Mean Annual Temp (°C)	DD >5	Location	Coordinates
<i>Pinus taeda</i>									
North Carolina	Moist lowland Atlantic coastal plane	15	1,376	722	122	16.9	4465	Jacksonville Onslow County NC, USA	34.84, -77.41
Alabama	Mesic, upland coastal plane	68	1,407	510	329	17	4502	Tuscaloosa, AL, USA	33.04, -87.51
Lost Pines	Xeric, disjunct, Texas pop.	168	900	412	623	20.2	5570	Bastrop County TX, USA	30.11, -97.28
<i>Pseudotsuga menziesii</i> var. <i>menziesii</i>									
Coos Bay	Mesic, Low elevation	104 to 594	1,473 to 1,827	150 to 239	317 to 467	10.4 to 11.1	2175 to 2331	Coos Bay County OR, USA	43.18 to 43.48 ; 123.6 to 124.12
Cascades	Mesic, High elevation Cascades	400 to 1,712	1,581 to 1,717	284 to 446	121 to 156	3.4 to 9	806 to 1841	Lewis/King County Cascades, WA	47.00 to 48.35, 121.47 to 122.15
New Mexico	Xeric, Lincoln National Forest	2, 062	533	352	717	9.8	2218	Lincoln County Ruidoso NM, USA	33.34, -105.67

Table 2. Loblolly pine average predawn and midday leaf water potentials (Ψ), summarized for all droughts and treatments by provenance. Droughts are D1 (first drought) and D2 (second drought). Treatments are ND (no drought), ED (early drought), LD (late drought), and 2D (two drought). 95% confidence intervals shown in parentheses. Watered signifies ND and LD averaged for the end of D1 and recovery after D1, and Droughted signifies 2D and ED averaged for the end of D1 and end of D1 recovery.

Drought	Provenance	Treatment	Predawn Ψ (MPa)	Midday Ψ (MPa)	n
End of D1 (2-3 Sept)	North Carolina	Watered	-0.44 (0.17)	-1.00 (0.49)	10
		Droughted	-1.75 (0.46)	-2.20 (0.28)	10
	Alabama	Watered	-0.53 (0.21)	-0.86 (0.20)	10
		Droughted	-1.71 (0.24)	-2.11 (0.38)	10
	Lost Pines	Watered	-0.59 (0.33)	-0.91 (0.32)	10
		Droughted	-1.64 (0.28)	-2.00 (0.25)	10
Recovery after D1 (14 Sept)	North Carolina	Watered	--	-0.55 (0.18)	6
		Droughted	--	-0.75 (0.51)	6
	Alabama	Watered	--	-0.82 (0.28)	6
		Droughted	--	-0.93 (0.53)	6
	Lost Pines	Watered	--	-0.97 (0.57)	6
		Droughted	--	-1.19 (0.52)	6
End of D2 (29 Sept)	North Carolina	ND	-0.52 (0.18)	-0.95 (0.20)	4
		ED	-0.75 (0.37)	-0.84 (0.37)	7
		LD	-2.66 (0.72)	-2.47 (0.52)	6
		2D	-2.30 (0.65)	-2.34 (0.44)	7
	Alabama	ND	-0.63 (0.35)	-0.751 (0.20)	4
		ED	-0.66 (0.11)	-0.836 (0.32)	4
		LD	-2.30 (0.52)	-2.43 (0.67)	6
		2D	-2.21 (0.40)	-2.69 (0.37)	6
	Lost Pines	ND	-0.62 (0.13)	-0.89 (0.34)	4
		ED	-0.54 (0.001)	-1.23 (0.39)	4
		LD	-2.40 (0.42)	-2.67 (0.64)	6
		2D	-2.14 (0.54)	-1.84 (0.47)	7

Table 3. Douglas-fir average predawn and midday leaf water potentials (Ψ), summarized for all droughts and treatments by provenance. Droughts are D1 (first drought) and D2 (second drought). Treatments are ND (no drought), ED (early drought), LD (late drought), and 2D (two drought). 95% confidence intervals in parentheses. Watered signifies ND and LD averaged for the end of D1 and recovery after D1, and Droughted signifies 2D and ED averaged for the end of D1 and end of D1 recovery.

Drought	Provenance	Treatment	Predawn Ψ (MPa)	Midday Ψ (MPa)	n
End of D1 (28-29 Aug)	Coos Bay	Watered	-0.63 (0.15)	-1.11 (0.15)	10/11
		Droughted	-1.75 (0.60)	-2.35 (0.51)	12
	Cascades	Watered	-0.55 (0.057)	-1.13 (0.22)	12/10
		Droughted	-1.65 (0.59)	-2.28 (0.51)	12
	New Mexico	Watered	-0.54 (0.081)	-0.95 (0.15)	12/11
		Droughted	-1.05 (0.35)	-1.90 (0.27)	12
End of D1 Recovery (10 Sept)	Coos Bay	Watered	--	-1.92 (0.30)	6
		Droughted	--	-1.85 (0.37)	6
	Cascades	Watered	--	-2.11 (0.26)	6
		Droughted	--	-1.52 (0.29)	6
	New Mexico	Watered	--	-1.87 (0.49)	6
		Droughted	--	-1.09 (0.29)	6
End of D2 (7-8 Oct)	Coos Bay	ND	-0.78 (0.21)	-0.81 (0.14)	5
		ED	-0.86 (0.12)	-0.89 (0.24)	5
		LD	-3.43 (0.33)	-2.58 (0.63)	9/7
		2D	-2.92 (0.64)	-2.64 (0.50)	8/7
	Cascades	ND	-0.70 (0.14)	-0.80 (0.14)	5
		ED	-0.90 (0.10)	-0.83 (0.13)	5
		LD	-2.79 (0.88)	-2.98 (0.90)	7/9
		2D	-2.62 (0.85)	-3.05 (0.71)	7
	New Mexico	ND	-0.85 (0.11)	-0.74 (0.13)	5
		ED	-0.96 (0.13)	-0.75 (0.04)	5
		LD	-1.75 (0.38)	-1.75 (0.36)	10/8
		2D	-2.48 (0.53)	-2.60 (0.47)	7

Table 4. Statistical analyses (ANOVA F-Tests) for loblolly pine (*Pinus taeda*) maximum electron transport rates (ETR_{max}). Treatments are ND (no drought), ED (early drought), LD (late drought), and 2D (two drought). Bold signifies statistical significance at $p < 0.05$.

Date	Test	F-value	p-value (p)	df
Mid-drought D2 (22 Sept)	Treatment	3.20	0.027	3, 71
	Provenance	1.56	0.216	2, 71
	Treatment x provenance	1.60	0.158	6, 71
Near end of D2 (28 Sept)	Treatment	158.5	2 x 10⁻¹⁶	3, 45
	Provenance	0.81	0.45	2, 45
	Treatment x Provenance	0.31	0.9289	6, 45
D2 recovery (10 Oct)	Treatment	23.9	7.92 x 10⁻¹¹	3, 72
	Provenance	3.09	0.051	2, 72
	Treatment x provenance	0.40	0.877	6, 72

Table 5. Statistical analyses (ANOVA F-tests) for Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) maximum electron transport rates (ETR_{max}). Treatments are ND (no drought), ED (early drought), LD (late drought), and 2D (two drought). Bold signifies statistical significance at $p < 0.05$.

Date	Test	F-value	p-value (p)	df
Mid-drought D2 (2 Oct)	Treatment	54.2	2.2×10^{-16}	3, 96
	provenance	22.9	7.51×10^{-9}	2, 96
	Treatment x provenance	5.84	3.18×10^{-5}	6, 96
End of D2 (5 Oct)	Treatment	43.0	1.19×10^{-13}	3, 48
	Provenance	12.5	4.32×10^{-5}	2, 48
	Treatment x provenance	3.84	3.31×10^{-3}	6, 48
D2 recovery (15 Oct)	Treatment	2.84	0.0460	3, 57
	Provenance	6.07	4.10×10^{-3}	2, 57
	Treatment x provenance	0.94	0.471	6, 57

Table 6. Pairwise comparisons for loblolly pine maximum electron transport rates (ETR_{max}). Treatments are ND (no drought), ED (early drought), LD (late drought), and 2D (two drought). Estimates are in $\mu\text{mol electrons m}^{-2} \text{ s}^{-1}$, with the treatment on the right side of the “versus” subtracted from the treatment on the left side of the “versus” in the comparison column. Bold signifies statistical significance at $p < 0.05$.

Date	Provenance	Comparison	t-value	p-value (<i>p</i>)	df	Estimate (95% CI)	n
Mid-drought D2 (22 Sept)	All	2D vs LD	2.17	0.0331	3, 79	8.28 (0.68, 15.9)	21
		LD vs ND	-2.92	4.58 x 10⁻³	3, 79	-11.1 (-18.7, 3.54)	21
		2D vs ND	-0.76	0.450	3, 79	-2.86 (-10.4, 4.64)	21
		ED vs ND	-1.13	0.260	3, 79	-4.28 (-11.8, 3.22)	21
	North Carolina	2D vs LD	2.49	0.0153	72	15.8 (3.12, 28.4)	7
	Alabama	2D vs LD	1.33	0.187	72	8.79 (-4.37, 22.0)	7
	Lost Pines	2D vs LD	-0.009	0.993	72	-0.057 (-12.7, 12.6)	7
Near end of D2 (28 Sept)	All	2D vs LD	1.11	0.272	3, 53	4.07 (-3.28, 11.4)	15
		LD vs ND	-16.4	2 x 10⁻¹⁶	3, 53	-60.0 (-67.3, -52.6)	15
		2D vs ND	-15.5	2 x 10⁻¹⁶	3, 53	-55.9 (-63.1, -48.7)	15
		ED vs ND	0.90	0.372	3, 53	3.36 (-4.13, 10.8)	15
	North Carolina	2D vs LD	0.52	0.603	45	3.59 (-10.3, -17.5)	5
	Alabama	2D vs LD	0.80	0.425	45	5.24 (-7.88, 18.4)	5
	Lost Pines	2D vs LD	0.59	0.559	45	3.84 (-9.28, 17.0)	5
D2 recovery (10 Oct)	All	2D vs LD	2.78	6.83 x 10⁻³	3, 80	13.8 (3.92, 23.8)	21
		LD vs ND	-4.29	5.15 x 10⁻¹⁰	3, 80	-35.3 (-45.2, -25.3)	21
		2D vs ND	-4.30	4.87 x 10⁻⁵	3, 80	-21.4 (-31.3, -11.5)	21
		ED vs ND	-0.11	0.911	3, 80	-0.56 (-10.5, 9.36)	21
	North Carolina	2D vs LD	2.11	0.038	72	18.2 (1.01, 35.3)	7
	Alabama	2D vs LD	0.61	0.541	72	5.29 (-11.9, 22.4)	7
	Lost Pines	2D vs LD	2.10	0.039	72	18.1 (0.94, 35.2)	7

Table 7. Pairwise comparisons for Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) maximum electron transport rates (ETR_{max}). Treatments are ND (no drought), ED (early drought), LD (late drought), and 2D (two drought). Estimates are in $\mu\text{mol electrons m}^{-2} \text{ s}^{-1}$, with the treatment on the right side of the “versus” subtracted from the treatment on the left side of the “versus” in the comparison column. Bold signifies statistical significance at $p < 0.05$.

Date	Provenance	Comparison	t-value	p-value (<i>p</i>)	df	Estimate (95% CI)	n
Mid-drought D2 (2 Oct)	All	2D vs LD	0.17	0.869	104	0.900 (-9.93, 11.7)	27
		LD vs ND	-7.33	5.12 x 10⁻¹¹	104	-40.0 (-50.9, -29.2)	27
		2D vs ND	7.14	1.15 x 10⁻¹⁰	104	-39.1 (-50.0, -28.3)	27
		ED vs ND	-0.71	0.481	104	-3.87 (-14.7, 7.0)	27
	Coos Bay	2D vs LD	1.32	0.191	96	9.56 (-4.85, 24.0)	9
	Cascades	2D vs LD	0.44	0.664	96	3.17 (-11.2, 17.6)	9
	New Mexico	2D vs LD	-1.38	0.170	96	-10.0 (-24.4, 4.38)	9
Near end of D2 (5 Oct)	All	2D vs LD	-0.28	0.78	3, 56	-2.10 (-17.3, 13.1)	15
		LD vs ND	-6.10	1.04 x 10⁻⁷	3, 56	-46.3 (-61.5, -31.1)	15
		2D vs ND	-6.38	3.66 x 10⁻⁸	3, 56	-48.4 (-63.6, -33.2)	15
		ED vs ND	-0.22	0.83	3, 56	-1.64 (-16.8, 13.6)	15
	Coos Bay	2D vs LD	0.20	0.841	48	2.02 (-18.2, 22.2)	5
	Cascades	2D vs LD	1.00	0.321	48	10.6 (-10.1, 30.2)	5
	New Mexico	2D vs LD	-1.83	0.073	48	-18.4 (-38.6, 1.80)	5
D2 recovery (15 Oct)	All	LD vs 2D	-0.04	0.976	3, 65	-0.161 (-7.58, 7.26)	18
		LD vs ND	-2.20	0.032	3, 65	-8.28 (-15.8, -0.752)	18
		2D vs ND	-2.24	0.029	3, 65	-8.44 (-16.0, -0.91)	18
		ED vs ND	-0.69	0.495	3, 65	-2.67 (-10.4, 5.09)	18
	Coos Bay	2D vs LD	1.18	0.245	57	7.05 (-4.96, 19.1)	6
	Cascades	2D vs LD	-0.081	0.936	57	-0.483 (-12.5, 11.5)	6
	New Mexico	2D vs LD	-1.18	0.245	57	-7.05 (-19.1, 4.96)	6

Figures

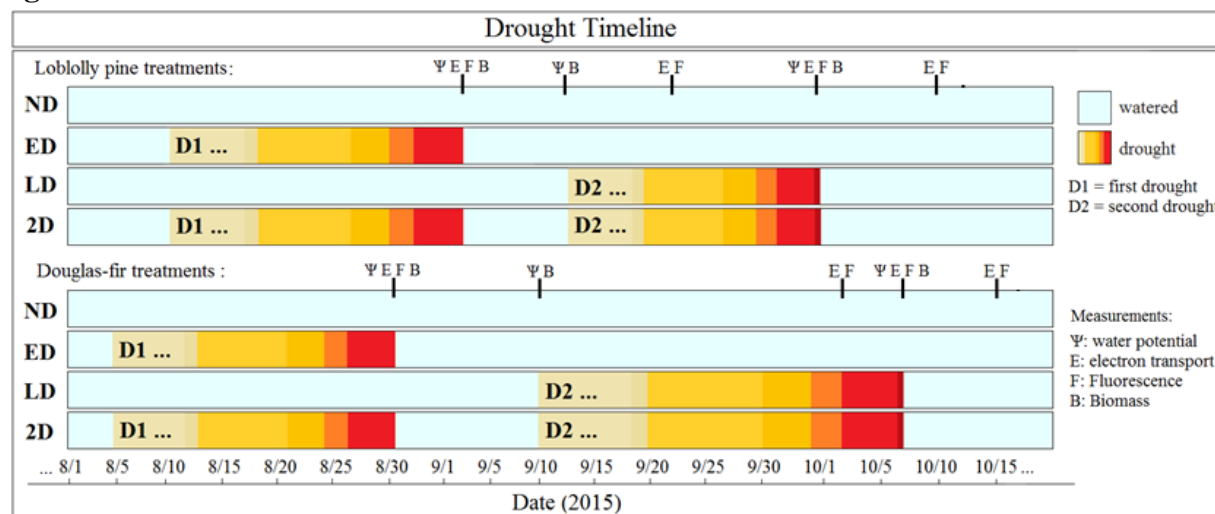


Figure 1. Timeline of dates of drought treatments for loblolly pine and Douglas-fir during the summer of 2015. Treatment listed on left and dates listed on bottom. Treatments are ND (no drought), ED (early drought), LD (late drought), and 2D (two drought). The first drought (D1) for loblolly pine was 10 Aug to 3 Sept, and the second drought (D2) was from 13 Sept to 1 Oct. For Douglas-fir, D1 was from Aug 5 to Aug 30, and D2 was from 10 Sept to 8 Oct. Color changes shown here for drought do not represent quantitative values.

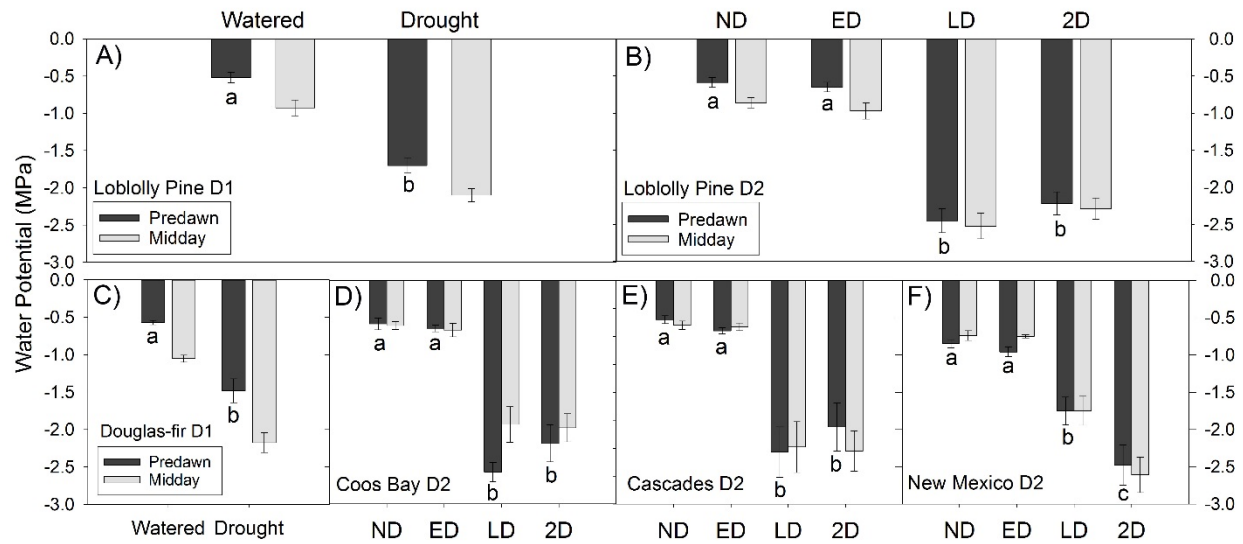


Fig 2. Seedling needle water potentials (Ψ) at the end of the first drought (D1) and the second drought (D2). Loblolly pine water potentials at the end of the first drought by treatment (A) and end of second drought by treatment (B). Douglas-fir water potentials at the end of the first drought (D1) for treatments all together (C) and at the end of the second drought (D2) by provenance (D, E, F). Treatments are ND (no drought), ED (early drought), LD (late drought), and 2D (two drought). Error bars represent standard error. For D1, ND and LD treatments have been pooled to represent 'Watered' treatments and ED and 2D treatments have been pooled to represent 'Drought' treatments because until that date those classes had received the same experimental manipulations. The same letter within a panel denotes no significant difference at $p < 0.05$.

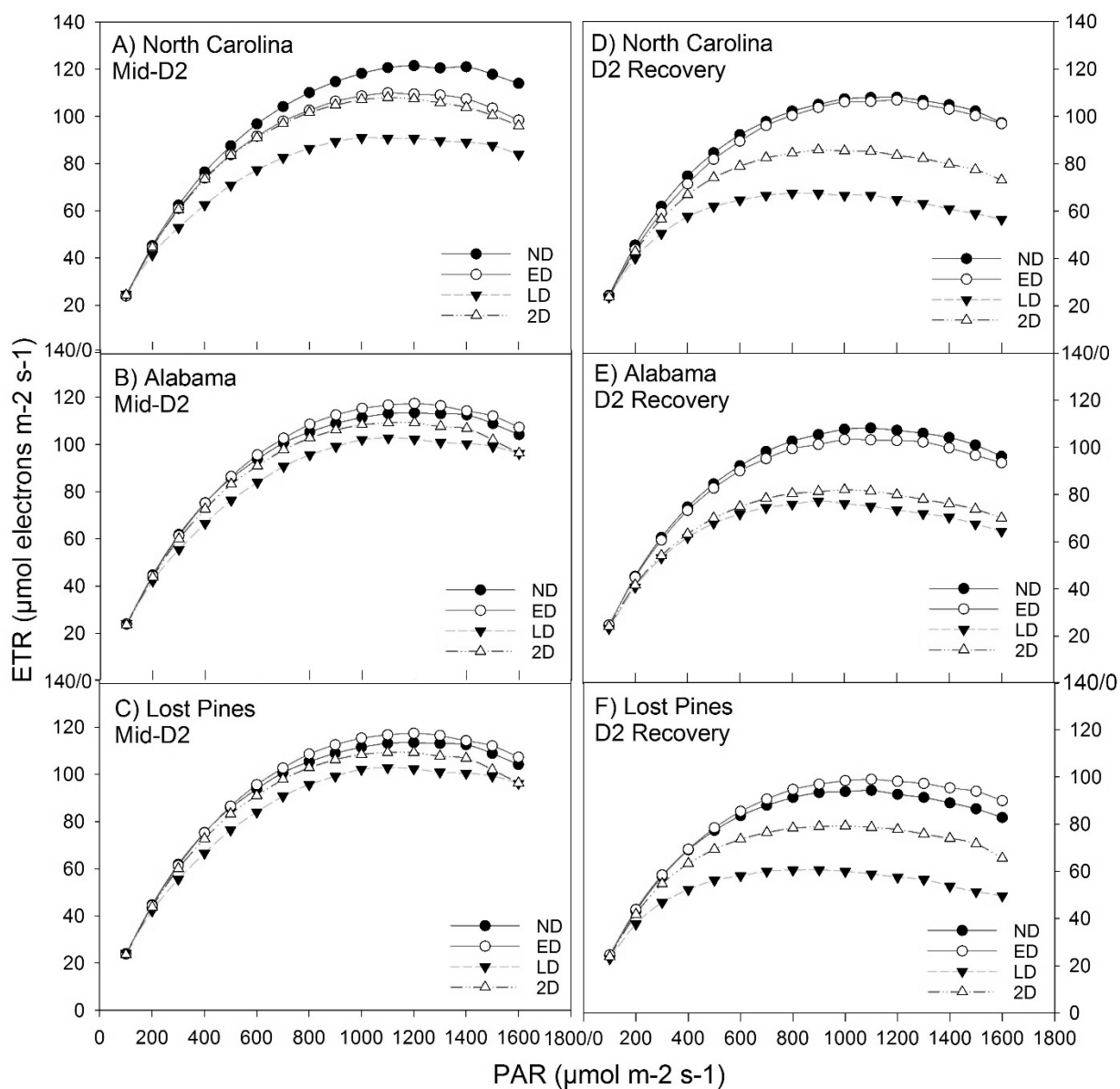


Fig 3. Rapid light curves showing electron transport rates (ETR) vs. light level (PAR) for loblolly pine seedlings of the three provenances. A-C, during mid-drought 2 (22 Sept); D-F during the recovery from the second drought (Oct 9). Each symbol represents a mean for measurements on 7 seedlings. Treatments are ND (no drought), ED (early drought), LD (late drought), and 2D (two drought).

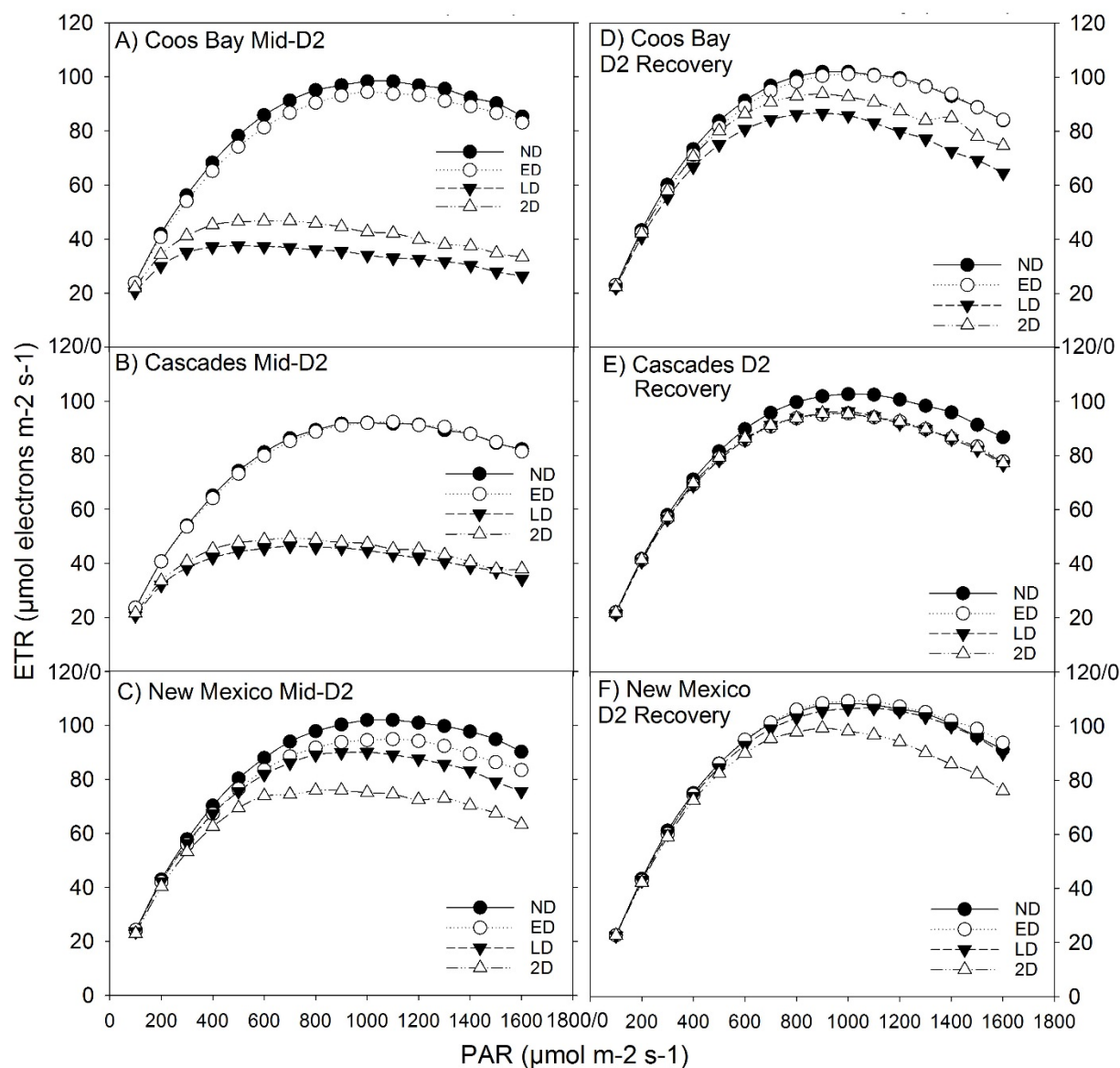


Fig 4. Rapid light curves showing electron transport rates (ETR) vs. light level (PAR) for Douglas-fir seedlings of the three provenances A-C during mid-drought 2 (2 Oct); D-F during the recovery from the second drought in figures D-F (15 Oct). Each symbol represents a mean for measurements on 6 seedlings for 2 Oct, and on 9 seedlings for 15 Oct. Treatments are ND (no drought), ED (early drought), LD (late drought), and 2D (two drought).

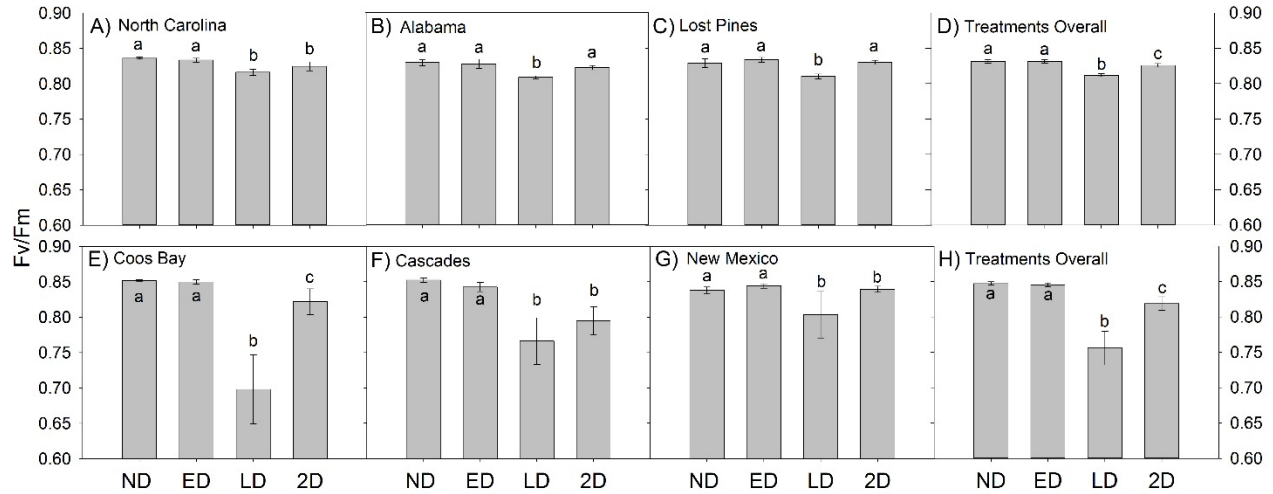


Fig 5. Dark-adapted fluorescence (F_v/F_m) of loblolly pine seedlings (A-D) by treatment for each provenance measured mid-drought (27 Sept) during the second drought ($n = 7$) and of Douglas-fir seedlings (E-H) by treatment for each provenance at the end (8 Oct) of the second drought ($n = 5$). Treatments are ND (no drought), ED (early drought), LD (late drought), and 2D (two drought). The same letter within a panel denotes no significant difference at $p < 0.05$.

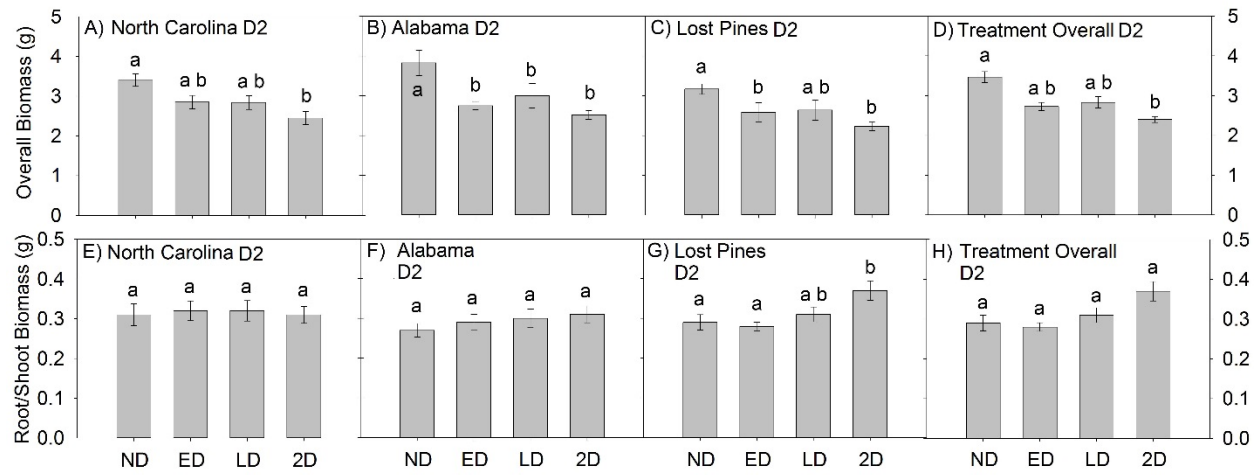


Fig 6. Loblolly pine (A-D) total overall biomass and (E-H) root/shoot ratio at the end of the second drought of for each of the provenances and the provenances pooled. Treatments are ND (no drought), ED (early drought), LD (late drought), and 2D (two drought). Error bars show standard error. The same letter within a panel denotes no significant difference at $p < 0.05$.

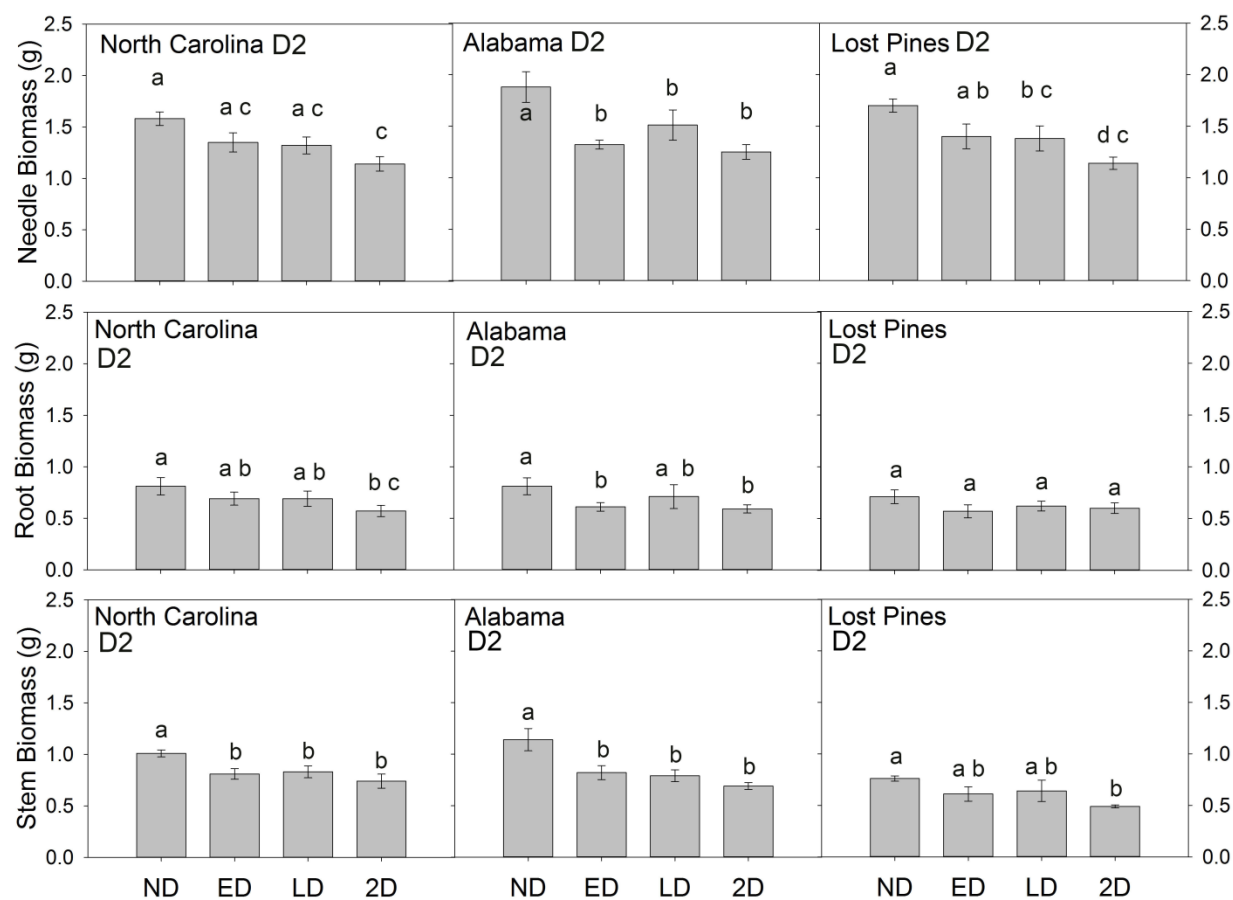


Fig 7. Loblolly pine needle (top), root (middle), and stem (bottom) biomass at the end of the second drought ($n = 7$). Treatments are ND (no drought), ED (early drought), LD (late drought), and 2D (two drought). Error bars show standard error. The same letter within a panel denotes no significant difference at $p < 0.05$.

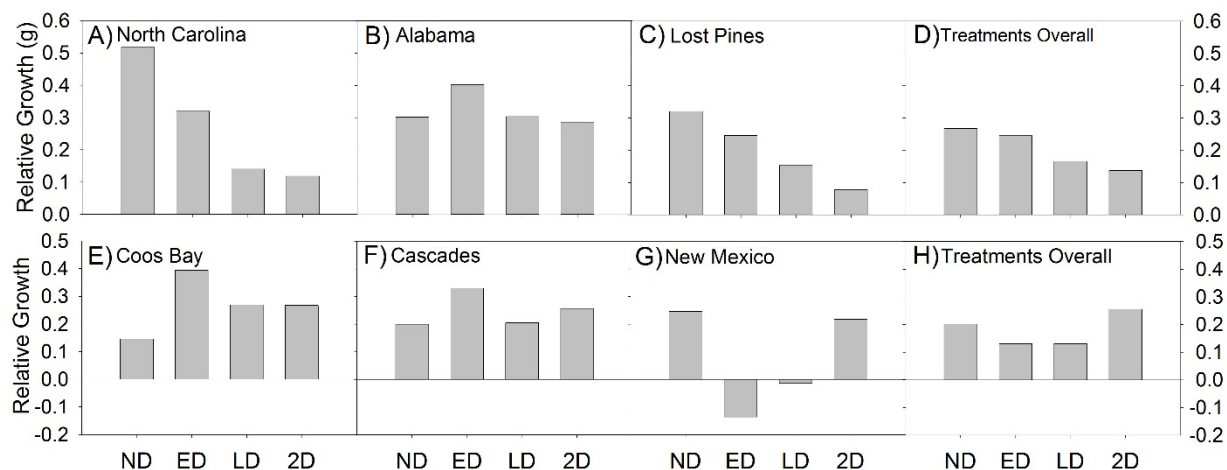


Fig 8. Relative growth rates of loblolly pine (A-D) and Douglas-fir (E-H). Treatments are ND (no drought), ED (early drought), LD (late drought), and 2D (two drought). Data shows relative differences only, not statistical differences.

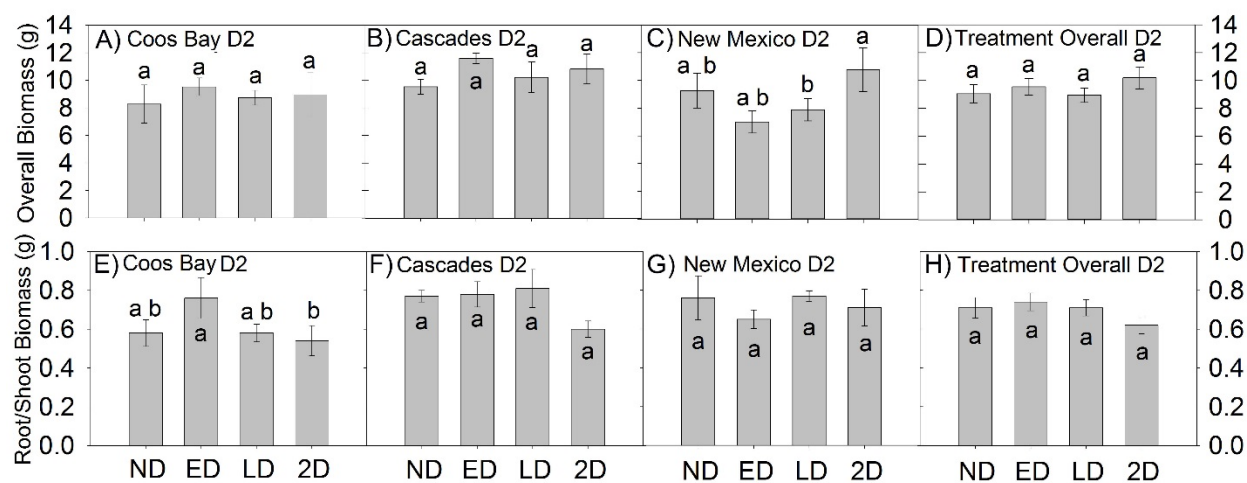


Fig 9. Douglas-fir (A-D) total overall biomass and (E-H) root/shoot ratio at the end of the second drought of for each of the provenances and the provenances pooled. Treatments are ND (no drought), ED (early drought), LD (late drought), and 2D (two drought). Error bars show standard error. The same letter within a panel denotes no significant difference at $p < 0.05$.

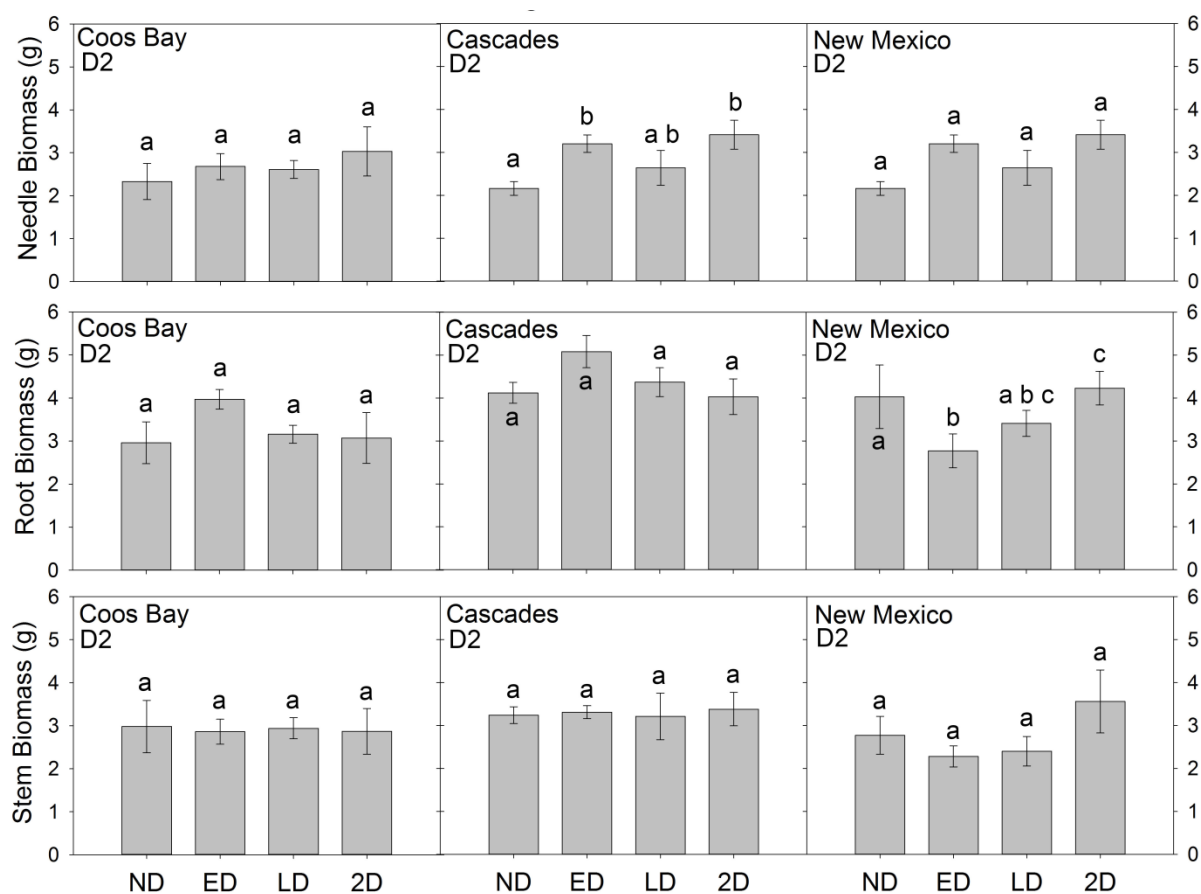


Fig 10. Douglas-fir needle (top), root (middle), and stem (bottom) biomass at the end of the second drought ($n = 5$). Treatments are ND (no drought), ED (early drought), LD (late drought), and 2D (two drought). Error bars show standard error. The same letter within a panel denotes no significant difference at $p < 0.05$.

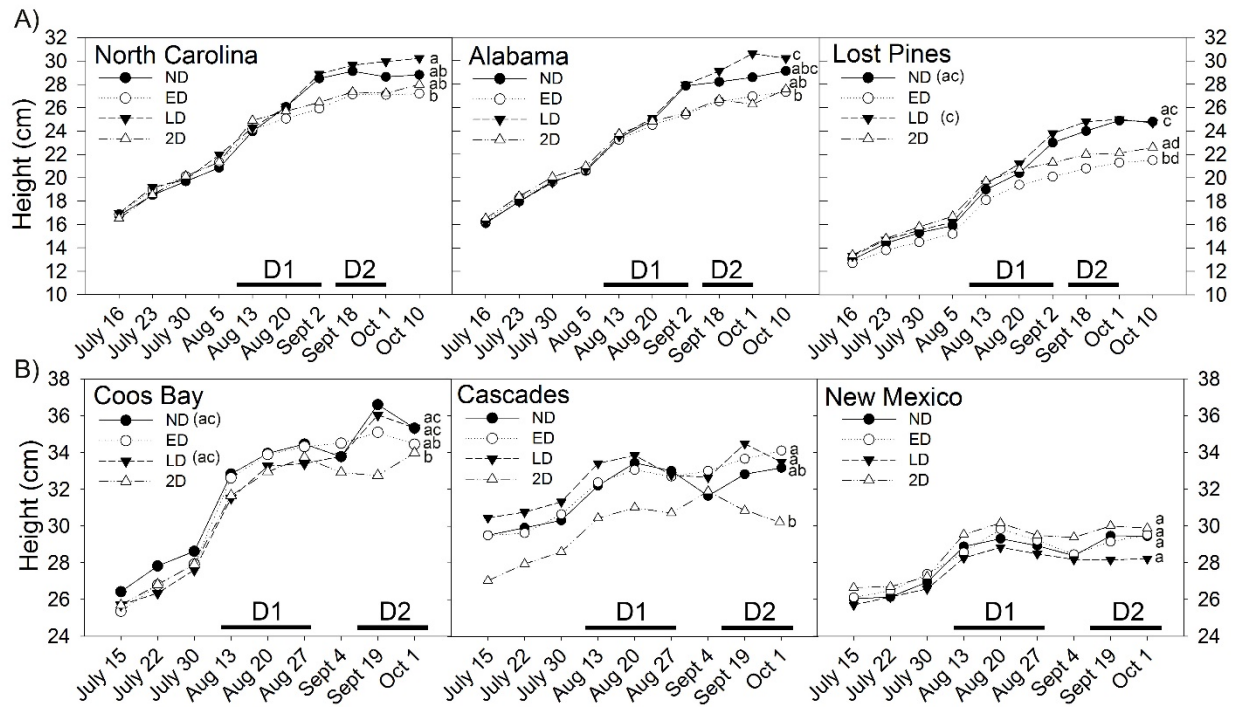


Fig 11. Heights of loblolly (A) and Douglas-fir (B) of the three provenances, measured through the summer of 2015. D1 shows first drought period, D2s shows the second drought period. Treatments are ND (no drought), ED (early drought), LD (late drought), and 2D (two drought) Sample size decreases over time for Douglas-fir, as some seedlings were harvested over the summer for the experiment (See Fig A8). For loblolly pine, sample size remains 7 (7 rows per treatment/provenance). The same letter within a panel denotes no significant difference at $p < 0.05$.

3. CONCLUSIONS

Given the increasing likelihood of drought and climate-change related stresses in the future, continued study of seedling responses to drought is vital in understanding and predicting forest dynamics (IPCC 2007, Dai 2011, 2013, Anderegg et al. 2013, 2015). Numerous studies have shown that drought can lead to reductions in photosynthesis and growth in trees and in seedlings, affecting gross primary productivity (Bréda et al. 2006, Breshears et al. 2009, Klos et al. 2009). Mortality and associated effects of drought on trees have been observed across many continents (Allen et al. 2010). Nonetheless, there remain uncertainties in how seedlings will cope with increasing stress, how species ranges may shift, and whether seedlings may or may not be able to adapt to new conditions and rapid change (St Clair and Howe 2007).

Beyond reductions in photosynthesis and growth, drought often leads to changes in allocation of photosynthate, including changes in allocation to aboveground and belowground biomass and other morphological adaptations. Effects of drought may extend to future years, and have long-lasting effects. Moreover, seedling responses may be further exacerbated by temperature stress (Allen et al. 2010, Anderegg et al. 2013, Williams et al. 2013), which was not addressed by the current study, but has an effect on seedling survival and performance in many drought and future climate change scenarios. Therefore, the water stress component of drought on its own will not fully predict drought stress implications for seedlings (Kolb and Robberecht 1996, Mittler 2006, Atkinson and Urwin 2012). For example, under drought stress, it is advantageous to close stomata to conserve water, but under temperature stress it can be advantageous to open stomata and take advantage of evaporative cooling (Kolb and Robberecht 1996). Similarly, many studies have considered the potential enhancements of elevated

atmospheric CO₂ in terms of productivity; however, given concurrent increases in biotic and abiotic stresses, benefits of elevated CO₂ may be greatly outweighed in many parts of the world that become increasingly prone to climatic extremes, and integrative studies are all the more important (Bauweraerts et al. 2014). Furthermore, species often respond differently to stresses, and even responses within species may show considerable differences. Another area of uncertainty is the extent to which seedling responses to drought represent the responses of saplings or mature trees, and vice-versa. Many studies only focus on specific age classes, but understanding and then integrating the responses at all ages is important to predict regeneration, succession, species composition, species ranges, and persistence of ecosystems on a landscape. If seedlings are unable to regenerate or persist on a landscape, then forests in those regions will decline or shift in composition towards species able to survive and reproduce (Kobe 1996).

Beyond individual tree health or forest composition, climate change and large-scale forest mortality or lack of regeneration can drastically alter landscapes, species ranges, microclimates, hydrologic cycles and forest products (Chapin et al. 2000, Dale et al. 2001, Parmesan and Yohe 2003, Allen 2007, Bonan 2008, Campbell et al. 2009). Yet, despite many advancements in recent years, the mechanisms of tree and seedling mortality are still not fully understood. A better understanding of effects of drought at the seedling level may be important for informed management decisions, and for better predicting effects of drought on resources that humans and many other species directly rely on.

This study investigated the responses of loblolly pine (*Pinus taeda*) and Douglas-fir (*Pseudotsuga menziesii*) seedlings from various provenances to drought stress in a greenhouse scenario. The goals of the study were to evaluate the responses of provenances associated with varying site climates (characterized by differences in mean annual precipitation, mean summer precipitation, and climatic moisture deficit) and also to study possible acclimation in response to drought exposure at the seedling level and across provenances. The study yielded evidence for differences in drought performance by provenance for both species. However, differences were not as distinct as expected, and further study of the respective provenances is necessary for more conclusive results. Questions remain as to the extent of drought performance differences among the respective provenances and among broader provenances. That is, this study included only three provenances for each species, limiting the extent to which differences can truly be associated more extensively to site climate conditions. Furthermore, this study did not include mortality, as droughts were designed to be sub-lethal in order to study subsequent acclimation. Mortality during more severe droughts may differ among the provenances.

More in-depth studies, investigating osmotic adjustment, hydraulic conductivity, anatomy, non-structural carbohydrate dynamics, stomatal conductance and other relevant measures may also provide greater understanding of intraspecific differences in performance across provenances. Given that the experiment was carried out in greenhouse conditions, results may not extend to field conditions or natural conditions. Low light levels in the greenhouse (often between 200 and 450 PAR) may also have influenced seedling responses. Both loblolly pine and Douglas-fir are relatively shade intolerant species, and light levels have been shown to

alter stress responses (Valladares and Pearcy 2002). For example, lower than usual light levels for a species can reduce carbon gain. Reductions in carbon gain can negatively affect a seedling's ability to cope with drought, further limiting photosynthate available for cell maintenance, repair, and growth. Especially in a drought scenario, where stomata are closed and photosynthesis further reduced, any additional impairments on a seedling's capacity to take full advantage of watered-conditions or to photosynthesize may reduce drought performance. While greenhouse studies provide many benefits, including better control of variables and environmental conditions, results from greenhouses may not always extend to natural conditions. Likewise, seedlings in this study were grown in containers, which likely affected root growth and seedling responses to drought. In raised beds or in field conditions, root systems may be able to expand or pursue deeper soil moisture sources; in containers, roots are bound to a small volume. Many studies have shown increased allocation to root biomass after drought, and containers may have inhibited greater allocations to root biomass. For example, this study did not see particularly large alterations of root biomass or of biomass overall across treatments, and this is likely an artefact of containers. Similarly, containerized seedlings may be more prone to temperature effects, with container walls more prone to heating (as seen in infrared imagery taken in addition to this study).

With respect to the second goal of the study, to investigate potential for drought acclimation, the study yielded some evidence that acclimation occurred in response to drought. Seedlings in 'late drought' treatments showed patterns of lower electron transport rate (ETR) and lower F_v/F_m during drought in comparison to 'two drought' seedlings during drought which had

experienced a previous drought, suggesting acclimation. Yet, given the limitations of the measures undertaken in this study, further study would be necessary to explore the exact forms of acclimation that led to these differences in ETR and F_v/F_m . Moreover, small samples sizes (as are common in labor-intensive and time intensive physiological studies) combined with high variation within measurements can reduce the ability to detect statistical differences. In future studies, focusing on one species alone and increasing sample size may be more effective in order to draw stronger conclusions, even though understanding of interspecific drought responses are also important. In this regard, the study was also unable to draw conclusions across the two species, as the ages were deemed to be too different for reliable comparison. Using species of the same age or using two ages of the same species would have possibly improved the strength of the study.

Questions also remain as to the extent of plasticity and acclimation among various provenances. This study was able to yield tentative evidence for differences in plasticity among the provenances, with provenances from more mesic site climates (such as the North Carolina provenance of loblolly pine and the Coos Bay provenance of Douglas-fir) showing more acclimation than provenances from more xeric site climates (such as the Alabama provenance from loblolly pine and the New Mexico provenance from Douglas-fir). Yet, it is difficult to fully disentangle these results, given that the Lost Pines provenance, often thought to be highly drought resistant, also showed acclimation. Either the Lost Pines are not as drought resistant as thought, or there are other factors influencing plasticity more strongly than site climate associations with provenance or drought resistance. Because plant material was provided and

selected by the industrial partner in this study, the extent to which site climate conditions can be fully linked for some of the provenances (primarily in the case of Douglas-fir) is limited to the range of site information disclosed by the industrial partner. The respective provenances did vary in site climate, but especially for Douglas-fir, two of the provenances (Coos Bay and Cascades provenances) had similar mean annual precipitation but different numbers of degree days. Because the Cascades provenance comes from a high elevation source, it may be influenced by somewhat more stressful conditions than the more coastal inland Coos Bay provenance. Yet, based on site climate information for the range of coordinates for the Cascades provenances, it is difficult to know with certainty if there might be larger or smaller differences in site climate conditions between the provenances.

This study attempted to provide important information on seedling responses to drought among provenances, as well as possibilities for drought acclimation. Although statistical evidence was not always found, nevertheless this study found intriguing patterns that encourage further study. If species such as Douglas-fir are in fact unable to adapt genetically at fast enough rates to match changing climates, acclimation may be one of few strategies available to populations for persisting on a landscape besides assisted migration. Predicting whether acclimation may prove a realistic strategy, and how this acclimation varies across a species range, will be important in understanding forests in a changing world.

References

- Allen, C. 2007. Interactions across spatial scales among forest dieback, fire, and erosion in northern New Mexico landscapes. *Ecosystems* 10:797-808.
- Allen, C.D., Macalady, A.K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M., Kitzberger, T., Rigling, A., Breshears, D.D., Hogg, E.H. 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and Management* 259: 660–684.
- Anderegg, W., Kane, J., Anderegg, L. 2013. Consequences of widespread tree mortality triggered by drought and temperature stress. *Nature Climate Change* 3: 30-36.
- Anderegg, W., Hicke, J., Fisher, R., Allen, C., Aukema, J., Bentz, B., Hood, S., Lichstein, J., Macalady, A., McDowell, N., Pan, Y., Raffa, K., Sala, A., Shaw, J., Stephenson, L., Tague, C., Zeppel, M. 2015. Tree mortality from drought, insects, and their interactions in a changing climate. *New Phytologist* 208 (3): 674-683.
- Atkinson, N., Urwin, P. 2012. The interaction of plant biotic and abiotic stresses: from genes to the field. *Journal of Experimental Botany* 63: 3523-3543.
- Bauweraerts, I., Ameye, M., Wertin, T., McGuire, M., Teskey, R., Steppe, K. 2014. Water availability is the decisive factor for the growth of two tree species in the occurrence of consecutive heat waves. *Agricultural and Forest Meteorology* 189-190: 19-29.
- Bonan, G. 2008. Forests and climate change: forcings, feedbacks, and the climate benefits of forests. *Science* 320: 1444-1449.
- Bréda, N., Huc, R., Granier, A., Dreyer, E. 2006. Temperate forest trees and stands under severe drought: a review of ecophysiological responses, adaptation processes and long-term consequences. *Annals of Forest Science* 63 (6): 625-644.
- Breshears, D.D., Myers O.B., Meyer C.W., Barnes F.J., Zou C.B., Allen C.D., McDowell N.G., Pockman W.T. 2009. Tree die-off in response to global change-type drought: mortality insights from a decade of plant water potential measurements. *Frontiers in Ecology and the Environment* 7(4):185-189.
- Campbell, J.L., Rustad L.E., Boyer E.W., Christopher S.F., Driscoll C.T., Fernandez I.J., Groffman P.M., Houle D., Kiebusch J, Magill A.H., Mitchell M.J., Ollinger S.V. 2009. Consequences of climate change for biogeochemical cycling in forests of northeastern North America. *Canadian Journal of Forest Research* 39:264–84.
- Chapin, F.S., Zavaleta, E., Eviner, V., Naylor, R., Vitousek, P., Reynolds, H., Hooper, D., Lavorel, S., Sala, O., Hobbie, S., Mack, M., Diaz, S. 2000. Consequences of changing biodiversity. *Nature* 405:234-242.

- Dai, A. 2011. Drought under global warming: a review. *Wiley Interdisciplinary Reviews: Climate Change* 2 (1): 45-65.
- Dai, A. 2013. Increasing drought under global warming in observations and models. *Nature Climate Change Letters* 3: 52-58.
- Dale, V., Joyce, L., McNulty, S., Neilson, R., Ayres, M., Flannigan, M., Hanson, P., Irland, L., Lugo, A., Peterson, C., Simberloff, D., Swanson, F., Stocks, B., Wotton, B.M. 2001. Climate change and forest disturbances. *BioScience* 51 (9): 723-734.
- IPCC, 2007. Climate change 2007: the physical science basis. In: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M., Miller, H.L. (Eds.), Contribution of Working Group I to the Fourth Assessment. Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom/New York, NY, USA, pp 996.
- Klos, R., Wang, G., Bauerle, W., Rieck, J. 2009. Drought impact on forest growth and mortality in the southeast USA: an analysis using Forest Health and Monitoring data. *Ecological Applications* 19 (3): 699-708.
- Kolb, P., Robberecht, R. 1996. High temperature and drought stress effects on survival of *Pinus ponderosa* seedlings. *Tree Physiology* 16(8): 665-72.
- Mittler, R. 2006. Abiotic stress, the field environment and stress combination. *Trends in Plant Science* 11:15–19.
- Parmesan, C., Yohe, G. 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* 421, 37–42
- St. Clair, B., Howe, G. 2007. Genetic maladaptation of coastal Douglas-fir seedlings to future climates. *Global Change Biology* 13:1441-1454.
- Valladares, F., Pearcy, R. 2002. Drought can be more critical in the shade than in the sun: a field study of carbon gain and photo-inhibition in a Californian shrub during a dry El Niño year. *Plant, Cell, and Environment* 25(6): 749-759.
- Williams, P., Allen, C., Macalady, A., Griffin, D., Woodhouse, C., Meko, D., Swetnam, T., Rauscher, S., Seager, R., Grissino-Mayer, H.D., Dean, J., Cook, E., Gangodagamage, C., Cai, M., McDowell, N. 2013. Temperature as a potent driver of regional forest drought stress and tree mortality. *Nature Climate Change* 3:292-297.

APPENDICES

Appendix A

This appendix contains tables and figures associated with Chapter 2 that we consider supplemental information. The methods for these tables and figures are included in Chapter 2, and all tables and figures are referenced there.

Table A1. Loblolly pine predawn water potentials (Ψ_p) statistical analysis (ANOVA F-tests) for the end of the first drought (D1) and the end of the second drought (D2). Bold signifies statistical significance at $p < 0.05$.

Date	Test	F-value	p-value (p)	df
End of D1	Treatment	29.1	1.33×10^{-10}	3, 45
	Provenance	0.007	0.993	2, 45
	Treat x Prov	0.868	0.526	6, 45
End of D2	Treatment	41.2	1.50×10^{-13}	3, 50
	Provenance	0.380	0.686	2, 50
	Treat x Prov	0.159	0.986	6, 50

Table A2. Douglas-fir predawn water potentials (Ψ_p) statistical analysis (ANOVA F-tests) for the end of the first drought (D1) and the end of the second drought (D2). Bold signifies statistical significance at $p < 0.05$.

Date	Test	F-value	p-value (p)	df
End of D1	Treatment	13.8	5.80×10^{-7}	3, 60
	Provenance	2.39	0.101	2, 60
	Treat x Prov	0.422	0.986	6, 60
End of D2	Treatment	41.5	2.37×10^{-15}	3, 68
	Provenance	6.39	0.00286	2, 68
	Treat x Prov	2.97	0.0122	6, 68

Table A3. Pairwise comparisons of loblolly pine predawn water potentials (Ψ_p) at the end of the first drought (D1) and at the end of the second drought (D2). CI represent 95% confidence intervals. Bold signifies statistical significance at $p < 0.05$. Treatments are ND (no drought), ED (early drought), LD (late drought), and 2D (two drought). Estimates are in MPa, with the treatment on the right side of the “versus” subtracted from the treatment on the left side of the “versus” in the comparison column.

Date	Provenance	Comparison	t-value	p-value (p)	Estimate (MPa)	Upper CI	Lower CI	df	n
End of D1	All	2D vs LD	-6.62	1.86×10^{-8}	-1.19	-0.83	-1.56	53	21
		2D vs ED	0.758	0.452	0.129	0.47	-0.21	53	21
		2D vs ND	-7.55	5.84×10^{-10}	-1.29	-0.94	-1.63	53	21
		ND vs ED	-6.80	9.67×10^{-9}	-1.16	-0.82	-1.50	53	21
		ND vs LD	-0.503	0.617	-0.091	0.27	-0.453	53	21
		ED vs LD	-5.90	2.58×10^{-7}	-1.07	-0.70	-1.43	53	21
	North Carolina	2D vs LD	-4.16	1.42×10^{-4}	-1.34	-0.69	-1.98	45	7
		2D vs ED	-0.86	0.393	-0.26	0.35	-0.87	45	7
		2D vs ND	-5.05	8×10^{-6}	-1.53	-0.92	-2.14	45	7
		ND vs ED	4.19	1.28×10^{-4}	1.27	1.88	0.66	45	7
		ND vs LD	0.61	0.547	0.19	0.84	-0.45	45	7
		ED vs LD	-3.35	0.00166	-1.07	-0.43	-1.72	45	7
	Alabama	2D vs LD	-4.72	2.4×10^{-5}	-1.52	-0.87	-2.16	45	7
		2D vs ED	-0.98	0.332	-0.30	0.31	-0.91	45	7
		2D vs ND	-3.86	3.62×10^{-4}	-1.17	-0.56	-1.78	45	7
		ND vs ED	2.88	6.11×10^{-3}	0.87	1.48	0.26	45	7
		ND vs LD	-1.08	0.286	-0.35	0.30	-0.99	45	7
		ED vs LD	-3.79	4.43×10^{-4}	-1.22	-0.57	-1.87	45	7
	Lost Pines	2D vs LD	-2.28	0.0272	-0.73	-0.09	-1.38	45	7
		2D vs ED	0.56	0.575	0.17	0.78	-0.44	45	7
		2D vs ND	-3.82	4.05×10^{-4}	-1.16	-0.55	-1.77	45	7
		ND vs ED	4.39	6.9×10^{-5}	1.33	1.94	0.72	45	7
		ND vs LD	1.32	0.194	0.42	1.07	-0.22	45	7
		ED vs LD	-2.82	0.00721	-0.90	-0.26	-1.55	45	7
End of D2	All	2D vs LD	-1.28	0.204	-0.234	0.131	-0.60	58	21
		2D vs ED	-7.66	2.27×10^{-10}	-1.57	-1.16	-1.98	58	21
		2D vs ND	7.96	7.07×10^{-11}	1.63	2.04	1.22	58	21
		ND vs ED	0.270	0.788	0.0617	0.52	-0.40	58	21
		ND vs LD	8.92	1.79×10^{-12}	1.86	2.04	1.22	58	21
		ED vs LD	-8.63	5.53×10^{-12}	-1.80	-1.38	-2.22	58	21
	North Carolina	2D vs LD	-1.08	0.285	-0.36	0.31	-1.02	50	7
		2D vs ED	4.18	1.18×10^{-4}	1.55	2.30	0.81	50	7
		2D vs ND	4.78	1.6×10^{-5}	1.78	2.52	1.03	50	7
		ND vs ED	-0.53	0.596	-0.22	0.62	-1.07	50	7
		ND vs LD	-5.57	1.0×10^{-6}	-2.13	-1.36	-2.90	50	7
		ED vs LD	-4.99	7.78×10^{-6}	-1.91	-1.14	-2.68	50	7
	Alabama	2D vs LD	-0.26	0.796	-0.09	0.60	-0.78	50	7
		2D vs ED	4.04	1.82×10^{-4}	1.55	2.32	0.78	50	7
		2D vs ND	4.13	1.37×10^{-4}	1.58	2.35	0.81	50	7
		ND vs ED	-0.08	0.936	-0.03	0.81	-0.88	50	7

		ND vs LD	-4.36	6.4×10^{-5}	-1.67	-0.90	-2.44	50	7
		ED vs LD	-4.28	8.6×10^{-5}	-1.64	-0.87	-2.41	50	7
	Lost Pines	2D vs LD	-0.78	0.441	-0.26	0.41	-0.92	50	7
		2D vs ED	4.30	7.9×10^{-5}	1.60	2.35	0.85	50	7
		2D vs ND	4.10	1.50×10^{-4}	1.53	2.27	0.78	50	7
		ND vs ED	0.17	0.863	0.07	0.92	-0.77	50	7
		ND vs LD	-4.65	2.4×10^{-5}	-1.78	-1.01	-2.55	50	7
		ED vs LD	-4.84	1.3×10^{-5}	-1.85	-1.09	-2.62	50	7

Table A4. Pairwise comparisons of Douglas-fir predawn water potentials (Ψ_p) at the end of the first drought (D1) and at the end of the second drought (D2). CI represent 95% confidence intervals. Bold signifies statistical significance at $p < 0.05$. Treatments are ND (no drought), ED (early drought), LD (late drought), and 2D (two drought). Estimates are in MPa, with the treatment on the right side of the “versus” subtracted from the treatment on the left side of the “versus” in the comparison column.

Date	Provenance	Comparison:	t-value	p-value (<i>p</i>)	Est- imate (MPa)	Upper CI	Lower CI	df	n
End of D1	All	2D vs LD	5.31	1.28x10⁻⁶	1.18	1.63	0.74	68	18
		2D vs ED	-2.52	0.0142	-0.56	-0.116	-1.00	68	18
		2D vs ND	5.40	9.24x10⁻⁷	1.20	1.64	0.76	68	18
		ND vs ED	2.88	0.0053	0.64	1.08	0.20	68	18
		ND vs LD	0.084	0.933	0.019	0.46	-0.43	68	18
		ED vs LD	2.89	0.00671	0.622	1.07	0.18	68	18
	Coos Bay	2D vs LD	3.67	0.00052	1.39	2.14	0.63	60	6
		2D vs ED	1.53	0.13087	0.58	1.33	-0.18	60	6
		2D vs ND	3.79	0.00036	1.43	2.19	0.67	60	6
		ND vs ED	-2.25	0.02788	-0.85	-0.10	-1.61	60	6
		ND vs LD	-0.12	0.908	-0.04	0.71	-0.80	60	6
		ED vs LD	2.14	0.0366	0.81	1.56	0.05	60	6
	Cascades	2D vs LD	4.18	0.00010	1.58	2.33	0.82	60	6
		2D vs ED	2.58	0.0124	0.97	1.73	0.22	60	6
		2D vs ND	4.25	0.00008	1.60	2.36	0.85	60	6
		ND vs ED	-1.67	0.101	-0.63	0.13	-1.39	60	6
		ND vs LD	-0.07	0.944	-0.03	0.73	-0.78	60	6
		ED vs LD	1.60	0.115	0.60	1.36	-0.15	60	6
	New Mexico	2D vs LD	0.33	0.739	0.13	0.88	-0.63	60	6
		2D vs ED	1.54	0.130	0.58	1.34	-0.18	60	6
		2D vs ND	1.50	0.139	0.57	1.32	-0.19	60	6
		ND vs ED	-1.17	0.249	-0.44	0.32	-1.20	60	6
		ND vs LD	0.04	0.971	0.01	0.77	-0.74	60	6
		ED vs LD	1.20	0.234	0.45	1.21	-0.30	60	6
End of D2	All	2D vs LD	-0.076	0.939	-0.018	0.441	-0.477	68	15-30
		2D vs ED	-6.59	5.16x10⁻⁹	-1.77	-1.23	-2.30	68	15-30
		2D vs ND	7.07	6.50x10⁻¹⁰	1.89	2.43	1.36	68	15-30
		ND vs ED	0.433	0.667	0.129	0.721	-0.464	68	15-30
		ND vs LD	1.91	3.05 x 10⁻¹⁰	1.91	2.44	1.39	68	15-30
		ED vs LD	-6.76	2.52x10⁻⁹	-1.78	-1.26	-2.31	68	15-30
	Coos Bay	2D vs LD	-1.48	0.144	-0.51	0.18	-1.21	68	5-9
		2D vs ED	5.06	3.42 x 10⁻⁶	2.06	2.87	1.25	68	5-9
		2D vs ND	5.24	1.73x10⁻⁶	2.13	2.95	1.32	68	5-9
		ND vs ED	-0.16	0.873	-0.07	0.83	-0.97	68	5-9
		ND vs LD	-6.64	6.28 x 10⁻⁹	-2.65	-1.85	-3.44	68	5-9
		ED vs LD	-6.46	1.33x10⁻⁸	-2.57	-1.78	-3.37	68	5-9
	Cascades	2D vs LD	-1.23	0.223	-0.46	0.28	-1.19	68	5-8

		2D vs ED	4.22	7.36x10⁻⁵	1.72	2.53	0.91	68	5-8
		2D vs ND	4.71	1.28x10⁻⁵	1.92	2.73	1.11	68	5-8
		ND vs ED	-0.44	0.663	-0.20	0.70	-1.10	68	5-8
		ND vs LD	-5.67	3.16x10⁻⁶	-2.37	-1.54	-3.21	68	5-8
		ED vs LD	-5.20	2.01x10⁻⁶	-2.18	-1.34	-3.01	68	5-8
	New Mexico	2D vs LD	3.72	4.05x10⁻⁴	1.52	2.33	0.70	68	5-10
		2D vs ED	2.14	0.0357	0.73	1.40	0.05	68	5-10
		2D vs ND	4.00	1.57x10⁻⁴	1.63	2.44	0.82	68	5-10
		ND vs ED	-0.25	0.800	-0.12	0.79	-1.02	68	5-10
		ND vs LD	-2.31	0.0239	-0.90	-0.12	-1.69	68	5-10
		ED vs LD	-2.02	0.0477	-0.79	-0.01	-1.57	68	5-10

Table A5. Statistical analysis (ANOVA F-Tests) and pairwise comparisons of dark-adapted fluorescence (F_v/F_m) for loblolly pine on two dates during the second drought (D2). (n = 7 measurements per provenance x treatment) Bold signifies statistical significance at $p < 0.05$. Treatments are ND (no drought), ED (early drought), LD (late drought), and 2D (two drought).

Date	Provenance	Test	F-value	p-value (<i>p</i>)	df
Mid-D2 (23 Sept)	All	Treatment	8.80	4.79 x 10⁻⁵	3, 72
		Provenance	2.32	0.106	2, 72,
		Treatment x Provenance	0.86	0.532	6, 72
Mid-D2 (27 Sept)	All	Treatment	14.3	2.10 x 10⁻⁷	3, 72
		Provenance	1.75	0.118	2, 72,
		Treatment x Provenance	0.506	0.802	6, 72
Date:	Provenance	Comparison:	t-value	p-value (<i>p</i>)	df
Mid-D2 (23 Sept)	All	2D vs LD	2.58	0.0118	80
	North Carolina	2D vs LD	-1.71	1.00	72
	Alabama	2D vs LD	2.63	0.010	72
	Lost Pines	2D vs LD	1.89	0.064	72
Near end of D2 (27 Sept)	All	2D vs LD	4.04	1.23 x 10⁻⁴	80
	North Carolina	2D vs LD	1.37	0.174	72
	Alabama	2D vs LD	2.27	0.026	72
	Lost Pines	2D vs LD	3.29	0.002	72

Table A6. Statistical analysis (ANOVA F-tests) and pairwise comparisons of dark-adapted fluorescence (F_v/F_m) for Douglas-fir on two dates during the second drought (D2). (n = 6 measurements per provenance x treatment) Bold signifies statistical significance at $p < 0.05$. Treatments are ND (no drought), ED (early drought), LD (late drought), and 2D (two drought).

Date	Provenance	Test	F-value	p-value (p)	df
Mid D2 (3 Oct)	All	Treatment	5.13	0.00319	3, 60
		Provenance	2.57	0.085	2, 60
		Treatment x Provenance	2.92	0.0145	6, 60
End of D2 (8 Oct)	All	Treatment	12.2	4.61x10⁻⁶	3, 48
		Provenance	1.54	0.225	2, 48
		Treatment x Provenance	2.04	0.079	6, 48
Date:	Provenance	Comparison:	t-value	p-value (p)	df
Mid D2 (3 Oct)	All	2D vs LD	0.777	0.440	68
	Coos Bay	2D vs LD	2.99	0.004	60
	Cascade	2D vs LD	-1.07	0.287	60
	New Mexico	2D vs LD	-0.435	0.665	60
End of D2 (8 Oct)	All	2D vs LD	3.42	0.00116	56
	Coos Bay	2D vs LD	4.14	1.40 x 10⁻⁴	48
	Cascade	2D vs LD	0.965	0.339	48
	New Mexico	2D vs LD	1.20	0.236	48

Table A7. Statistical analysis (ANOVA F-tests) of loblolly pine total biomass (roots, stems, needles combined), root/shoot biomass, and needles, roots and stem biomass harvested at the end of the second drought (D2). Bold signifies statistical significance at $p < 0.05$. Treatments are ND (no drought), ED (early drought), LD (late drought), and 2D (two drought).

Biomass	Test	F-value	p-value (<i>p</i>)	df	n
Total	Treatment	14.5	1.90×10^{-7}	3, 71	7
	provenance	3.46	0.037	2, 71	7
	Treat x Prov	0.376	0.892	6, 71	7
Root to Shoot	Treatment	2.00	0.122	3, 71	7
	provenance	1.35	0.267	2, 71	7
	Treat x Prov	1.13	0.356	6, 71	7
Needles	Treatment	16.24	3.91×10^{-8}	3, 71	7
	Provenance	2.00	0.144	2, 71	7
	Treat x Prov	0.627	0.708	6, 71	7
Roots	Treatment	4.24	8.15×10^{-3}	3, 71	7
	Provenance	1.10	0.338	2, 71	7
	Treat x Prov	0.336	0.916	6, 71	7
Stems	Treatment	13.6	4.09×10^{-7}	3, 71	7
	Provenance	16.3	1.46×10^{-6}	2, 71	7
	Treat x Prov	0.723	0.632	6, 71	7

Table A8. Statistical analysis (ANOVA F-tests) of Douglas-fir total biomass (roots, stems, needles combined), root/shoot biomass, and needles, roots and stem biomass harvested at the end of the second drought (D2). Bold signifies statistical significance at $p < 0.05$. Treatments are ND (no drought), ED (early drought), LD (late drought), and 2D (two drought).

Biomass	Test	F-value	p-value (<i>p</i>)	df	n
Total	Treatment	0.834	0.481	3, 50	5
	provenance	3.06	0.056	2, 50	5
	Treat x Prov	1.10	0.377	6, 50	5
Root to Shoot	Treatment	1.32	0.278	3, 50	5
	provenance	3.31	0.045	2, 50	5
	Treat x Prov	1.00	0.434	6, 50	5
Needles	Treatment	3.29	0.028	3, 50	5
	Provenance	1.59	0.213	2, 50	5
	Treat x Prov	0.960	0.462	6, 50	5
Roots	Treatment	0.405	0.750	3, 50	5
	Provenance	6.00	4.61 x 10⁻³	2, 50	5
	Treat x Prov	1.82	0.115	6, 50	5
Stems	Treatment	0.581	0.630	3, 50	5
	Provenance	1.48	0.236	2, 50	5
	Treat x Prov	0.541	0.775	6, 50	5

Table A9. Pairwise comparisons of loblolly pine biomass at the end of the second drought (D2) for the three respective provenances. Biomass was measured in grams (g). CI represent 95% confidence intervals. Bold signifies statistical significance at $p < 0.05$. Treatments are ND (no drought), ED (early drought), LD (late drought), and 2D (two drought). Estimates are in grams (g), with the treatment on the right side of the “versus” subtracted from the treatment on the left side of the “versus” in the comparison column.

Comparison	Biomass	Provenance	t-value	p-value (<i>p</i>)	Estimate (g)	n
2D vs LD	Needles	North Carolina	-1.29	0.203	-0.176 (-0.450, 0.097)	7
		Alabama	-1.88	0.064	-0.259 (-0.532, 0.015)	7
		Lost Pines	-1.77	0.080	-0.243 (-0.517, 0.030)	7
	Roots	North Carolina	-1.26	0.211	-0.121 (-0.312, 0.070)	7
		Alabama	-1.26	0.211	-0.121 (-0.312, 0.071)	7
		Lost Pines	-0.197	0.844	-0.019 (-0.210, 0.172)	7
	Stems	North Carolina	-1.01	0.318	-0.091 (-0.272, 0.090)	7
		Alabama	-1.10	0.276	-0.010 (-0.281, 0.081)	7
		Lost Pines	-1.64	0.105	-0.149 (-0.330, 0.032)	7
ND vs 2D	Needles	North Carolina	-3.06	3.09×10^{-3}	-0.437 (-0.722, -0.153)	7
		Alabama	-4.60	1.82×10^{-5}	-0.630 (-0.904, -0.360)	7
		Lost Pines	-4.10	1.10×10^{-4}	-0.562 (-0.835, -0.288)	7
	Roots	North Carolina	-2.37	2.04×10^{-2}	-0.236 (-0.435, -0.038)	7
		Alabama	-2.33	2.27×10^{-2}	-0.223 (-0.414, -0.032)	7
		Lost Pines	-1.13	0.261	-0.109 (-0.300, 0.082)	7
	Stems	North Carolina	-2.91	4.79×10^{-3}	-0.275 (-0.464, -0.087)	7
		Alabama	-5.01	3.88×10^{-6}	-0.454 (-0.635, -0.273)	7
		Lost Pines	-2.98	3.95×10^{-3}	-0.270 (-0.451, -0.089)	7
ND vs LD	Needles	North Carolina	-1.83	0.072	-0.261 (-0.546, 0.024)	7
		Alabama	-2.71	0.008	-0.372 (-0.645, -0.098)	7
		Lost Pines	-2.32	0.023	-0.319 (-0.592, -0.045)	7
	Roots	North Carolina	-1.16	0.250	-0.116 (-0.314, 0.083)	7
		Alabama	-1.07	0.289	-0.102 (-0.293, 0.089)	7
		Lost Pines	-0.94	0.352	-0.90 (-0.281, 0.101)	7
	Stems	North Carolina	-1.95	0.056	-1.84 (-0.372, 0.005)	7
		Alabama	-3.91	2.10×10^{-4}	-0.355 (-0.536, -0.174)	7
		Lost Pines	-1.34	0.186	-0.121 (-0.302, 0.060)	7
ND vs ED	Needles	North Carolina	-1.58	0.117	-0.226 (-0.511, 0.058)	7
		Alabama	-4.10	1.09×10^{-4}	-0.562 (-0.836, -0.289)	7
		Lost Pines	-2.16	0.345	-0.296 (-0.569, -0.022)	7
	Roots	North Carolina	-1.89	0.239	-0.118 (-0.317, 0.080)	7
		Alabama	-2.08	0.041	-0.199 (-0.390, -0.008)	7
		Lost Pines	-1.51	0.135	-0.145 (-0.336, 0.046)	7
	Stems	North Carolina	-2.15	0.035	-0.203 (-0.391, -0.015)	7
		Alabama	-3.49	8.27×10^{-4}	-0.317 (-0.498, -0.136)	7
		Lost Pines	-1.72	0.090	-0.156 (-0.337, 0.025)	7

Table A10. Pairwise comparisons of Douglas-fir needles, roots, and stem biomass at the end of the second drought (D2) for the three respective provenances. Biomass was measured in grams. CI represent 95% confidence intervals. Bold signifies statistical significance at $p < 0.05$.

Treatments are ND (no drought), ED (early drought), LD (late drought), and 2D (two drought). Estimates are in grams (g), with the treatment on the right side of the “versus” subtracted from the treatment on the left side of the “versus” in the comparison column.

Comparison	Biomass	Provenance	t-value	p-value (p)	Estimate (g)	df	n
2D vs LD	Needles	Coos Bay	0.889	0.379	0.412 (-1.35, 1.16)	50	5
		Cascades	1.58	0.121	0.764 (-1.65, 0.97)	50	5
		New Mexico	1.84	0.071	0.893 (-0.488, 2.14)	50	5
	Roots	Coos Bay	-0.151	0.881	-0.094 (-1.35, 1.16)	50	5
		Cascades	-0.521	0.605	-0.340 (-1.65, 0.97)	50	5
		New Mexico	1.26	0.213	0.834 (-0.488, 2.14)	50	5
	Stems	Coos Bay	-0.127	0.899	-0.076 (-1.28, 1.12)	50	5
		Cascades	0.266	0.791	0.167 (-1.09, 1.42)	50	5
		New Mexico	1.86	0.069	1.16 (-0.093, 2.41)	50	5
ND vs 2D	Needles	Coos Bay	1.43	0.158	0.700 (-0.279, 1.67)	50	5
		Cascades	2.57	0.013	1.24 (0.270, 2.22)	50	5
		New Mexico	1.13	0.264	0.508 (-0.394, 1.41)	50	5
	Roots	Coos Bay	0.158	0.875	0.103 (-1.21, 1.41)	50	5
		Cascades	-0.146	0.884	-0.096 (-1.41, 1.22)	50	5
		New Mexico	0.342	0.733	0.207 (-1.01, 1.42)	50	5
	Stems	Coos Bay	-0.184	0.854	-0.115 (-1.37, 1.14)	50	5
		Cascades	0.216	0.830	0.135 (-1.12, 1.39)	50	5
		New Mexico	1.37	0.178	0.789 (-0.371, 1.95)	50	5
ND vs LD	Needles	Coos Bay	0.610	0.545	0.283 (-0.649, 1.215)	50	5
		Cascades	0.991	0.326	0.480 (-0.493, 1.45)	50	5
		New Mexico	-0.859	0.394	-0.386 (-1.29, 0.516)	50	5
	Roots	Coos Bay	0.316	0.754	0.198 (-1.06, 1.45)	50	5
		Cascades	0.375	0.710	0.245 (-1.07, 1.56)	50	5
		New Mexico	-1.02	0.313	-0.617 (-1.83, 0.598)	50	5
	Stems	Coos Bay	-0.652	0.948	-0.039 (-1.24, 1.16)	50	5
		Cascades	-0.050	0.960	-0.031 (-1.28, 1.22)	50	5
		New Mexico	-0.644	0.523	-0.372 (-1.53, 0.788)	50	5
ND vs ED	Needles	Coos Bay	0.728	0.470	0.353 (-0.621, 1.33)	50	5
		Cascades	2.14	0.037	1.04 (0.066, 2.013)	50	5
		New Mexico	-1.04	0.304	-0.499 (-1.46, 0.466)	50	5
	Roots	Coos Bay	1.55	0.128	1.01 (-0.30, 2.32)	50	5
		Cascades	1.47	0.149	0.957 (-0.354, 2.27)	50	5
		New Mexico	-1.95	0.057	-1.26 (-2.56, 0.040)	50	5
	Stems	Coos Bay	-0.191	0.849	-0.119 (-1.37, 1.13)	50	5
		Cascades	0.110	0.912	0.069 (-1.18, 1.32)	50	5
		New Mexico	-0.790	0.433	-0.489 (-1.73, 0.753)	50	5

Table A11. Pairwise comparisons of loblolly pine total biomass (roots, stems, and needles) at the end of the second drought (D2). Biomass was measured in grams (g). CI represent 95% confidence intervals. Bold signifies statistical significance at $p < 0.05$. Treatments are ND (no drought), ED (early drought), LD (late drought), and 2D (two drought). Estimates are in grams (g), with the treatment on the right side of the “versus” subtracted from the treatment on the left side of the “versus” in the comparison column.

Provenance	Comparison	t-value	p-value (p)	Estimate (g)	Upper CI	Lower CI	df	n
All	2D vs LD	-2.58	0.0118	2.828	-0.073	-0.755	79	21
	2D vs ED	1.97	0.053	0.325	0.654	-0.004	79	21
	2D vs ND	-6.39	1.07x10⁻⁸	-1.69	-0.736	-1.40	79	21
	ND vs ED	-4.45	2.82x10⁻⁵	-0.744	-0.411	-1.077	79	21
	ND vs LD	-3.84	2.44x10⁻⁴	-0.643	-0.310	-0.976	79	21
	ED vs LD	0.543	-0.611	-0.101	0.228	-0.430	79	21
North Carolina	2D vs LD	-1.37	0.176	-0.39	0.18	-0.96	71	7
	2D vs ED	-1.41	0.162	-0.40	0.17	-0.97	71	7
	2D vs ND	-3.21	0.0020	-0.95	-0.36	-1.54	71	7
	ND vs ED	1.85	0.0683	0.55	1.14	-0.04	71	7
	ND vs LD	1.89	0.0622	0.56	1.15	-0.03	71	7
	ED vs LD	0.04	0.964	0.01	0.58	-0.55	71	7
Alabama	2D vs LD	-1.69	0.0963	-0.48	0.09	-1.05	71	7
	2D vs ED	-0.81	0.421	-0.23	0.34	-0.80	71	7
	2D vs ND	-4.60	1.78x10⁻⁵	-1.31	-0.74	-1.87	71	7
	ND vs ED	3.79	3.10x10⁻⁴	1.08	1.64	0.51	71	7
	ND vs LD	2.92	0.00473	0.83	1.40	0.26	71	7
	ED vs LD	-0.88	0.384	-0.25	0.32	-0.82	71	7
Lost Pines	2D vs LD	-1.45	0.152	-0.41	0.16	-0.98	71	7
	2D vs ED	-1.21	0.229	-0.34	0.22	-0.91	71	7
	2D vs ND	-3.31	0.00147	-0.94	-0.37	-1.51	71	7
	ND vs ED	2.10	0.0395	0.60	1.16	0.03	71	7
	ND vs LD	1.86	0.0665	0.53	1.10	-0.04	71	7
	ED vs LD	-0.23	0.815	-0.07	0.50	-0.63	71	7

Table A12. Pairwise comparisons of loblolly pine root/shoot biomass (root divided by sum of stems and needles) harvested at the end of the second drought (D2). Biomass was measured in grams (g). CI represent 95% confidence intervals. Bold signifies statistical significance at $p < 0.05$. Treatments are ND (no drought), ED (early drought), LD (late drought), and 2D (two drought). Estimates are in grams (g), with the treatment on the right side of the “versus” subtracted from the treatment on the left side of the “versus” in the comparison column.

Provenance	Comparison	t-value	p-value (p)	Estimate (g)	Upper CI	Lower CI	df	n
All	2D vs LD	0.861	0.392	0.015	0.050	-0.020	79	21
	2D vs ED	-1.75	0.083	-0.031	0.004	-0.066	79	21
	2D vs ND	2.24	0.028	0.040	0.075	0.004	79	21
	ND vs ED	0.507	0.613	0.009	0.0445	-0.026	79	21
	ND vs LD	1.39	0.169	0.025	0.060	-0.011	79	21
	ED vs LD	-0.892	0.375	-0.016	0.019	-0.051	79	21
North Carolina	2D vs LD	-0.431	0.668	-0.013	0.047	-0.073	71	7
	2D vs ED	-0.458	0.648	-0.014	0.046	-0.074	71	7
	2D vs ND	-0.108	0.915	-0.003	0.059	-0.066	71	7
	ND vs ED	-0.332	0.741	-0.010	0.052	-0.073	71	7
	ND vs LD	-0.307	0.760	-0.010	0.053	-0.072	71	7
	ED vs LD	0.027	0.979	0.001	0.061	-0.059	71	7
Alabama	2D vs LD	0.173	0.863	0.005	0.065	-0.055	71	7
	2D vs ED	0.645	0.521	0.019	0.080	-0.041	71	7
	2D vs ND	1.254	0.214	0.038	0.098	-0.022	71	7
	ND vs ED	-0.609	0.544	-0.018	0.042	-0.079	71	7
	ND vs LD	-1.081	0.283	-0.033	0.028	-0.093	71	7
	ED vs LD	-0.472	0.639	-0.014	0.046	-0.075	71	7
Lost Pines	2D vs LD	1.763	0.0822	0.053	0.114	-0.007	71	7
	2D vs ED	2.878	0.00528	0.087	0.147	0.027	71	7
	2D vs ND	2.714	0.00835	0.082	0.142	0.022	71	7
	ND vs ED	0.165	0.870	0.005	0.065	-0.055	71	7
	ND vs LD	-0.950	0.345	-0.029	0.032	-0.089	71	7
	ED vs LD	-1.115	0.269	-0.034	0.027	-0.094	71	7

Table A13. Pairwise comparisons of Douglas-fir total biomass (roots, stems, and needles) at the end of the second drought (D2). Biomass was measured in grams (g). CI represent 95% confidence intervals. Bold signifies statistical significance at $p < 0.05$. Treatments are ND (no drought), ED (early drought), LD (late drought), and 2D (two drought). Estimates are in grams (g), with the treatment on the right side of the “versus” subtracted from the treatment on the left side of the “versus” in the comparison column.

Provenance	Comparison	t-value	p-value (<i>p</i>)	Estimate (g)	Upper CI	Lower CI	df	n
All	2D vs LD	1.37	0.177	1.25	3.08	-0.58	58	15-21
	2D vs ED	-0.669	0.506	-0.633	1.26	-2.53	58	15-21
	2D vs ND	1.25	0.216	1.13	2.94	-0.68	58	15-21
	ND vs ED	0.539	0.592	0.495	2.34	-1.35	58	15-21
	ND vs LD	-0.137	0.892	-0.123	1.66	-1.90	58	15-21
	ED vs LD	0.661	0.511	0.617	2.48	-1.25	58	15-21
Coos Bay	2D vs LD	0.16	0.871	0.24	3.22	-2.74	50	5-7
	2D vs ED	-0.36	0.719	-0.56	2.55	-3.67	50	5-7
	2D vs ND	0.44	0.661	0.68	3.80	-2.43	50	5-7
	ND vs ED	-0.80	0.425	-1.25	1.87	-4.36	50	5-7
	ND vs LD	-0.30	0.767	-0.44	2.54	-3.42	50	5-7
	ED vs LD	0.54	0.590	0.80	3.78	-2.18	50	5-7
Cascades	2D vs LD	0.38	0.705	0.59	3.70	-2.52	50	5-7
	2D vs ED	-0.50	0.616	-0.78	2.33	-3.89	50	5-7
	2D vs ND	0.83	0.412	1.28	4.40	-1.83	50	5-7
	ND vs ED	-1.33	0.189	-2.07	1.05	-5.18	50	5-7
	ND vs LD	-0.45	0.656	-0.69	2.42	-3.81	50	5-7
	ED vs LD	0.89	0.380	1.37	4.48	-1.74	50	5-7
New Mexico	2D vs LD	2.28	0.0268	3.75	7.05	0.45	50	5-7
	2D vs ED	1.86	0.0693	2.88	5.99	-0.24	50	5-7
	2D vs ND	1.05	0.300	1.50	4.39	-1.38	50	5-7
	ND vs ED	1.46	0.150	2.25	5.33	-0.84	50	5-7
	ND vs LD	0.96	0.343	1.37	4.26	-1.51	50	5-7
	ED vs LD	-0.53	0.597	-0.87	2.43	-4.18	50	5-7

Table A14. Pairwise comparisons of Douglas-fir root/shoot biomass (root divided by sum of stems plus needles) at the end of the second drought (D2). CI represent 95% confidence intervals. Biomass was measured in grams (g). Bold signifies statistical significance at $p < 0.05$. Treatments are ND (no drought), ED (early drought), LD (late drought), and 2D (two drought). Estimates are in grams (g), with the treatment on the right side of the “versus” subtracted from the treatment on the left side of the “versus” in the comparison column.

Provenance	Comparison	t-value	p-value	Estimate (g)	Upper CI	Lower CI	df	n
All	2D vs LD	-1.42	0.160	-0.095	0.039	-0.228	58	15-21
	2D vs ED	1.75	0.085	0.121	0.258	-0.017	58	15-21
	2D vs ND	-1.45	0.152	-0.095	0.036	-0.227	58	15-21
	ND vs ED	0.376	0.709	0.025	0.159	-0.109	58	15-21
	ND vs LD	-0.10	0.992	-0.001	0.129	-0.130	58	15-21
	ED vs LD	0.380	0.705	0.026	0.162	-0.110	58	15-21
Coos Bay	2D vs LD	-0.407	0.686	-0.044	0.173	-0.261	50	5-7
	2D vs ED	-1.953	0.0564	-0.220	0.006	-0.447	50	5-7
	2D vs ND	-0.417	0.678	-0.047	0.180	-0.274	50	5-7
	ND vs ED	-1.536	0.131	-0.173	0.053	-0.400	50	5-7
	ND vs LD	0.028	0.978	0.003	0.220	-0.214	50	5-7
	ED vs LD	1.632	0.109	0.176	0.393	-0.041	50	5-7
Cascades	2D vs LD	-1.836	0.0723	-0.207	0.019	-0.434	50	5-7
	2D vs ED	-1.654	0.104	-0.187	0.040	-0.413	50	5-7
	2D vs ND	-1.488	0.143	-0.168	0.059	-0.394	50	5-7
	ND vs ED	-0.165	0.869	-0.019	0.208	-0.245	50	5-7
	ND vs LD	-0.348	0.729	-0.039	0.187	-0.266	50	5-7
	ED vs LD	-0.182	0.856	-0.021	0.206	-0.247	50	5-7
New Mexico	2D vs LD	0.522	0.604	0.062	0.303	-0.178	50	5-7
	2D vs ED	-0.525	0.602	-0.059	0.167	-0.286	50	5-7
	2D vs ND	-0.484	0.630	-0.051	0.159	-0.260	50	5-7
	ND vs ED	1.011	0.317	0.113	0.338	-0.112	50	5-7
	ND vs LD	-0.082	0.935	-0.009	0.201	-0.218	50	5-7
	ED vs LD	-1.016	0.314	-0.122	0.119	-0.362	50	5-7

Table A15. Statistical analysis (ANOVA F-tests) of loblolly pine initial and final heights. Bold signifies statistical significance at $p < 0.05$.

Date	Test	F-value	p-value (p)	df
Initial heights (16 July)	Treatment	0.100	0.960	3, 72
	Provenance	94.88	2×10^{-16}	2, 72
	Treatment x Provenance	0.432	0.855	6, 72
Final heights (10 Oct)	Treatment	8.96	4.06×10^{-5}	3, 72
	Provenance	51.0	1.63×10^{-14}	2, 72
	Treatment x Provenance	0.24	0.963	6, 72

Table A16. Statistical analysis (ANOVA F-tests) of Douglas-fir initial and final heights. Bold signifies statistical significance at $p < 0.05$.

Date	Test	F-value	p-value (p)	df
Initial heights (15 July)	Treatment	1.35	0.258	3, 731
	Provenance	38.16	2.2×10^{-16}	2, 731
	Treatment x Provenance	2.94	0.00754	6, 731
Final heights (9 Oct)	Treatment	1.48	0.224	3, 126
	Provenance	5.97	0.00333	2, 126
	Treatment x Provenance	1.69	0.129	6, 126

Table A17. Pairwise comparisons of loblolly pine initial heights. Bold signifies statistical significance. CI represent 95% confidence intervals. Heights are in cm. Bold signifies statistical significance at $p < 0.05$. Treatments are ND (no drought), ED (early drought), LD (late drought), and 2D (two drought). Estimates are in cm, with the treatment on the right side of the “versus” subtracted from the treatment on the left side of the “versus” in the comparison column.

Date	Provenance	Comparison	t-value	p-value (p)	Est-imate (cm)	Upper CI	Lower CI	df	n
16 July	All	2D vs LD	0.014	0.989	0.008	1.23	-1.21	80	21
		2D vs ED	-0.227	0.821	-0.139	1.08	-1.35	80	21
		2D vs ND	0.210	0.834	0.129	1.35	-1.09	80	21
		ND vs ED	-0.017	0.987	-0.010	1.21	-1.23	80	21
		ND vs LD	0.196	0.845	0.120	1.34	-1.10	80	21
		ED vs LD	-0.213	0.832	-0.131	1.09	-1.35	80	21
	North Carolina	2D vs LD	-0.63	0.533	-0.37	0.80	-1.53	72	7
		2D vs ED	-0.79	0.430	-0.46	0.70	-1.63	72	7
		2D vs ND	-0.64	0.527	-0.37	0.79	-1.53	72	7
		ND vs ED	-0.16	0.875	-0.09	1.07	-1.25	72	7
		ND vs LD	0.01	0.993	0.01	1.17	-1.16	72	7
		ED vs LD	0.17	0.868	0.10	1.26	-1.07	72	7
	Alabama	2D vs LD	0.56	0.575	0.33	1.49	-0.83	72	7
		2D vs ED	0.38	0.704	0.22	1.39	-0.94	72	7
		2D vs ND	0.70	0.489	0.41	1.57	-0.76	72	7
		ND vs ED	-0.31	0.755	-0.18	0.98	-1.35	72	7
		ND vs LD	-0.13	0.895	-0.08	1.09	-1.24	72	7
		ED vs LD	0.18	0.857	0.11	1.27	-1.06	72	7
	Lost Pines	2D vs LD	0.11	0.915	0.06	1.23	-1.10	72	7
		2D vs ED	1.13	0.264	0.66	1.82	-0.51	72	7
		2D vs ND	0.60	0.549	0.35	1.51	-0.81	72	7
		ND vs ED	0.52	0.602	0.31	1.47	-0.86	72	7
		ND vs LD	-0.50	0.622	-0.29	0.87	-1.45	72	7
		ED vs LD	-1.02	0.311	-0.59	0.57	-1.76	72	7

Table A18. Pairwise comparisons of loblolly pine final heights. Bold signifies statistical significance at $p < 0.05$. CI represent 95% confidence intervals. Heights are in cm. Treatments are ND (no drought), ED (early drought), LD (late drought), and 2D (two drought). Estimates are in cm, with the treatment on the right side of the “versus” subtracted from the treatment on the left side of the “versus” in the comparison column.

Date	Provenance	Comparison	t-value	p-value (p)	Est-imate (cm)	Upper CI	Lower CI	df	n
10 Oct	All	2D vs LD	2.41	0.018	2.43	4.44	0.420	80	21
		2D vs ED	0.728	0.468	0.735	2.74	-1.27	80	21
		2D vs ND	-1.56	0.123	-1.57	0.437	-3.58	80	21
		ND vs ED	-2.29	0.025	-2.31	-0.30	-4.31	80	21
		ND vs LD	0.850	0.398	0.86	2.86	-1.15	80	21
		ED vs LD	3.14	0.0024	3.16	5.17	1.16	80	21
	North Carolina	2D vs LD	-1.90	0.0609	-2.25	0.11	-4.60	72	7
		2D vs ED	0.81	0.420	0.96	3.31	-1.40	72	7
		2D vs ND	-0.74	0.459	-0.88	1.47	-3.23	72	7
		ND vs ED	1.56	0.124	1.84	4.19	-0.52	72	7
		ND vs LD	-1.16	0.250	-1.37	0.98	-3.72	72	7
		ED vs LD	-2.72	0.00828	-3.20	-0.85	-5.56	72	7
	Alabama	2D vs LD	-2.29	0.0250	-2.70	-0.35	-5.06	72	7
		2D vs ED	0.19	0.853	0.22	2.57	-2.13	72	7
		2D vs ND	-1.34	0.186	-1.58	0.78	-3.93	72	7
		ND vs ED	1.52	0.133	1.80	4.15	-0.56	72	7
		ND vs LD	-0.95	0.343	-1.13	1.23	-3.48	72	7
		ED vs LD	-2.48	0.0157	-2.92	-0.57	-5.27	72	7
	Lost Pines	2D vs LD	-1.98	0.0519	-2.33	0.02	-4.69	72	7
		2D vs ED	0.87	0.386	1.03	3.38	-1.32	72	7
		2D vs ND	-1.91	0.0598	-2.26	0.10	-4.61	72	7
		ND vs ED	2.78	0.00686	3.29	5.64	0.93	72	7
		ND vs LD	-0.06	0.949	-0.08	2.28	-2.43	72	7
		ED vs LD	-2.85	0.00572	-3.36	-1.01	-5.71	72	7

Table A19. Pairwise comparisons of Douglas-fir initial heights. Bold signifies statistical significance. CI represent 95% confidence intervals. Heights are in cm. Bold signifies statistical significance at $p < 0.05$. Treatments are ND (no drought), ED (early drought), LD (late drought), and 2D (two drought). Estimates are in cm, with the treatment on the right side of the “versus” subtracted from the treatment on the left side of the “versus” in the comparison column.

Date	Provenance	Comparison	t-value	p-value (p)	Estimate (cm)	Upper CI	Lower CI	df	n
July 15	All	2D vs LD	-1.50	0.135	-0.758	0.236	-1.75	739	186
		2D vs ED	1.14	0.256	0.573	1.56	-0.416	739	186
		2D vs ND	-1.76	0.0784	-0.884	0.101	-1.87	739	186
		ND vs ED	-0.621	0.535	-0.311	0.673	-1.30	739	186
		ND vs LD	-0.250	0.803	-0.126	0.864	-1.16	739	186
		ED vs LD	-0.366	0.715	-0.185	0.809	-1.18	739	186
	Coos Bay	2D vs LD	-0.03	0.979	-0.02	1.61	-1.65	731	62
		2D vs ED	0.40	0.692	0.33	1.96	-1.30	731	62
		2D vs ND	-1.16	0.245	-0.97	0.67	-2.61	731	62
		ND vs ED	1.56	0.118	1.30	2.94	-0.33	731	62
		ND vs LD	1.14	0.253	0.95	2.59	-0.68	731	62
		ED vs LD	-0.42	0.672	-0.35	1.27	-1.97	731	62
	Cascades	2D vs LD	-3.92	9.82x10⁻⁵	-3.26	-1.63	-4.89	731	62
		2D vs ED	-3.03	2.55x10⁻³	-2.48	-0.87	-4.09	731	62
		2D vs ND	-3.01	2.70x10⁻³	-2.47	-0.86	-4.09	731	62
		ND vs ED	-0.01	0.995	0.00	1.60	-1.61	731	62
		ND vs LD	-0.95	0.344	-0.79	0.84	-2.41	731	62
		ED vs LD	-0.95	0.345	-0.78	0.84	-2.40	731	62
	New Mexico	2D vs LD	0.64	0.525	0.53	2.15	-1.10	731	62
		2D vs ED	1.14	0.256	0.94	2.57	-0.69	731	62
		2D vs ND	0.92	0.358	0.74	2.33	-0.84	731	62
		ND vs ED	-0.27	0.791	-0.22	1.39	-1.83	731	62
		ND vs LD	0.24	0.810	0.20	1.81	-1.41	731	62
		ED vs LD	0.49	0.621	0.42	2.06	-1.23	731	62

Table A20. Pairwise comparisons of Douglas-fir final heights. Bold signifies statistical significance. CI represent 95% confidence intervals. Heights are in cm. Bold signifies statistical significance at $p < 0.05$. Treatments are ND (no drought), ED (early drought), LD (late drought), and 2D (two drought). Estimates are in cm, with the treatment on the right side of the “versus” subtracted from the treatment on the left side of the “versus” in the comparison column.

Date	Provenance	Comparison	t-value	p-value (p)	Estimate (cm)	Upper CI	Lower CI	df	n
Oct 9	All	2D vs LD	-2.00	0.052	-3.32	0.032	-6.68	134	24-48
		2D vs ED	1.21	0.231	2.13	5.61	-1.36	134	24-48
		2D vs ND	-1.48	0.141	-2.13	0.778	-5.41	134	24-48
		ND vs ED	-0.121	0.904	-0.190	2.90	-3.28	134	24-48
		ND vs LD	0.678	0.499	1.01	3.94	-1.93	134	24-48
		ED vs LD	-0.106	0.482	-1.20	2.16	-4.55	134	24-48
	Coos Bay	2D vs LD	-2.02	0.0452	-5.84	-0.13	-11.55	126	8-16
		2D vs ED	-0.83	0.408	-2.40	3.31	-8.11	126	8-16
		2D vs ND	-2.09	0.0389	-5.46	-0.28	-10.64	126	8-16
		ND vs ED	1.21	0.228	3.06	8.07	-1.95	126	8-16
		ND vs LD	-0.15	0.883	-0.38	4.63	-5.39	126	8-16
		ED vs LD	-1.23	0.223	-3.44	2.12	-9.00	126	8-16
	Cascades	2D vs LD	-2.50	0.0139	-6.80	-1.41	-12.18	126	8-16
		2D vs ED	-2.04	0.0436	-5.88	-0.17	-11.59	126	8-16
		2D vs ND	-1.27	0.206	-3.32	1.85	-8.50	126	8-16
		ND vs ED	-1.01	0.314	-2.56	2.45	-7.57	126	8-16
		ND vs LD	-1.48	0.141	-3.47	1.17	-8.11	126	8-16
		ED vs LD	-0.35	0.730	-0.91	4.31	-6.14	126	8-16
	New Mexico	2D vs LD	0.79	0.434	2.34	8.23	-3.55	126	8-16
		2D vs ED	1.02	0.310	2.86	8.42	-2.70	126	8-16
		2D vs ND	0.61	0.54	1.53	6.48	-3.42	126	8-16
		ND vs ED	0.30	0.763	0.81	6.14	-4.52	126	8-16
		ND vs LD	0.53	0.596	1.33	6.28	-3.62	126	8-16
		ED vs LD	0.17	0.862	0.52	6.41	-5.37	126	8-16

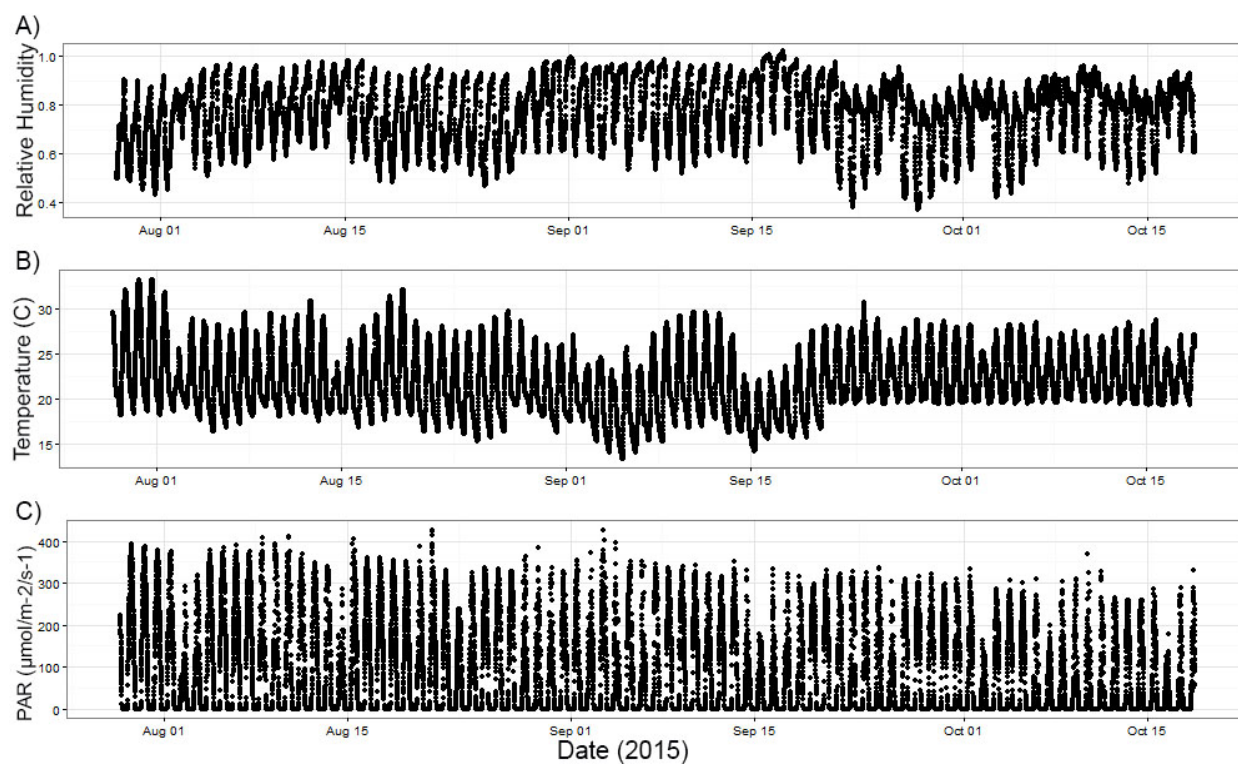


Fig A1. Relative humidity (A), temperature (B), and light levels PAR (C) of Oregon State greenhouse over the summer of 2015, as measured with a Decagon weather station VP-3 sensor, PAR sensor and EM-50 datalogger.

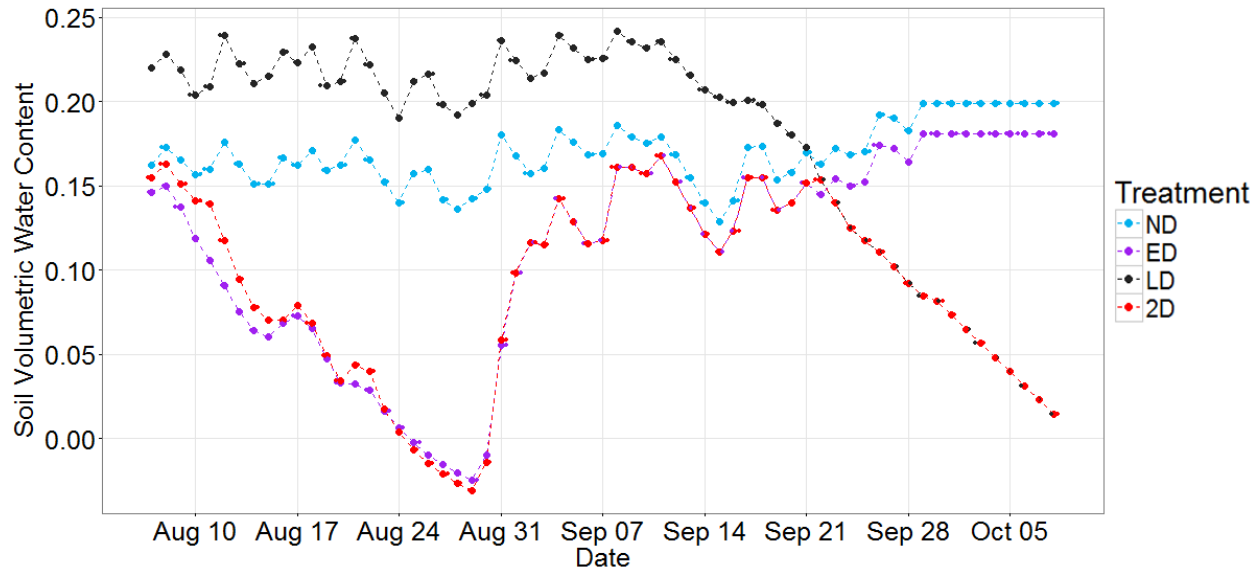


Fig A2. Volumetric water content (ratio of water volume to soil volume) measured only for Douglas-fir seedlings over the course of the experiment with Decagon EC-5 soil moisture sensors and averaged for each treatment (3 sensors per water stress treatment). Treatments are ND (no drought), ED (early drought), LD (late drought), and 2D (two drought). Due to malfunctions with some of the sensors, some values have been interpolated. Figure shows general trend of declining soil moisture.

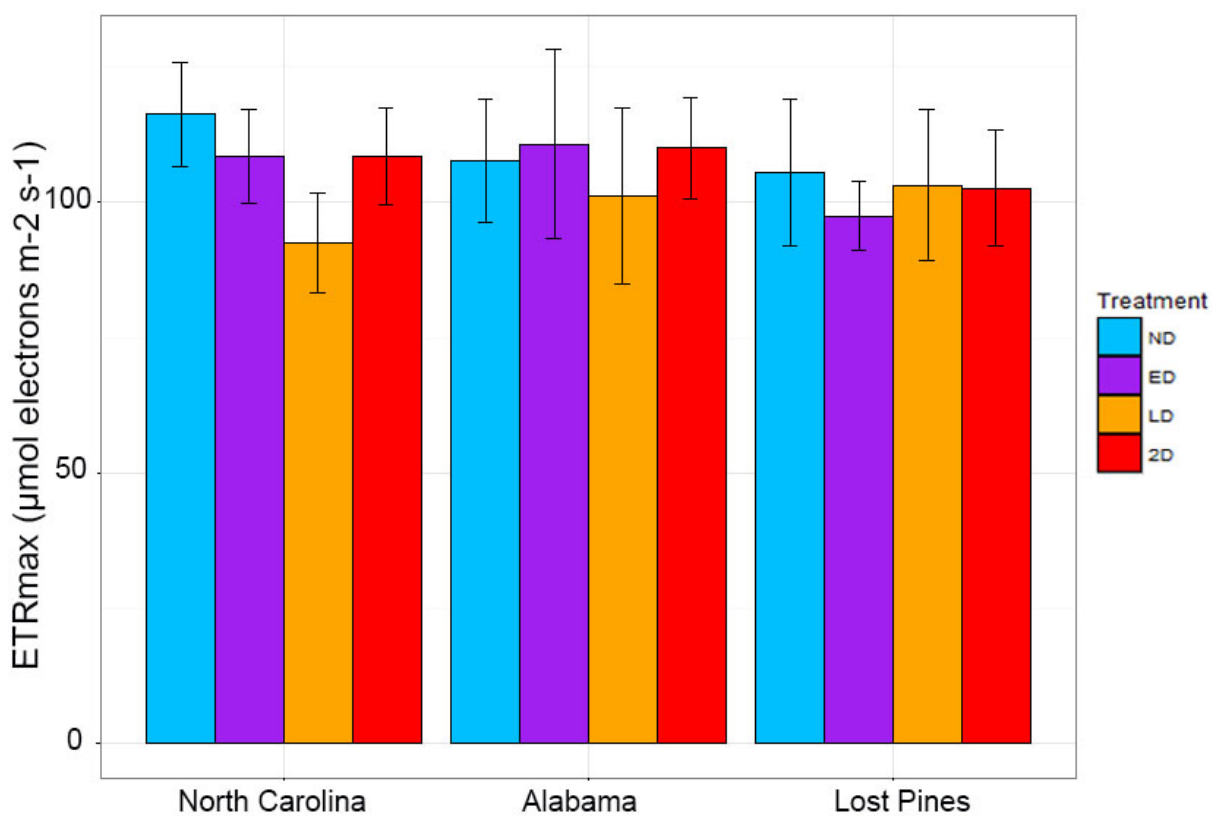


Fig A3. Maximum electron transport rate (ETR_{max}) for loblolly pine seedlings measured mid-drought during the second drought (22 Sept 2015, n=7). Treatments are ND (no drought), ED (early drought), LD (late drought), and 2D (two drought). Error bars show standard deviation.

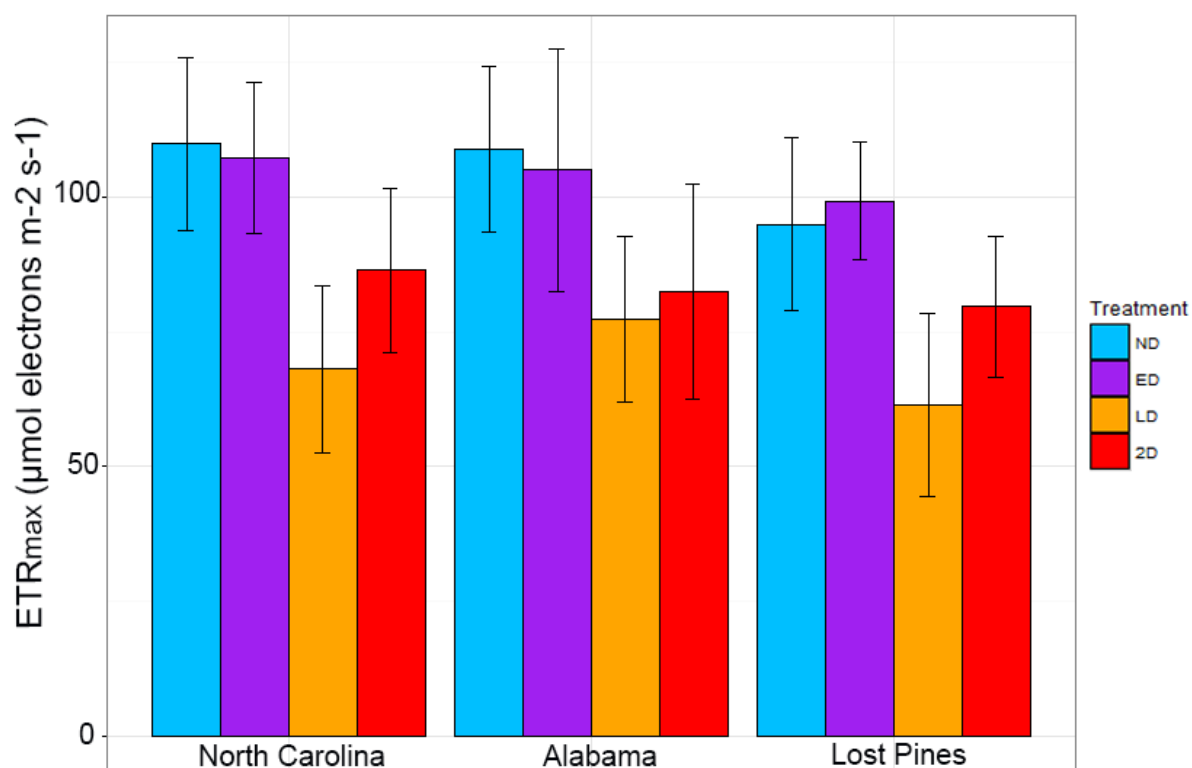


Fig A4. Maximum electron transport rates (ETR_{max}) for the four drought treatments by provenance for loblolly pine seedlings measured during the recovery period following the second drought (10 Oct, 2015, $n = 7$). Treatments are ND (no drought), ED (early drought), LD (late drought), and 2D (two drought). Error bars show standard deviation.

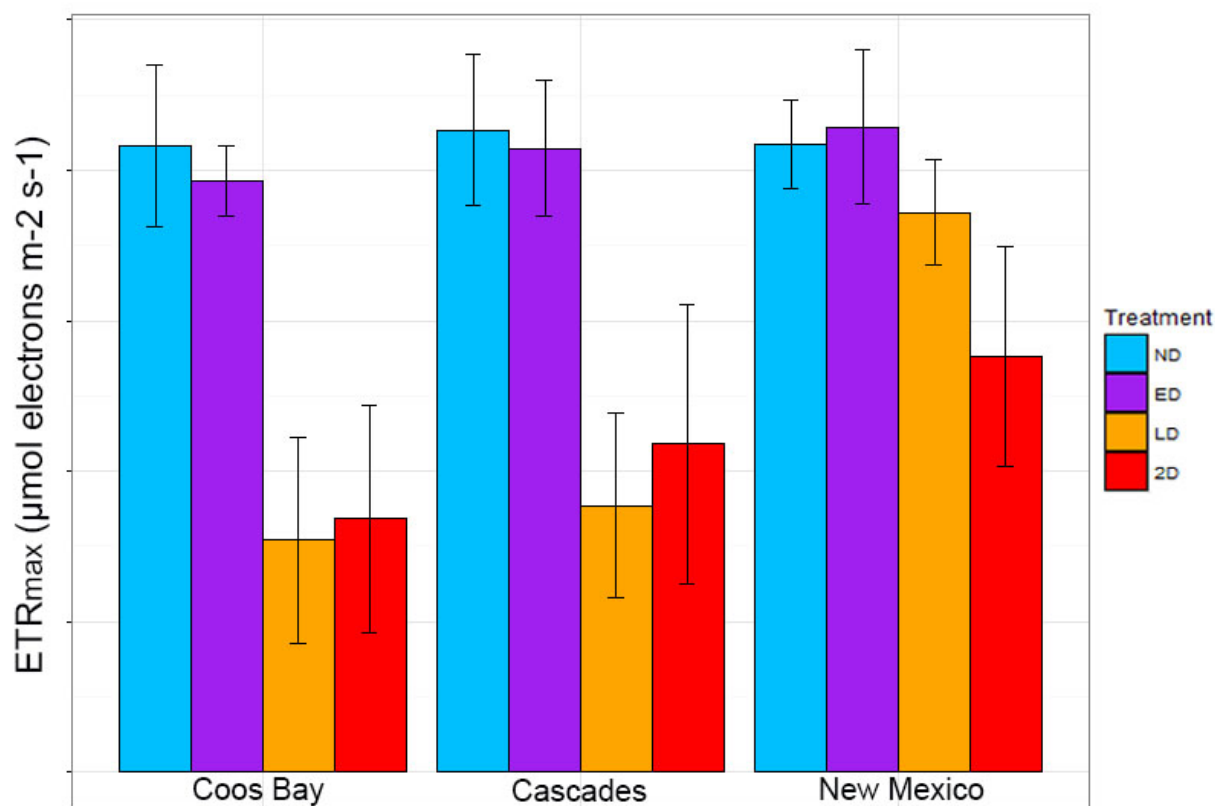


Fig A5. Maximum electron transport rates (ETR_{max}) for the four drought treatments by provenance for Douglas-fir measured near the end of the second drought (5 Oct, 2015, $n = 5$). Treatments are ND (no drought), ED (early drought), LD (late drought), and 2D (two drought). Error bars show standard deviation.

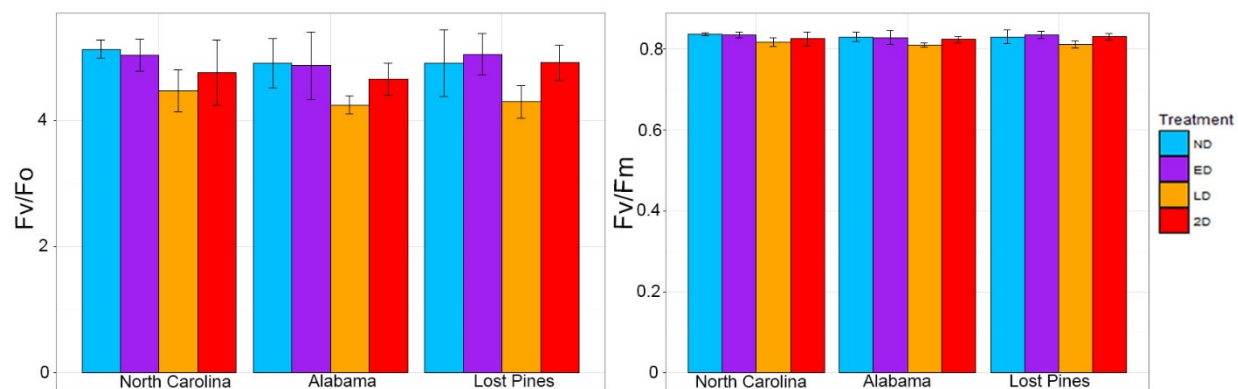


Fig A6. Mean dark-adapted fluorescence calculated as F_v/F_o (left) and F_v/F_m (right) for Loblolly pine seedlings ($n = 5$) during the second drought (27 Sept 2015). Treatments are ND (no drought), ED (early drought), LD (late drought), and 2D (two drought). Error bars show standard deviation.

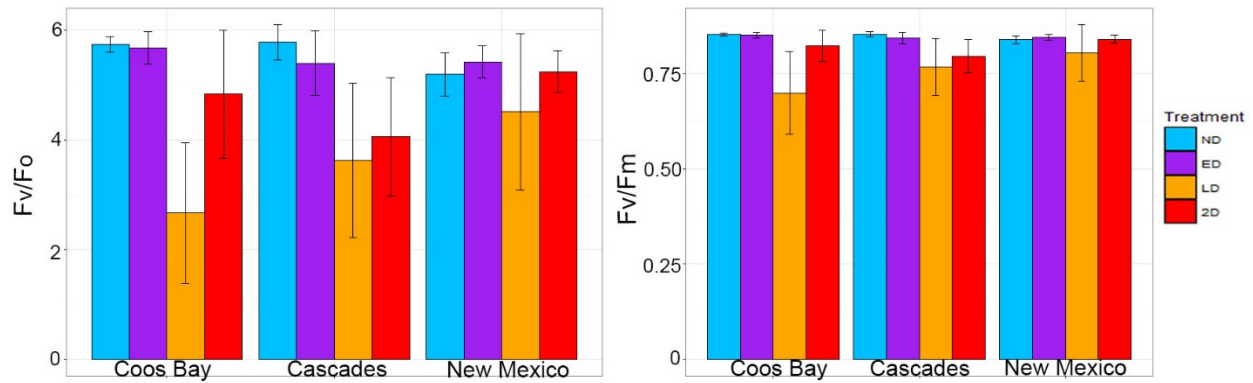


Fig A7. Mean dark-adapted fluorescence calculated as F_v/F_o (left) and F_v/F_m (right) for Douglas-fir ($n = 5$) seedlings measured during the second drought (8 Oct 2015). Treatments are ND (no drought), ED (early drought), LD (late drought), and 2D (two drought). Error bars show standard deviation.

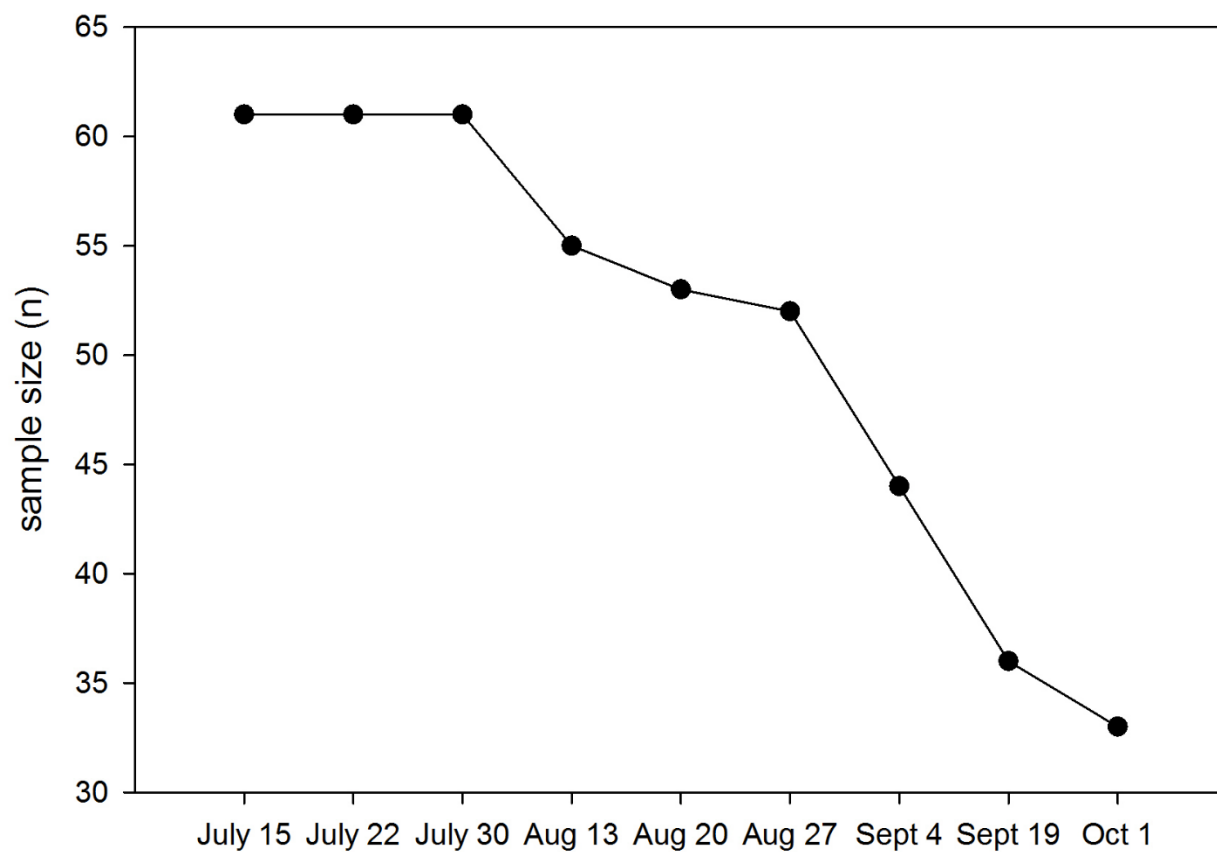


Fig A8. Douglas-fir heights sample size over the course of the experiment (summer 2015). Shows decrease in average number of samples for the combination of treatment/provenance as seedlings were harvested over time. Data for loblolly pine are not graphed, but decreased from 10 seedlings down to 4 seedlings per row over the course of the experiment and the number of rows remained 7 per combination of treatment/provenance.

Appendix B

Data, figures, and tables in Appendix B are not included or referred to in the manuscript, but were taken as part of the experiment. Methods for these measurements are provided here.

Infrared images: Infrared images were taken during drought treatments using an FLIR compact A-series infrared camera (Wilsonville, OR, USA). Seedlings were selected haphazardly from each species/treatment combination, and then moved to a stage backed by a large cardboard sheet to decrease background thermal variation, and then allowed to equilibrate until there were no signs of remaining thermal effects from handling. Images were taken during mid-afternoon (about 1-3 pm PST). Images were not analyzed, and were saved as Research IR files to be used with FLIR imagery software for analysis of regions of interest (ROI) and as JPEGs.

Infrared temperatures: Seedling temperatures were monitored during both drought treatments and during recovery periods using a Fluke 568 infrared thermometer (Everett, WA, USA) in both species and all treatments. Temperatures were taken during the afternoon (about 1-3pm PST) holding the infrared thermometer parallel to the ground, approximately 10 cm from the targeted seedling, with the laser pointer directed at the targeted seedling, approximately in the upper two-thirds portion of the seedling. Temperatures were taken in all treatments in all provenances on the same days. Two to three measurements were taken per seedling and averaged for a target of five seedlings from each treatment and provenance.

A/C_i curves: A/C_i curves (CO₂ assimilation rate 'A' vs calculated internal CO₂ concentration 'C_i') were measured using a LI-COR 6400 portable infrared gas-exchange system

(LI-COR, Lincoln, NE, USA) at the end of recovery following the second drought (mid-October 2015) in the late drought treatment and controls for loblolly pine, and in the controls for Douglas-fir. Sample sizes were 5 for each combination of treatment and provenance. Measurements were made on youngest, fully mature needles. Cuvette conditions were set at a flow rate of $400 \mu\text{mol m}^{-2} \text{s}^{-1}$, block temperature of 20°C , PAR $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$ for loblolly pine, and PAR $900 \mu\text{mol m}^{-2} \text{s}^{-1}$ for Douglas-fir (based on light saturation points from previously measured light curves). Relative humidity was maintained between 45% and 75%. A/C_i curves were measured by altering cuvette CO_2 levels in the following sequence: 400, 50, 100, 150, 250, 350, 500, 700, 900, 1200, 700, 400, 380 μmol . Conditions were allowed to stabilize at each CO_2 level before matching, and logging 3 times (once every 5 seconds). Logged values were averaged. Measured needles were collected, and needle area and length was calculated using ImageJ (version 1.51 e5). These were used to adjust A/C_i curves for needle area and length.

Blue rings: Stem samples for all treatments/provenances from both species were collected at the end of October. Stem sections of approximately 4 cm in length were taken approximately 2.5 cm above the stem/soil interface. Samples were allowed to air dry and stored in paper bags until used for microscopy. To prepare for microscopy, samples were re-hydrated by placing in filtered acidified water (pH 2.0) for 48 hours. As samples were being sectioned and slides prepared, samples were stored in filtered acidified water (pH 2.0) in a refrigerator for about one to two weeks during this process. Filtered acidified water was replaced with fresh filtered acidified water after about one week. Thin sections (about $40 \mu\text{m}$ thick) were made using a sliding microtome, and stained using a mixture of safranin and Astra-blue, which shows

locations with both lignin and cellulose as purple and locations with little lignin as blue. Stain was prepared by mixing 0.8 grams of Safranin powder in 100 ml of distilled water, 0.5 grams of Astra-blue powder in 100 ml of distilled water, and 2 ml of acetic acid. A drop of stain was placed onto the section while it sat at the edge of a glass plate. Stain was allowed to sit for about one minute, and then flushed gently with filtered water until the flushed water ran clear. Stained sections were mounted on glass slides using glycerin, within about 20 minutes of staining. Images of the slides were made using a VHX-1000E Keyence Digital Microscope (Keyence, Osaka, Japan) with a 2200 VH-Z1000W universal zoom lens. Last, images were analyzed for segments of ring that stained blue. If a segment was blue and it occupied an arc of 360° , the sample was said to have a blue ring. Number of samples with and without blue rings was recorded, summarized in this appendix. Example images showing blue rings are provided in this appendix. A record of which loblolly pines were sectioned and which ones showed blue rings is in an archive file referenced in Appendix C.

Table B1. Counts of stem cross sections in which blue rings were observed in loblolly pine. (See Fig B6 for examples of blue rings and images.) No blue rings were found in any Douglas-fir samples. Sample size is 5 for North Carolina and Alabama provenance and 6 for the Lost Pines provenance. Treatments are no drought (ND), early drought (ED), late drought (LD), and two drought (2D).

Provenance	Treatment	Blue ring Observed	No blue ring observed	Blue rings (% of observations)
North Carolina	ND	0	5	0%
	ED	0	5	0%
	LD	4	1	80%
	2D	2	3	40%
Alabama	ND	1	4	20%
	ED	0	5	0%
	LD	3	2	60%
	2D	0	5	0%
Lost Pines	ND	0	6	0%
	ED	0	6	0%
	LD	2	4	33%
	2D	0	5	0%

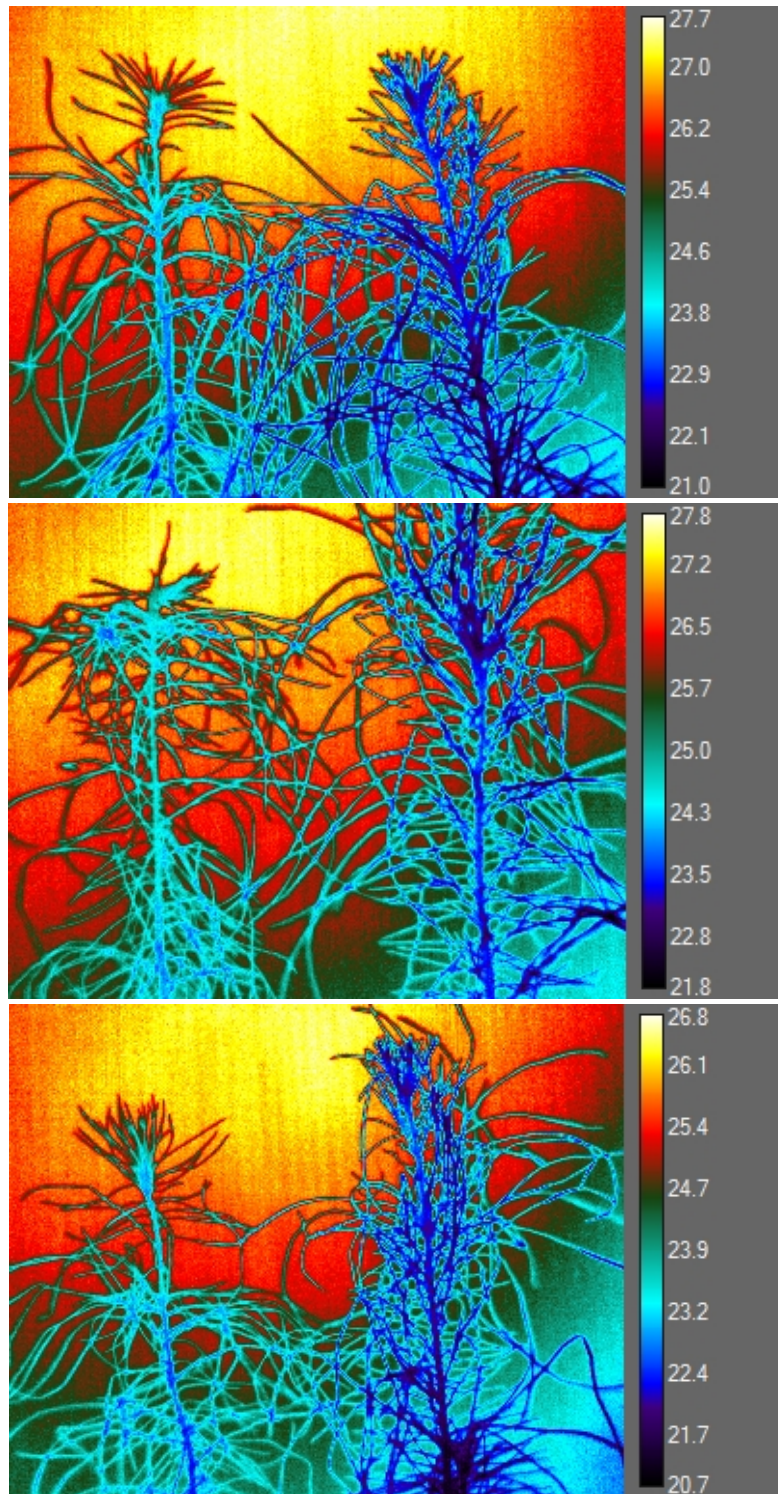


Fig B1. Thermal images of droughted (left) and no-drought loblolly pine seedlings (right) at the end of the first drought (around 3 Sept) from Lost Pines (top), Alabama (middle) and North Carolina (bottom) provenances. Temperatures on right (°C).

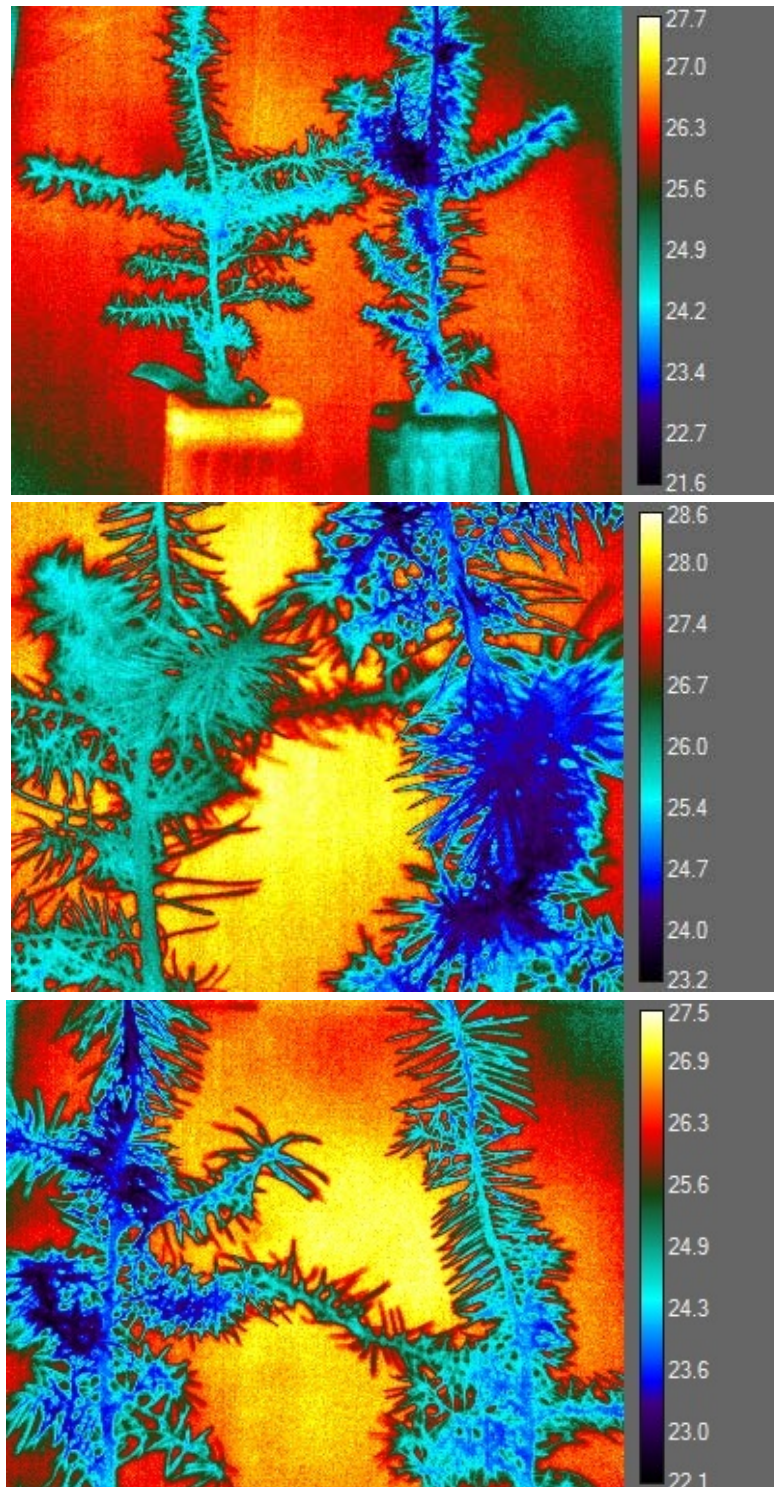


Fig B2. Thermal images of droughted (left) and no-drought Douglas-fir seedlings (right) at the end of the first drought (around 25 August) from Coos Bay (top), Coos Bay as a close up (middle) and Cascades (bottom) provenances. Temperatures on right (°C). In the Cascades image (bottom) the droughted seedling is on the right and watered seedling on left.

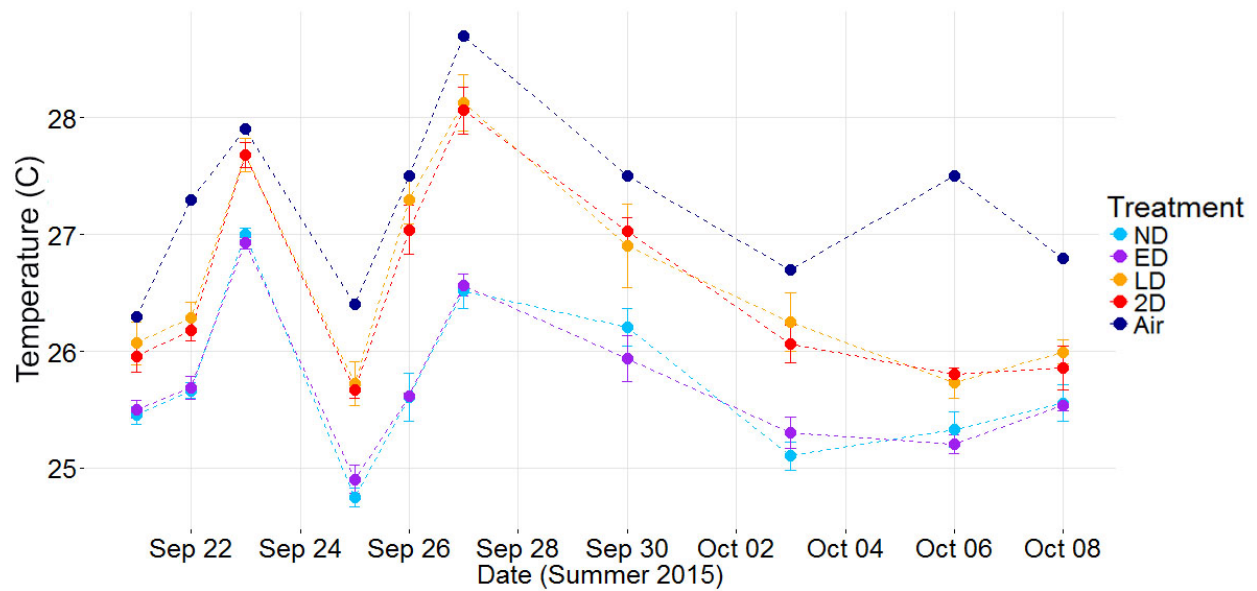


Fig B3. Canopy temperatures for loblolly pine taken with an infrared thermometer, all provenances combined. Dots represent means and error bars show standard error. Colors (as shown in the legend on the figure) are for drought treatments. Treatments are ND (no drought), ED (early drought), LD (late drought), and 2D (two drought). Air temperature shows the air temperature measured by a decagon weather station in the greenhouse. The first drought (D1) occurred for loblolly pine between Aug 10 and Sept 3 and occurred for the second drought (D2) between 13 Sept and 1 Oct 2015.

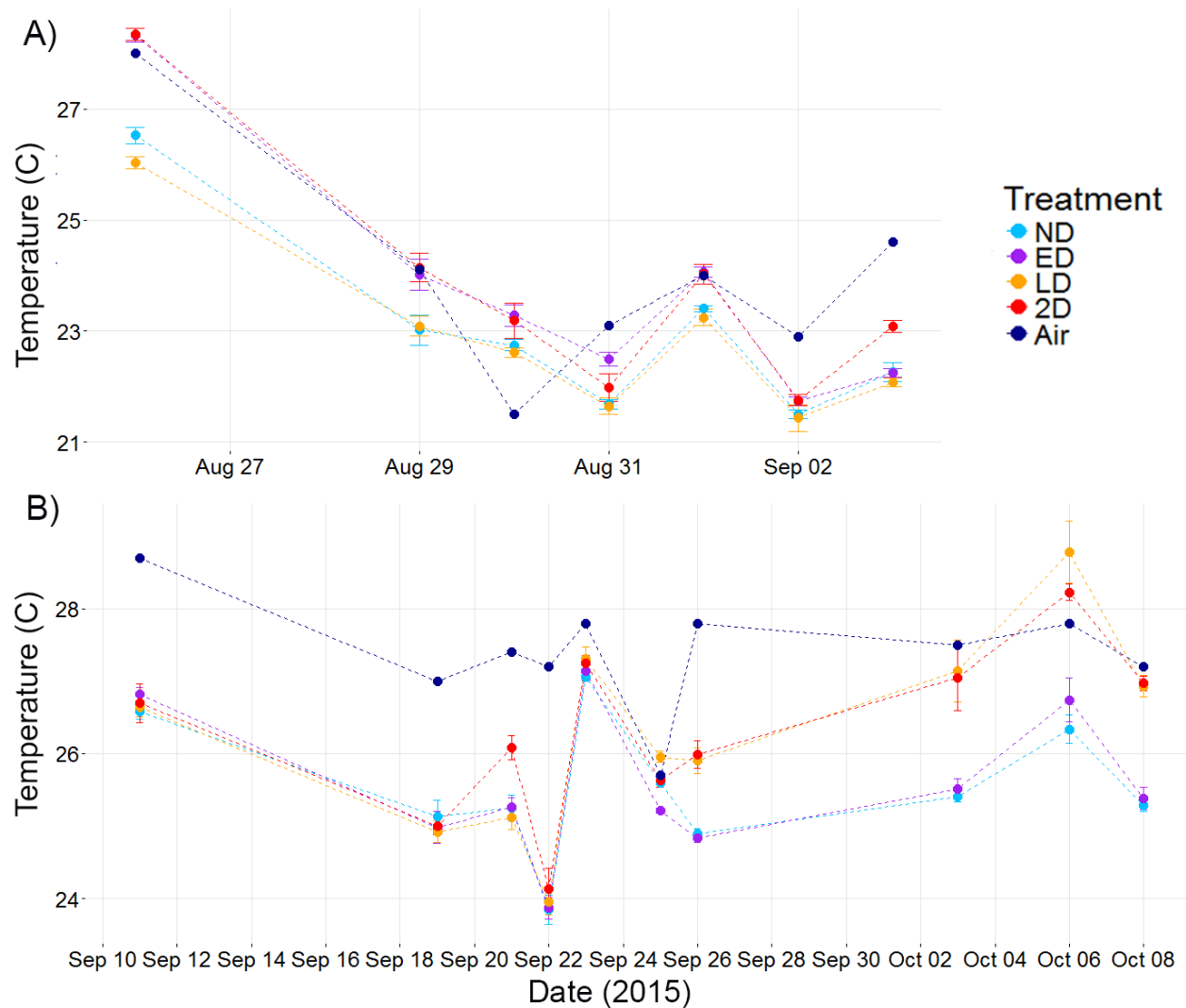


Fig B4. Canopy temperatures for Douglas-fir taken with an infrared thermometer, all provenances combined. Dots represent means and error bars show standard error. Colors (as show in the legend on the figure) are for drought treatments. Treatments are ND (no drought), ED (early drought), LD (late drought), and 2D (two drought). Air temperature shows the air temperature measured by a decagon weather station in the greenhouse. The first drought (D1) occurred for Douglas-fir between 5 Aug and 30 Aug and is shown in A. The second drought (D2) occurred between 10 Sept and 8 Oct 2015 and is shown in B.

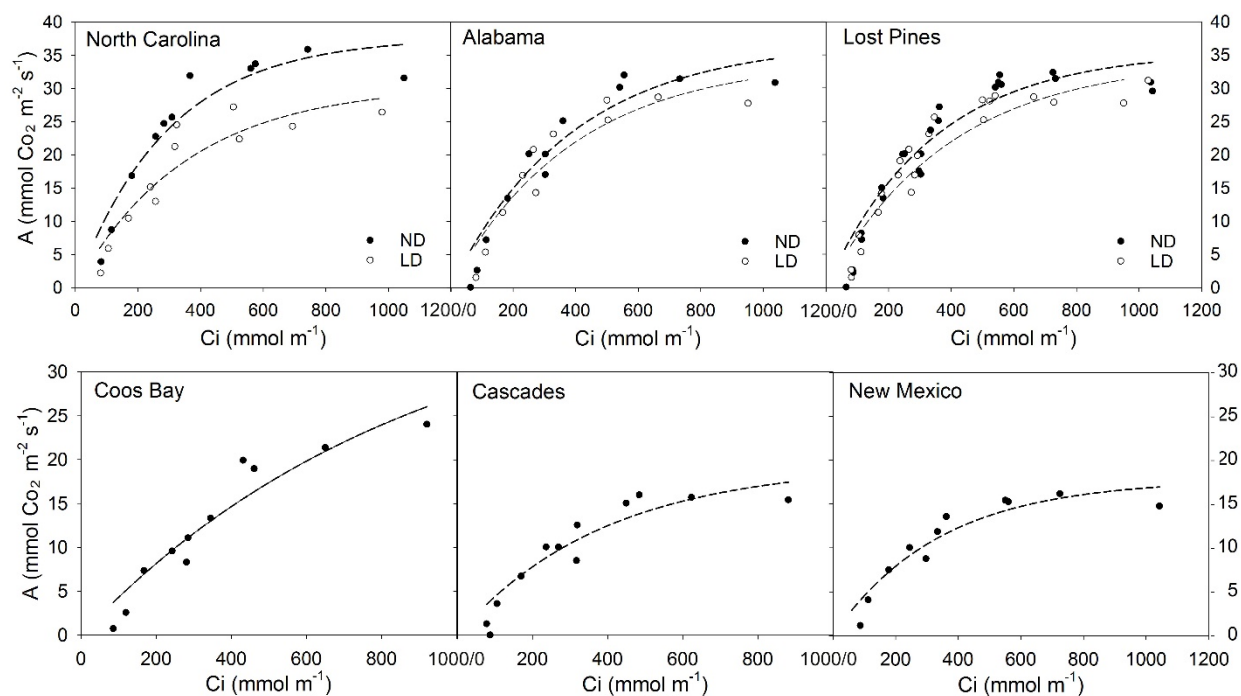
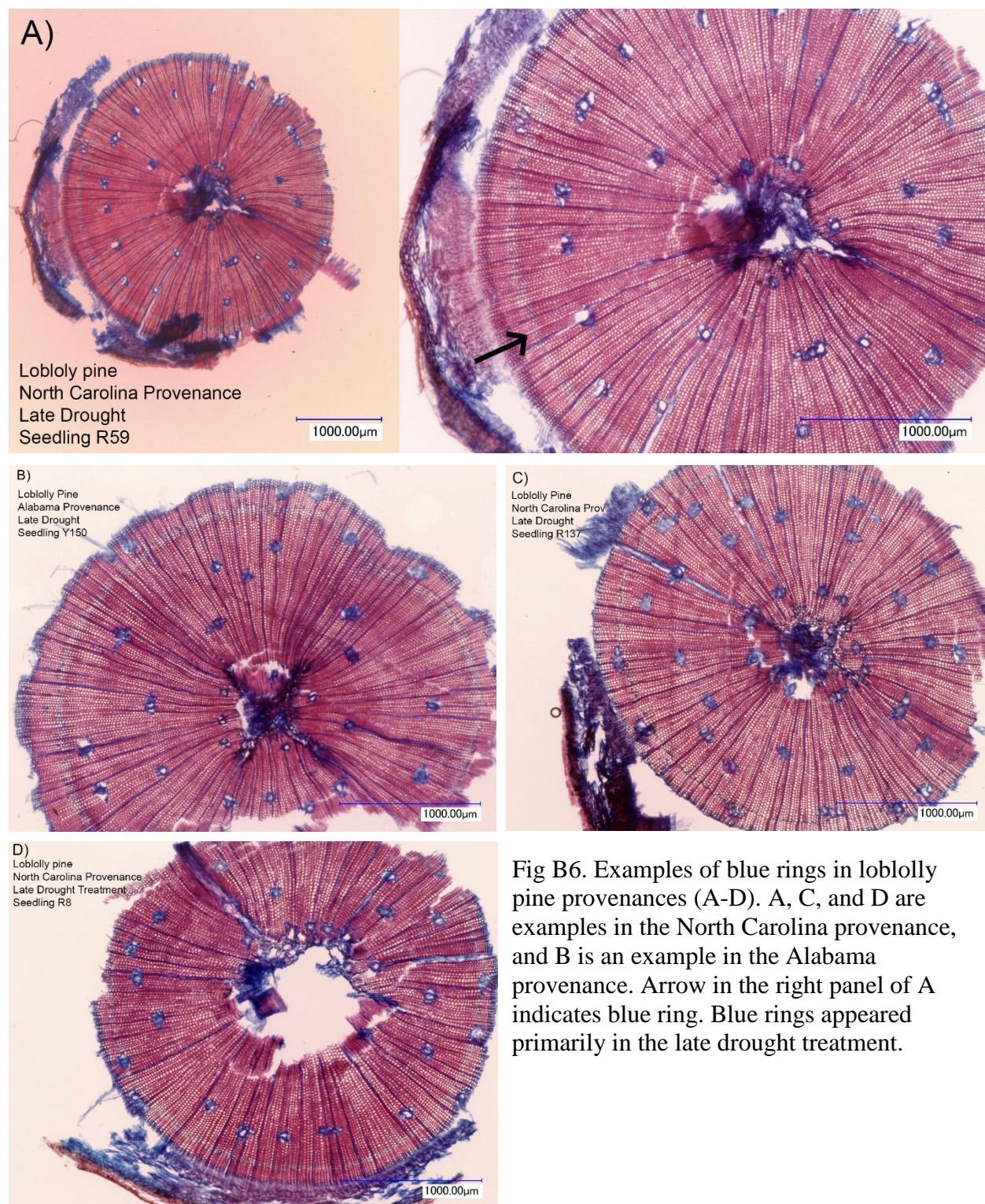


Fig B5. A/C_i curves (assimilation over internal CO_2 concentration) for loblolly pine provenances (top row) and for Douglas-fir provenances (bottom row) for no drought (ND) and late drought (LD) treatments (loblolly pine) or for ND (Douglas-fir). Measured at the end of the second drought.



Appendix C

This appendix provides a description of data files (Table 1) and R files (Table 2) from this thesis. Some of the experiments are not described in the thesis; in those cases, the Readme file explains the methods as well as the data. Files are archived in Box associated with Barbara Lachenbruch. For permissions to access, please contact Barbara Lachenbruch.

Table 1. Archived data files (.xlsx, csv, .jpeg, ResearchIR) files along with Read me files providing details and further description. All data are on GoogleDocs. Files are saved as both .xlsx and .csv unless otherwise stated. Excel (.xlsx) files and .csv files contain the same data, saved as different file types (.xlsx and .csv). In this table, Excel (.xlsx) files are given followed by associated .csv files. Because .csv files cannot contain separate worksheets, the separate worksheets in Excel files are saved as individual .csv files.

Data Filename	Documentation Filename (.doc)	Description
<i>A/Ci</i>		
ACI Curves_Doug fir.xlsx	ACI Curves_Doug fir_Readme	Raw output from the Li-Cor 6400 for A/Ci curves for Douglas-fir control treatments (ND) by provenance, as well as worksheets that show only A/Ci specific variables and incorporate corrections for leaf area.
ACI Curves_Doug fir_ACi data_doufir_allprov.csv	ACI Curves_Doug fir_ACi data_doufir_allprov_Readme	A/Ci specific data (Photo, Cond, Ci, CO ₂) for Douglas-fir controls (ND).
ACI Curves_Doug fir_RAW_df_oct16.csv	ACI Curves_Doug fir_RAW_df_oct16_Readme	Raw output from the Li-Cor 6400 measuring A/Ci curves on 16 Oct 2015 for Douglas-fir. Photo, Cond, and Ci are highlighted in color as pertinent data. (Also saved as a worksheet in ACI Curves_Doug fir.xlsx)
ACI Curves_Doug fir_RAW_df_oct17.csv	ACI Curves_Doug fir_RAW_df_oct17_Readme	Raw output from the Li-Cor 6400 measuring A/Ci curves on 17 Oct 2015 for Douglas-fir. Photo, Cond, and Ci are highlighted in color as pertinent data. (Also saved as a worksheet in ACI Curves_Doug fir.xlsx)
ACI Curves_loblolly.xlsx	ACI Curves_loblolly_Readme	Raw output from the Li-Cor 6400 for A/Ci curves for loblolly pine control treatments (ND) and late drought treatments (LD) by provenance, as well as worksheets that show only A/Ci specific variables and incorporate corrections for leaf area.
ACI Curves_loblolly_Aci specific data.csv	ACI Curves_loblolly_Aci specific data_Readme	A/Ci specific data (Photo, Cond, Ci, Tleaf, PARi, CO ₂) for loblolly pine control (ND) and late drought (LD) treatments. (Also saved as a worksheet in ACI Curves_loblolly.xlsx)

ACI Curves_loblolly_leafareas.csv	ACI Curves_loblolly_leafareas.csv	Leaf areas used to correct A/Ci curves for loblolly pine. (Also saved as a worksheet in ACI Curves_loblolly.xlsx)
ACI Curves_loblolly_ACicorrectedforleafareas.csv	ACI Curves_loblolly_ACicorrectedforleafareas_Readme	A/Ci specific data corrected for appropriate leaf area based on leaf areas shown in ACI Curves_loblolly_leafareas.csv (Also saved as a worksheet in ACI Curves_loblolly.xlsx)
ACI Curves_loblolly_AcforR.csv	ACI Curves_loblolly_AcforR.csv	A/Ci specific data (Photo, Cond, Ci, Tleaf, PARi, CO ₂) for loblolly pine control (ND) and late drought (LD) treatments formatted to be run in the program R. (Also saved as a worksheet in ACI Curves_loblolly.xlsx)
ACI Curves_loblolly_RAW_oct13 file.csv	ACI Curves_loblolly_RAW_oct13 file_Readme	Raw output from the Li-Cor 6400 measuring A/Ci curves on 13 Oct 2015 for loblolly pine. Photo, Cond, and Ci are highlighted in color as pertinent data. (Also saved as a worksheet in ACI Curves_loblolly.xlsx)
ACI Curves_loblolly_RAW_oct14 file.csv	ACI Curves_loblolly_RAW_oct14 file_Readme	Raw output from the Li-Cor 6400 measuring A/Ci curves on 14 Oct 2015 for loblolly pine. Photo, Cond, and Ci are highlighted in color as pertinent data. (Also saved as a worksheet in ACI Curves_loblolly.xlsx)
ACI Curves_loblolly_RAW_oct15 file.csv	ACI Curves_loblolly_RAW_oct15 file_Readme	Raw output from the Li-Cor 6400 measuring A/Ci curves on 15 Oct 2015 for loblolly pine. Photo, Cond, and Ci are highlighted in color as pertinent data. (Also saved as a worksheet in ACI Curves_loblolly.xlsx)
ACI Curves_loblolly_RAW_oct16 file.csv	ACI Curves_loblolly_RAW_oct16 file_Readme	Raw output from the Li-Cor 6400 measuring A/Ci curves on 16 Oct 2015 for loblolly pine. Photo, Cond, and Ci are highlighted in color as pertinent data. (Also saved as a worksheet in ACI Curves_loblolly.xlsx)
Biomass		
biomassforR.xlsx	biomassforR_Readme	Needle, stem, and root biomass (g) for loblolly pine and Douglas-fir measured at the end of the first drought (D1END), before the second drought (D1RECOV), and at the end of the second drought (D2). Above/Belowground, Root/Shoot, and overall total biomass are also calculated.
biomassforR_loblolly.csv	biomassforR_loblolly_Readme	Needle, stem, and root biomass (g) for loblolly pine measured at the end of the first drought (D1END), before the second drought (D1RECOV), and at the end of the second drought (D2). Above/Belowground, Root/Shoot, and overall total biomass are also calculated. Formatted to be run in R. (Also saved as a worksheet in biomassforR.xlsx)

biomassforR_dougfir.csv	biomassforR_dougfir_Readme	Needle, stem, and root biomass (g) for loblolly pine measured at the end of the first drought (D1END), before the second drought (D1RECOV), and at the end of the second drought (D2). Above/Belowground, Root/Shoot, and overall total biomass are also calculated. Formatted to be run in R. (Also saved as a worksheet in biomassforR.xlsx)
biomass_relativegrowth.xlsx	Biomass_relativegrowthrate_Readme	Relative growth rates in a worksheet for Douglas fir and a separate worksheet for loblolly pine. (Calculated from data in biomassforR.xlsx)
biomass_relativegrowth_loblolly.csv	biomass_relativegrowth_loblolly_Readme	Relative growth rates for loblolly pine. (Calculated from data in biomassforR_loblolly.csv) (Also saved as a worksheet in biomass_relativegrowth.xlsx)
biomass_relativegrowth_Douglasfir.csv	biomass_relativegrowth_douglasfir_Readme	Relative growth rates for Douglas-fir. (Calculated from data in biomassforR_douglasfir.csv) (Also saved as a worksheet in biomass_relativegrowth.xlsx)
<i>Blue Rings and Microscopy</i>		
Douglas fir microscopy images (folder of JPEGs)	Douglasfir_microscopy_images_Readme	Folder JPEGs of images taken for microscopy (stained stem samples) for Douglas-fir
Douglas fir tracheid diameters (folder of csvs)	Douglasfir_tracheiddiameters_Readme	Folder contains .csv files of tracheid diameters for Douglas-fir for individual seedling samples by lines. (Unlike with other .csv files, there is no corresponding Excel file for the .csv files in this folder).
Loblolly pine microscopy images (folder of JPEGs)	loblollypine_microscopy_images_Readme	Folder contains JPEGs of images taken for microscopy (stained stem samples) for loblolly pine
Loblolly pine tracheid diameters.xlsx	Loblolly pine tracheid diameters_Readme	Tracheid diameters for loblolly pine, with separate worksheets for the three provenances. Radii, Radii ⁴ , Avg Diameter, Avg Radius, and Water transport is calculated only for Lost Pines (Blue, abbreviated as B) provenance.
Loblolly pine tracheid diameters_B.csv	Loblolly pine tracheid diameters_B_Readme	Tracheid diameters for Lost Pines provenance loblolly pine, with separate worksheets for the three provenances. Radii, Radii ⁴ , Total tracheid number, Avg Diameter, Avg Radius, and Water transport is calculated. (Also saved as a worksheet in Loblolly pine tracheid diameters.xlsx)
Loblolly pine tracheid diameters_R.csv	Loblolly pine tracheid diameters_R_Readme	Tracheid diameters for North Carolina provenance loblolly pine. (Also saved as a worksheet in Loblolly pine tracheid diameters.xlsx)
Loblolly pine tracheid diameters_Y.csv	Loblolly pine tracheid diameters_Y_Readme	Tracheid diameters for Alabama provenance loblolly pine. (Also saved as a worksheet in Loblolly pine tracheid diameters.xlsx)

Bluerings_observations_loblolly.xlsx	Bluerings_observations_loblolly_Readme	Observations and count of blue rings in loblolly pine stained stem sections. Also includes example image of blue ring, and bar graph of count of blue ring observations. (No blue rings were observed in Douglas-fir, only in loblolly pine)
Bluerings_observations_loblolly.csv	Bluerings_observations_loblolly_CSV_Readme	Observations and count of blue rings in loblolly pine stained stem sections. Also includes example image of blue ring, and bar graph of count of blue ring observations. (No blue rings were observed in Douglas-fir, only in loblolly pine)
<i>Electron Transport Rate (ETR)</i>		
midD2DFETR.xlsx	midD2DFETR_Readme	Maximum ETR measured during the second drought (D2) prepared to be run in R for Douglas-fir. Measured Oct 2, 2015.
midD2DFETR.csv	midD2DFETR_CSV_Readme	Maximum ETR measured during the second drought (D2) prepared to be run in R for Douglas-fir. Measured Oct 2, 2015.
endofD2DFETR.xlsx	endofD2DFETR_Readme	Maximum ETR measured during the second drought (D2) prepared to be run in R for Douglas-fir. Measured Oct 5, 2015.
endofD2DFETR.csv	endofD2DFETR_CSV_Readme	Maximum ETR measured during the second drought (D2) prepared to be run in R for Douglas-fir. Measured Oct 5, 2015.
D2recoveryDFETR.xlsx	D2recovDFETR_Readme	Maximum ETR measured during the second drought (D2) prepared to be run in R for Douglas-fir. Measured Oct 15, 2015.
D2recoveryDFETR.csv	D2recovDFETR_CSV_Readme	Maximum ETR measured during the second drought (D2) prepared to be run in R for Douglas-fir. Measured Oct 15, 2015.
ETR DF mid drought 2 Oct 2.xlsx	ETR DF mid drought 2 Oct 2_Readme	Rapid light curves for Douglas-fir showing ETR only (i.e. ETR with increasing PAR) measured on Oct 2, as organized from raw output.
ETR DF mid drought 2 Oct 2.csv	ETR DF mid drought 2 Oct 2_CSV_Readme	Rapid light curves for Douglas-fir showing ETR only (i.e. ETR with increasing PAR) measured on Oct 2, as organized from raw output.
ETR DF End of drought 2 Oct 5.xlsx	ETR DF End of drought 2 Oct 5_Readme	Rapid light curves for Douglas-fir showing ETR only (i.e. ETR with increasing PAR) measured on Oct 5, as organized from raw output.
ETR DF End of drought 2 Oct 5.csv	ETR DF End of drought 2 Oct 5_CSV_Readme	Rapid light curves for Douglas-fir showing ETR only (i.e. ETR with increasing PAR) measured on Oct 5, as organized from raw output.
ETR DF drought 2 recovery Oct 15.xlsx	ETR DF drought 2 recovery Oct 15_Readme	Rapid light curves for Douglas-fir showing ETR only (i.e. ETR with increasing PAR) measured on Oct 15, as organized from raw output.

ETR DF drought 2 recovery Oct 15.csv	ETR DF drought 2 recovery Oct 15_CSV_Readme	Rapid light curves for Douglas-fir showing ETR only (i.e. ETR with increasing PAR) measured on Oct 15, as organized from raw output.
DFRLC102_raw.csv	DFRLC102_raw_Readme	Raw output file from the iFL integrated gas exchange fluorometer showing rapid light curves for measurements of ETR for Douglas-fir. Measured on Oct 2, 2015.
DFRLC105_raw.csv	DFRLC105_raw_Readme	Raw output file from the iFL integrated gas exchange fluorometer showing rapid light curves for measurements of ETR for Douglas-fir. Measured on Oct 2, 2015.
DFRLC1015_raw.csv	DFRLC1015_raw_Readme	Raw output file from the iFL integrated gas exchange fluorometer showing rapid light curves for measurements of ETR for Douglas-fir. Measured on Oct 2, 2015.
midd2lobetr.xlsx	midd2lobetr_Readme	Raw data for ETR and data for maximum ETR prepared to be run in R for loblolly pine. Measured Sept 22, 2015.
midd2lobetr.csv	midd2lobetr_CSV_Readme	Raw data for ETR and data for maximum ETR prepared to be run in R for loblolly pine. Measured Sept 22, 2015.
endD2_MaxETRLOB.xlsx	endD2_MaxETRLOB_Readme	Raw data for ETR and data for maximum ETR prepared to be run in R for loblolly pine. Measured Sept 28, 2015.
endD2_MaxETRLOB.csv	endD2_MaxETRLOB_CSV_Readme	Raw data for ETR and data for maximum ETR prepared to be run in R for loblolly pine. Measured Sept 28, 2015.
D2recovery_lobmaxETR.xlsx	D2recovery_lobmaxETR_Readme	Raw data for ETR and data for maximum ETR prepared to be run in R for loblolly pine. Measured Oct 10, 2015.
D2recovery_lobmaxETR.csv	D2recovery_lobmaxETR_CSV_Readme	Raw data for ETR and data for maximum ETR prepared to be run in R for loblolly pine. Measured Oct 10, 2015.
ETR LOB mid drought 2 Sept 22.xlsx	ETR LOB mid drought 2 Sept 22_Readme	Rapid light curves for loblolly pine showing ETR only (i.e. ETR with increasing PAR) measured on Sept 22, as organized from raw output.
ETR LOB mid drought 2 Sept 22.csv	ETR LOB mid drought 2 Sept 22_CSV_Readme	Rapid light curves for loblolly pine showing ETR only (i.e. ETR with increasing PAR) measured on Sept 22, as organized from raw output.
ETR LOB end of drought 2 Sept 28.xlsx	ETR LOB end of drought 2 Sept 28_Readme	Rapid light curves for loblolly pine showing ETR only (i.e. ETR with increasing PAR) measured on Sept 28, as organized from raw output.
ETR LOB end of drought 2 Sept 28.csv	ETR LOB end of drought 2 Sept 28_CSV_Readme	Rapid light curves for loblolly pine showing ETR only (i.e. ETR with increasing PAR) measured on Sept 28, as organized from raw output.

ETR LOB drought 2 recovery Oct 10.xlsx	ETR LOB drought 2 recovery Oct 10_Readme	Rapid light curves for loblolly pine showing ETR only (i.e. ETR with increasing PAR) measured on Oct 10, as organized from raw output.
ETR LOB drought 2 recovery Oct 10.csv	ETR LOB drought 2 recovery Oct 10_CSV_Readme	Rapid light curves for loblolly pine showing ETR only (i.e. ETR with increasing PAR) measured on Oct 10, as organized from raw output.
LRLC9_22_raw.csv	LRLC9_22_raw_Readme	Raw output file from the iFL integrated gas exchange fluorometer showing rapid light curves for measurements of ETR for loblolly pine. Measured on Sept 22, 2015.
LRCL9_28_raw.csv	LRCL9_28_raw_Readme	Raw output file from the iFL integrated gas exchange fluorometer showing rapid light curves for measurements of ETR for loblolly pine. Measured on Sept 28, 2015.
LRLC1010_raw.csv	LRLC1010_raw_Readme	Raw output file from the iFL integrated gas exchange fluorometer showing rapid light curves for measurements of ETR for loblolly pine. Measured on Oct 10, 2015.
Fluorescence		
oct3FVFM_DF.xlsx	oct3FVFM_DF_Readme	Dark-adapted fluorescence (calculated F_v/F_m and F_v/F_o) prepared to be run in R for Douglas-fir measured on Oct 3, 2015.
oct3FVFM_DF.csv	oct3FVFM_DF_CSV_Readme	Dark-adapted fluorescence (calculated F_v/F_m and F_v/F_o) prepared to be run in R for Douglas-fir measured on Oct 3, 2015.
oct8FVFM_DF.xlsx	oct8FVFM_DF_Readme	Dark-adapted fluorescence (calculated F_v/F_m and F_v/F_o) prepared to be run in R for Douglas-fir measured on Oct 8, 2015.
oct8FVFM_DF.csv	oct8FVFM_DF_CSV_Readme	Dark-adapted fluorescence (calculated F_v/F_m and F_v/F_o) prepared to be run in R for Douglas-fir measured on Oct 8, 2015.
DFOct8FVFM_raw.csv	DFOct8FVFM_raw_Readme	Raw output file of dark-adapted fluorescence for Douglas-fir measured on Oct 8, 2015.
Sept23FVFM_lob.xlsx	Sept23FVFM_lob_Readme	Dark-adapted fluorescence (calculated F_v/F_m and F_v/F_o) prepared to be run in R for loblolly pine measured on Sept 23 2015.
Sept23FVFM_lob.csv	Sept23FVFM_lob_CSV_Readme	Dark-adapted fluorescence (calculated F_v/F_m and F_v/F_o) prepared to be run in R for loblolly pine measured on Sept 23 2015.
sept27FVFM_lob.xlsx	sept27FVFM_lob_Readme	Dark-adapted fluorescence (calculated F_v/F_m and F_v/F_o) prepared to be run in R for loblolly pine measured on Sept 27 2015.
sept27FVFM_lob.csv	sept27FVFM_lob_CSV_Readme	Dark-adapted fluorescence (calculated F_v/F_m and F_v/F_o) prepared to be run in R for loblolly pine measured on Sept 27 2015.
9_27_LOB.csv	9_27_LOB_Readme	Raw output file of dark-adapted fluorescence for loblolly pine measured on Sept 27, 2015.

Heights		
Lob_and_df_heightsforR.xlsx	Lob_and_df_heightsforR_Readme	Corrected for errors and organized heights for loblolly pine and Douglas-fir, prepared to run in R. Contains multiple worksheets, which are also saved as individual .csv files beginning with Lob_and_df_heightsforR in the file name.
Lob_and_df_heightsforR_dfheights.csv	Lob_and_df_heightsforR_dfheights_CSV_Readme	Corrected for errors and organized heights for Douglas-fir and Douglas-fir, prepared to run in R. (Also saved as a worksheet in Lob_and_df_heightsforR.xlsx)
Lob_and_df_heightsforR_lobheights.csv	Lob_and_df_heightsforR_lobheights_CSV_Readme	Corrected for errors and organized heights for loblolly pine (row averages), prepared to run in R. (Also saved as a worksheet in Lob_and_df_heightsforR.xlsx)
Douglasfir_RAWandunorganized.xlsx	Douglasfir_RAWandunorganzied_Readme	Individual seedling heights (raw) as entered and uncorrected for errors for Douglas-fir for the summer of 2015.
Douglasfir_RAWandunorganized.csv	Douglasfir_RAWandunorganzied_CSV_Readme	Individual seedling heights (raw) as entered and uncorrected for errors for Douglas-fir for the summer of 2015.
Loblollypine_RAWandunorganized.xlsx	Loblollypine_RAWandunorganized_Readme	Individual seedling heights (raw) as entered and uncorrected for errors for loblolly pine for the summer of 2015. Contains two worksheets, which are also saved as individual .csv files beginning with Loblollypine_RAWandunorganized in the file name.
Loblollypine_RAWandunorganized_loblolly.csv	Loblollypine_RAWandunorganized_loblolly_Readme	Individual seedling heights (raw) as entered and uncorrected for errors for loblolly pine for the summer of 2015.
Loblollypine_RAWandunorganized_withthrowlabels.csv	Loblollypine_RAWandunorganized_withthrowlabels_Readme	Individual seedling heights (raw) as entered and uncorrected for errors for loblolly pine for the summer of 2015. Row is added in the column 'Rep'.
Infrared Imagery		
July 25 (folder)	Infrared Imagery July 25_Readme	Loblolly pine infrared images and some Douglas-fir infrared images taken on July 25, 2015. Inside the main folder (July 25), there are two subfolders: one of JPEGs (July 25 JPEGs), and one for the ResearchIR program files specific to FLIR infrared cameras (July 25 Research IR Files)

Aug 17 (folder)	Infrared Imagery Aug 17_Readme	Loblolly pine and Douglas-fir infrared images taken on Aug 17, 2015. Inside the main folder (Aug 17), there are two subfolders: one for Douglas fir and one for Loblolly. Within the separate folders for each species, there are subfolders for image type: one for JPEGs (Aug 17 JPEGs), and one for files for the ResearchIR program specific to FLIR infrared cameras (Aug 17 Research IR files)
Aug 25 (folder)	Infrared Imagery Aug 25_Readme	Loblolly pine and Douglas-fir infrared images taken on Aug 25, 2015. Inside the main folder (Aug 25), there are two subfolders: one for Douglas fir and one for Loblolly pine. Within the separate folders for each species, there are subfolders for image type: one for JPEGs (Aug 25 JPEGs), and one for files for the ResearchIR program specific to FLIR infrared cameras (Aug 25 Research IR)
Sept 3 (folder)	Infrared Imagery Sept 3_Readme	Loblolly pine infrared images taken on Sept 3, 2015. There are subfolders for image type: one for JPEGs, and one for files for the ResearchIR program specific to FLIR infrared cameras (Research IR)
Sept 27 (folder)	Infrared Imagery Sept 27_Readme	Loblolly pine infrared images taken on Sept 27, 2015. There are subfolders for image type: one for JPEGs, and one for files for the ResearchIR program specific to FLIR infrared cameras (Research IR)
<i>Infrared Temperatures</i>		
Doug fir avg temperature timeline.xlsx	Doug fir avg temperature timeline_Readme	Average infrared temperatures for Douglas-fir as measured throughout the summer of 2015. This file contains 6 worksheets, showing average temperatures for D1, D2, D1 and D2 together, D1 and D2 temperatures organized for R, D1 temperatures for R, and D2 temperatures for R.
Doug fir avg temperature timeline_D1.csv	Doug fir avg temperature timeline_D1_Readme	Average infrared temperatures for Douglas-fir as measured for the first drought (D1). .
Doug fir avg temperature timeline_D2.csv	Doug fir avg temperature timeline_D2_Readme	Average infrared temperatures for Douglas-fir as measured for the second drought (D2). .
Doug fir avg temperature timeline_D1 and D2.csv	Doug fir avg temperature timeline_D1 and D2_Readme	Average infrared temperatures for Douglas-fir as measured for the first and second droughts .
Doug fir avg temperature timeline_forR.csv	Doug fir avg temperature timeline_forR_Readme	Average infrared temperatures for Douglas-fir as measured for the first and second droughts formatted for R. .

Doug fir avg temperature timeline_D1forR.csv	Doug fir avg temperature timeline_D1forR_Readme	Average infrared temperatures for Douglas-fir as measured for the first drought (D1) formatted for R. .
Doug fir avg temperature timeline_D2forR.csv	Doug fir avg temperature timeline_D2forR_Readme	Average infrared temperatures for Douglas-fir as measured for the second drought (D2) formatted for R. .
Doug fir temps_RAW.xlsx	Doug fir temps_RAW_Readme	Raw data (un-averaged) of infrared temperatures for Douglas-fir as measured throughout the summer of 2015 (July 28 to Oct 8). There are 19 separate worksheet for 19 measurement dates.
Doug fir temps_RAW_July28th.csv	Doug fir temps_RAW_CSVs_Readme	Raw data (unaveraged) of infrared temperatures for Douglas-fir measured July 28.
Doug fir temps_RAW_July30.csv	Doug fir temps_RAW_CSVs_Readme	Raw data (unaveraged) of infrared temperatures for Douglas-fir measured July 30.
Doug fir temps_RAW_August26th.csv	Doug fir temps_RAW_CSVs_Readme	Raw data (unaveraged) of infrared temperatures for Douglas-fir measured August 26.
Doug fir temps_RAW_August29th.csv	Doug fir temps_RAW_CSVs_Readme	Raw data (unaveraged) of infrared temperatures for Douglas-fir measured August 29.
Doug fir temps_RAW_August30th.csv	Doug fir temps_RAW_CSVs_Readme	Raw data (unaveraged) of infrared temperatures for Douglas-fir measured August 30.
Doug fir temps_RAW_August31st.csv	Doug fir temps_RAW_CSVs_Readme	Raw data (unaveraged) of infrared temperatures for Douglas-fir measured August 31 st
Doug fir temps_RAW_Sept2nd.csv	Doug fir temps_RAW_CSVs_Readme	Raw data (unaveraged) of infrared temperatures for Douglas-fir measured Sept 2.
Doug fir temps_RAW_Sept3rd.csv	Doug fir temps_RAW_CSVs_Readme	Raw data (unaveraged) of infrared temperatures for Douglas-fir measured Sept 3.
Doug fir temps_RAW_Sept11th.csv	Doug fir temps_RAW_CSVs_Readme	Raw data (unaveraged) of infrared temperatures for Douglas-fir measured Sept 11.
Doug fir temps_RAW_Sept19th.csv	Doug fir temps_RAW_CSVs_Readme	Raw data (unaveraged) of infrared temperatures for Douglas-fir measured Sept 19.
Doug fir temps_RAW_Sept21st.csv	Doug fir temps_RAW_CSVs_Readme	Raw data (unaveraged) of infrared temperatures for Douglas-fir measured Sept 21.
Doug fir temps_RAW_Sept22nd.csv	Doug fir temps_RAW_CSVs_Readme	Raw data (unaveraged) of infrared temperatures for Douglas-fir measured Sept 22.
Doug fir temps_RAW_Sept23rd.csv	Doug fir temps_RAW_CSVs_Readme	Raw data (unaveraged) of infrared temperatures for Douglas-fir measured Sept 23.
Doug fir temps_RAW_Sept25th.csv	Doug fir temps_RAW_CSVs_Readme	Raw data (unaveraged) of infrared temperatures for Douglas-fir measured Sept 25.
Doug fir temps_RAW_Sept26th.csv	Doug fir temps_RAW_CSVs_Readme	Raw data (unaveraged) of infrared temperatures for Douglas-fir measured Sept 26.

Doug fir temps_RAW_Oct3rd.csv	Doug fir temps_RAW_CSVs_Readme	Raw data (unaveraged) of infrared temperatures for Douglas-fir measured Oct 3.
Doug fir temps_RAW_Oct8th.csv	Doug fir temps_RAW_CSVs_Readme	Raw data (unaveraged) of infrared temperatures for Douglas-fir measured Oct 8.
Loblolly avg temperature timeline.xlsx	Loblolly avg temperature timeline_Readme	Average infrared temperatures for loblolly pine as measured throughout the summer of 2015 (averaged by row). This file contains 4 worksheets, showing temperatures in °F, in °C, organized with °F and °C, and formatted for R.
Loblolly avg temperature timeline_TempsF.csv	Loblolly avg temperature timeline_TempsF_Readme	Average infrared temperatures for loblolly pine as measured throughout the summer of 2015 (averaged by row) in °F. (Also saved as a worksheet in the file Loblolly avg temperature timeline.xlsx)
Loblolly avg temperature timeline_TempsC.csv	Loblolly avg temperature timeline_TempsC_Readme	Average infrared temperatures for loblolly pine as measured throughout the summer of 2015 (averaged by row) in °C. (Also saved as a worksheet in the file Loblolly avg temperature timeline.xlsx)
Loblolly avg temperature timeline_OrganizedTempData.csv	Loblolly avg temperature timeline_OrganizedTempData_Readme	Average infrared temperatures for loblolly pine as measured throughout the summer of 2015 (averaged by row) in °F and C, well-organized. (Also saved as a worksheet in the file Loblolly avg temperature timeline.xlsx)
Loblolly avg temperature timeline_forR.csv	Loblolly avg temperature timeline_forR_Readme	Average infrared temperatures for loblolly pine as measured throughout the summer of 2015 (averaged by row) in °C formatted for R. (Also saved as a worksheet in the file Loblolly avg temperature timeline.xlsx)
Loblolly temps_RAW.xlsx	Loblolly temps_RAW_Readme	Raw data (un-averaged) of infrared temperatures for loblolly pine as measured throughout the summer of 2015.
Loblolly temps_RAW_July28.csv	Loblolly temps_RAW_CSVs_Readme	Raw data (unaveraged) of infrared temperatures for loblolly pine measured July 28.
Loblolly temps_RAW_July30.csv	Loblolly temps_RAW_CSVs_Readme	Raw data (unaveraged) of infrared temperatures for loblolly pine measured July 30.
Loblolly temps_RAW_August26.csv	Loblolly temps_RAW_CSVs_Readme	Raw data (unaveraged) of infrared temperatures for loblolly pine measured August 26.
Loblolly temps_RAW_Sept1st.csv	Loblolly temps_RAW_CSVs_Readme	Raw data (unaveraged) of infrared temperatures for loblolly pine measured Sept 1.
Loblolly temps_RAW_Sept5th.csv	Loblolly temps_RAW_CSVs_Readme	Raw data (unaveraged) of infrared temperatures for loblolly pine measured Sept 5.

Loblolly temps_RAW_Sept21st.csv	Loblolly temps_RAW_CSVs_Readme	Raw data (unaveraged) of infrared temperatures for loblolly pine measured Sept 21.
Loblolly temps_RAW_Sept22nd.csv	Loblolly temps_RAW_CSVs_Readme	Raw data (unaveraged) of infrared temperatures for loblolly pine measured Sept 22.
Loblolly temps_RAW_Sept23rd.csv	Loblolly temps_RAW_CSVs_Readme	Raw data (unaveraged) of infrared temperatures for loblolly pine measured Sept 23.
Loblolly temps_RAW_Sept25th.csv	Loblolly temps_RAW_CSVs_Readme	Raw data (unaveraged) of infrared temperatures for loblolly pine measured Sept 25.
Loblolly temps_RAW_Sept26th.csv	Loblolly temps_RAW_CSVs_Readme	Raw data (unaveraged) of infrared temperatures for loblolly pine measured Sept 26.
Loblolly temps_RAW_Sept27th.csv	Loblolly temps_RAW_CSVs_Readme	Raw data (unaveraged) of infrared temperatures for loblolly pine measured Sept 27.
Loblolly temps_RAW_Sept30th.csv	Loblolly temps_RAW_CSVs_Readme	Raw data (unaveraged) of infrared temperatures for loblolly pine measured Sept 30.
Loblolly temps_RAW_Oct3rd.csv	Loblolly temps_RAW_CSVs_Readme	Raw data (unaveraged) of infrared temperatures for loblolly pine measured Oct 3.
Loblolly temps_RAW_Oct6th.csv	Loblolly temps_RAW_CSVs_Readme	Raw data (unaveraged) of infrared temperatures for loblolly pine measured Oct 6.
Loblolly temps_RAW_Oct8th.csv	Loblolly temps_RAW_CSVs_Readme	Raw data (unaveraged) of infrared temperatures for loblolly pine measured Oct 8.
Maximum Specific Conductivity (Ks)		
ks dougfir.xlsx	ks dougfir_Readme	ks measurements for Douglas-fir control (ND) and early drought (ED) treatments. This file contains 2 worksheets (one for each treatment).
ks dougfir_ED.csv	ks dougfir_ED_Readme	ks measurements for Douglas-fir from the ED treatment. (Also saved as a worksheet in the file ks dougfir.xlsx)
ks dougfir_ND.csv	ks dougfir_ND_Readme	ks measurements for Douglas-fir from the ND treatment. (Also saved as a worksheet in the file ks dougfir.xlsx)
ksforR_DF.xlsx	ksforR_DF_Readme	ks measurements for Douglas-fir control (ND and ED) treatments formatted to be run in R.
ks loblolly.xlsx	ks loblolly_Readme	ks measurements for all treatments of loblolly pine.
ks loblolly_2D.csv	ks loblolly_2D_Readme	ks measurements for the 2D treatment of loblolly pine. (Also saved as a worksheet in the file ks loblolly.xlsx)
ks loblolly_ED.csv	ks loblolly_ED_Readme	ks measurements for the ED treatment of loblolly pine. (Also saved as a worksheet in the file ks loblolly.xlsx)
ks loblolly_ND.csv	ks loblolly_ND_Readme	ks measurements for the ND treatment of loblolly pine. (Also saved as a worksheet in the file ks loblolly.xlsx)

ks loblolly_LD.csv	ks loblolly_LD_Readme	ks measurements for the LD treatment of loblolly pine. (Also saved as a worksheet in the file ks loblolly.xlsx)
ks loblolly_Comparisonoftreat.csv	ks loblolly_Comparisonoftreat_Readme	Comparison of average ks measurements for all treatments of loblolly pine. (Also saved as a worksheet in the file ks loblolly.xlsx)
Needle Areas		
Douglasfir scanned needle images (folder)	Douglasfir scanned needle images_Readme	Folder containing .jpeg images of scanned Douglas-fir needles harvested to go along with A/Ci curves.
Douglas fir Needle Areas.xlsx	Douglas fir Needle Areas_Readme	Needle areas measured with ImageJ for Douglas-fir. (No lengths)
Douglas fir Needle Areas.csv	Douglas fir Needle Areas_CSV_Readme	Needle areas measured with ImageJ for Douglas-fir. (No lengths)
Loblolly scanned needle images (folder)	Loblolly scanned needle images_Readme	Folder containing .jpeg images of scanned loblolly pine needles harvested to go along with A/Ci curves.
Loblolly Needle Area and lengths.xlsx	Loblolly Needle Area and lengths_Readme	Needle areas and lengths measured with ImageJ for loblolly pine. Average needle area, average needle length, and needle area for 0.8cm of length for the number of needles in the Li-Cor measuring chamber at the time of measurement are also calculated.
Loblolly Needle Area and lengths_Shane_original.csv	Loblolly Needle Area and lengths_Shane_original_Readme	Needle areas measured for loblolly pine using ImageJ. These area measurements were made by Shane Wilson. (Also saved as a worksheet in the file Loblolly Needle Area and lengths.xlsx)
Loblolly Needle Area and lengths_needleareaandlength.csv	Loblolly Needle Area and lengths_needleareaandlength._Readme	Needle areas and lengths measured for loblolly pine using ImageJ. Also shows number of needles in the Li-Cor chamber during A/Ci measurement, average needle areas, average needle lengths, and average areas for 0.8cm of length for needle in the Li-Cor chamber during A/Ci measurement. (Also saved as a worksheet in the file Loblolly Needle Area and lengths.xlsx)
Loblolly Needle Area and lengths_summary.csv	Loblolly Needle Area and lengths_summary_Readme	Summary of needle areas and lengths measured with ImageJ for loblolly pine. Average needle area, average needle length, and needle area for 0.8cm of length for the number of needles in the Li-Cor measuring chamber at the time of measurement are also calculated. (Also saved as a worksheet in the file Loblolly Needle Area and lengths.xlsx)
(no file)	ImageJ_needlearea_method.docx	Methodology for measuring needle areas in ImageJ.
Soil Moisture		
EC5_Soilmoisturesensors_data.xlsx	EC5_Soilmoisturesensors_data_Readme	Soil moisture data from EC-5 moisture sensors for Douglas-fir treatments throughout the summer of 2015.

EC5_Soilmoisturesensors_data_Raw.csv	EC5_Soilmoisturesensors_data_Raw_Readme	Raw output from datalogger for soil moisture data from EC-5 moisture sensors for Douglas-fir treatments throughout the summer of 2015. (Also saved as a worksheet in the file EC5_Soilmoisturesensors_data.xlsx)
EC5_Soilmoisturesensors_data_correctedforsoiltype.csv	EC5_Soilmoisturesensors_data_correctedforsoiltype_Readme	Soil moisture data from EC-5 moisture sensors for Douglas-fir treatments throughout the summer of 2015 corrected for potting soil. (Also saved as a worksheet in the file EC5_Soilmoisturesensors_data.xlsx)
EC5_Soilmoisturesensors_data_extrapolated.csv	EC5_Soilmoisturesensors_data_extrapolated_Readme	Soil moisture data from EC-5 moisture sensors for Douglas-fir treatments throughout the summer of 2015, extrapolated through measurement gaps. (Also saved as a worksheet in the file EC5_Soilmoisturesensors_data.xlsx)
EC5_Soilmoisturesensors_data_forR.csv	EC5_Soilmoisturesensors_data_forR_Readme	Soil moisture data from EC-5 moisture sensors for Douglas-fir treatments throughout the summer of 2015, formatted to be run in R. (Also saved as a worksheet in the file EC5_Soilmoisturesensors_data.xlsx)
Water Potentials		
Douglasfir_waterpotential_endofD1_Aug28.xlsx	Douglasfir_waterpotential_endofD1_Aug28_Readme	Douglas-fir predawn and midday water potentials from the end of the first drought, measured on Aug 28.
Douglasfir_waterpotential_endofD1_Aug28_Doug fir end of first drought.csv	Douglasfir_waterpotential_endofD1_Aug28_Doug fir end of first drought_Readme	Douglas-fir predawn and midday water potentials from the end of the first drought raw file as entered directly from measurements. Measured on Aug 28. (Also saved as a worksheet in the file Douglasfir_waterpotential_endofD1_Aug28.xlsx)
Douglasfir_waterpotential_endofD1_Aug28_forR.csv	Douglasfir_waterpotential_endofD1_Aug28_forR_Readme	Douglas-fir predawn and midday water potentials from the end of the first drought formatted for R. Measured on Aug 28. (Also saved as a worksheet in the file Douglasfir_waterpotential_endofD1_Aug28.xlsx)
Douglasfir_waterpotential_endofD1_Aug28_forR_organized.csv	Douglasfir_waterpotential_endofD1_Aug28_forR_organized_Readme	Douglas-fir predawn and midday water potentials from the end of the first drought formatted for R, and well-organized. Measured on Aug 28. (Also saved as a worksheet in the file Douglasfir_waterpotential_endofD1_Aug28.xlsx)
Douglasfir_waterpotential_D1recov_Sept 10.xlsx	Douglasfir_waterpotential_D1recov_Sept 10_Readme	Douglas-fir midday water potentials from the end of the recovery from the first drought, measured on Sept 10.
Douglasfir_waterpotential_D1recov_Sept 10.csv	Douglasfir_waterpotential_D1recov_Sept 10_CSV_Readme	Douglas-fir midday water potentials from the end of the recovery from the first drought, measured on Sept 10.
Douglasfir_waterpotential_endofD2_Oct7.xlsx	Douglasfir_waterpotential_endofD2_Oct7_Readme	Douglas-fir predawn and midday water potentials from the end of the second drought, measured on Oct 7.

Douglasfir_waterpotential_endofD2_Oct7_summary.csv	Douglasfir_waterpotential_endofD2_Oct7_summary_Readme	Douglas-fir predawn and midday water potentials from the end of the second drought, measured on Oct 7, raw data as entered and summarized. (This file is also saved as a worksheet in Douglasfir_waterpotential_endofD2_Oct7.xlsx)
Douglasfir_waterpotential_endofD2_Oct7_predawn.csv	Douglasfir_waterpotential_endofD2_Oct7_predawn_Readme	Douglas-fir predawn water potentials only, from the end of the second drought, measured on Oct 7. Formatted to be run in R.
Douglasfir_waterpotential_endofD2_Oct7_midday.csv	Douglasfir_waterpotential_endofD2_Oct7_midday_Readme	Douglas-fir midday water potentials only, from the end of the second drought, measured on Oct 7. Formatted to be run in R.
Douglasfir_waterpotential_endofD2_Oct7_middayandpredawn.csv	Douglasfir_waterpotential_endofD2_Oct7_predawnandmidday_Readme	Douglas-fir predawn and midday water potentials from the end of the second drought, measured on Oct 7. Formatted to be run in R.
Loblolly_waterpotential_endofD1_Sept2.xlsx	Loblolly_waterpotential_endofD1_Sept2_Readme	Loblolly pine predawn and midday water potentials from the end of the first drought, measured on Sept 2.
Loblolly_waterpotential_endofD1_Sept2_Sept2nd.csv	Loblolly_waterpotential_endofD1_Sept2_Sept2nd_Readme	Loblolly pine predawn and midday water potentials from the end of the first drought, measured on Sept 2. Raw data as entered and summarized. (Also saved as a worksheet in the file Loblolly_waterpotential_endofD1_Sept2.xlsx)
Loblolly_waterpotential_endofD1_Sept2_predawnandmidday.csv	Loblolly_waterpotential_endofD1_Sept2_predawnandmidday_Readme	Loblolly pine predawn and midday water potentials from the end of the first drought, measured on Sept 2, formatted to be run in R. (Also saved as a worksheet in the file Loblolly_waterpotential_endofD1_Sept2.xlsx)
Loblolly_waterpotential_endofD1_Sept2_predawnonly.csv	Loblolly_waterpotential_endofD1_Sept2_predawnonly_Readme	Loblolly pine predawn water potentials only, from the end of the first drought measured on Sept 2. Formatted to be run in R. (Also saved as a worksheet in the file Loblolly_waterpotential_endofD1_Sept2.xlsx)
Loblolly_waterpotential_D1recov_Sept14.xlsx	Loblolly_waterpotential_D1recov_Sept14_Readme	Loblolly pine midday water potentials from the end of the recovery from the first drought, measured on Sept 14.
Loblolly_waterpotential_D1recov_Sept14.csv	Loblolly_waterpotential_D1recov_Sept14_CSV_Readme	Loblolly pine midday water potentials from the end of the recovery from the first drought, measured on Sept 14.
Loblolly_waterpotential_endofD2_Sept29.xlsx	Loblolly_waterpotential_endofD2_Sept29_Readme	Loblolly pine predawn and midday water potentials from the end of the second drought.
Loblolly_waterpotential_endofD2_Sept29_summary.csv	Loblolly_waterpotential_endofD2_Sept29_summary_Readme	Loblolly pine predawn and midday water potentials from the end of the first drought, measured on Sept 29. Raw data as entered and summarized. (Also saved as a worksheet in the file Loblolly_waterpotential_endofD2_Sept29.xlsx)
Loblolly_waterpotential_endofD2_Sept29_predawn.csv	Loblolly_waterpotential_endofD2_Sept29_predawn_Readme	Loblolly pine predawn water potentials only, from the end of the first drought measured on Sept 29. Formatted to be run in R.

Loblolly_waterpotential_endofD2_Sept29_midday.csv	Loblolly_waterpotential_endofD2_Sept29_midday_Readme	Loblolly pine midday water potentials only, from the end of the first drought measured on Sept 29. Formatted to be run in R.
Loblolly_waterpotential_endofD2_Sept29_predawnandmidday.csv	Loblolly_waterpotential_endofD2_Sept29_predawnandmidday_Readme	Loblolly pine predawn and midday water potentials only, from the end of the first drought measured on Sept 29. Formatted to be run in R.
<i>Weather Station</i>		
Weatherstationdata_forR.xlsx	Weatherstationdata_forR_Readme	Raw data output from the EM-50 data logger for environmental monitoring of RH, Temperature, and PAR prepared to be run in R.
Weatherstationdata_forR.csv	Weatherstationdata_forR_CSV_Readme	Raw data output from the EM-50 data logger for environmental monitoring of RH, Temperature, and PAR prepared to be run in R.
EM33229_Weather_Station_raw.xlsx	EM33229_Weather_Station_raw_Readme	Raw data output from the EM-50 data logger for environmental monitoring of RH, Temperature, and PAR.
EM33229_Weather_Station_raw.csv	EM33229_Weather_Station_raw_CSV_Readme	Raw data output from the EM-50 data logger for environmental monitoring of RH, Temperature, and PAR.
<i>Photos</i>		
Photos (folder)	Photos_Readme	Example photos of loblolly pine and Douglas-fir seedlings, and the greenhouse.

Table 2. Descriptions and files for R, showing analysis and data exploration. All R files are annotated within the R code/script.

Filename (.r)	Description
<i>Biomass</i>	
dfbiomass	R code and analysis for Douglas-fir biomass. Analyzed for needles, stems, roots, and root/shoot ratio and total overall biomass.
Loblollybiomass	R code and analysis for loblolly pine biomass. Analyzed for needles, stems, roots, and root/shoot ratio and total overall biomass.
<i>Electron transport rates (ETR)</i>	
midD2DFETR	R code and analysis for Douglas-fir maximum electron transport rates as measured on mid-drought during the second drought (measured on Oct 2).
endofD2DFETR	R code and analysis for Douglas-fir maximum electron transport rates as measured on at the end of the second drought (measured on Oct 5).
D2recoveryDFETR	R code and analysis for Douglas-fir maximum electron transport rates as measured during the recovery from the second drought (measured on Oct 15).
midD2LOBETR	R code and analysis for loblolly pine maximum electron transport rates as measured on mid-drought during the second drought (measured on Sept 22).
Lob_maxETR_D2recovery	R code and analysis for loblolly pine maximum electron transport rates as measured on at the end of the second drought (measured on Sept 28).
Lob_maxETR_D2recovery	R code and analysis for loblolly pine maximum electron transport rates as measured during the recovery from the second drought (measured on Oct 10).
<i>Fluorescence</i>	
oct3D2DF_FVFM	R code and analysis for Douglas-fir fluorescence (Fv/Fm) as measured on Oct 3, 2015.
oct8D2DF_FVFM	R code and analysis for Douglas-fir fluorescence (Fv/Fm) as measured on Oct 8, 2015.
sept23midD2lobFVFM	R code and analysis for loblolly pine fluorescence (Fv/Fm) as measured on Sept 23, 2015.
sept27midD2lobFVFM	R code and analysis for loblolly pine fluorescence (Fv/Fm) as measured on Sept 27, 2015.
<i>Heights</i>	
Douglasfir_heights	R code for analysis of Douglas-fir heights measured through the summer 2015, analyzed for initial and final heights.
Loblollypine_heights	R code for analysis of loblolly pine heights measured through the summer 2015, analyzed for initial and final heights.

<i>Water Potentials</i>	
Douglasfir_waterpotential_endofD1_Aug28	R code for analysis of water potentials at the end of the first drought for Douglas-fir, measured on Aug 28.
Douglasfir_waterpotential_endofD2_Oct7	R code for analysis of water potentials at the end of the first drought for Douglas-fir, measured on Oct 7.
Loblolly_waterpotential_endofD1_Sept2	R code for analysis of water potentials at the end of the first drought for loblolly pine, measured on Sept 2.
Loblolly_waterpotential_endofD2_Sept29	R code for analysis of water potentials at the end of the first drought for loblolly pine, measured on Sept 29.