

**A range-wide common garden experiment of white spruce
indicates population differentiation in drought tolerance traits**

by

Lauren Marie Sinclair

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science
in
Forest Biology and Management

Department of Renewable Resources
University of Alberta

© Lauren Marie Sinclair
Spring 2019
Edmonton, Alberta

The author reserves all publication and other rights in association with the copyright in the thesis and neither the thesis nor any substantial portion thereof may be printed or otherwise reproduced in any material form whatsoever without the author's prior written permission

Abstract

In western regions of the Canadian boreal forest, mean annual temperature has increased by 1.5 to 2.5 °C, significantly exceeding the mean global temperature increase. Climate conditions have also become drier and together these climate trends have induced widespread growth reductions, dieback and mortality of tree populations due to drought stress, with white spruce having been identified as particularly vulnerable. Here I evaluate genetic population differentiation in a 40-year old provenance test and simultaneously conduct seedling trials with the original seed that has been stored for 40 years to investigate drought traits in controlled greenhouse environments.

Patterns of height growth differed between seedlings and adult trees. Seedling growth potential was highest in eastern provenances. In contrast, local sources matched or exceeded eastern trees in adult field trials in north central Alberta. This variation in growth indicates that eastern trees may be adapted to high competition at the seedling stage but are maladapted to the cooler and drier climates of western Canada. Fluorescence measurements used to investigate impairment of photosynthesis during drought indicated the far eastern Maritime provenances and far north western Yukon provenances were the first to seize photosynthesis under drought. Carbon isotopes showed strong variation between provenances and indicated greater stomatal response in Ontario trees, indicating no trade-off between growth and stomatal response in these trees. Maritime and Yukon provenances adapted to less drought-prone conditions showed little stomatal response. Surprisingly, Alberta provenances, which were expected to be drought tolerant, also showed relatively low stomatal response to drought.

We further observed associations of growth and drought traits with the origin climate of the seed sources. Populations vulnerable to drought were found at opposite ends of the spectrum:

warm and wet provenances from the maritime and very cold and dry provenances from northern Alberta and the Yukon, indicating that neither of these populations is selected for by drought (due to cold in the northwest and due to ample precipitation in the eastern maritime). The study highlights genetic variation in white spruce populations. This variation between populations indicates particular vulnerability of northern populations where climate shifts are likely to be most significant. These results indicate assisted migration should be possible to replace vulnerable Yukon populations with more drought tolerant populations from Ontario and Southern Alberta.

Preface

This research was funded by NSERC STPGP grant number 494071 awarded to Drs Andreas Hamann and Uwe G. Hacke, and an NSERC Discovery grant awarded to Dr. Uwe G. Hacke. All data collection were performed by me, with the assistance of Dr. Uwe G. Hacke. All analysis were performed by be, with the exception of isotopes which were sent to the Natural Resources Analytics Laboratory at the University of Alberta, Edmonton, Alberta, Canada. This thesis is an original work and no part of this thesis has been previously published.

Acknowledgements

This thesis has been made possible by all the wonderful people who have helped me along the way. Science is certainly not a task to tackle alone and I am beyond grateful to the people who have made this research possible.

I would first like to thank my supervisors, Dr. Uwe Hacke and Dr. Andreas Hamann, for their incredible patience and willingness to help me through what may have been one of the most challenging projects in my life. Uwe's constant support and input has been priceless, particularly as he willingly came to the greenhouse at 4 am to help with measurements many times. His support was particularly wonderful while writing and he has helped me to become a better writer and learn to enjoy the process. Andreas's ability to keep situations light hearted and encouragement of creative problem solving has been a saving grace many times and is a skill I hope to pick up someday soon.

Particular thanks also go to my lab mates, Rachel Hillabrand, Jaime Sebastian Azcona, Ryan Stanfield, Dave Love and Killian Fleurial for being incredibly friendly, helpful, and encouraging when I had no idea what was going on. Also to Dante Castellanos Acuna and Zihaohan Sang who are R wizards and statistics demi-gods. I have received incredible moral support from both new and old lab friends: Vinicius Goncalves Manvailier, Ashley Hynes, Sara Venskaitis, Jorge Daniel Grock Pereira, and Elisabeth Beaubien.

I also need to say an extremely big thank you to my roommates Kate Bezoooyen, Hilary Cameron, and Kiera Smith, for putting up with all my complaining, raging, procrastinating, and for encouraging me to finish over and over again with hugs and good company. A similar thank you goes out to all the members of my family, who have been invested in my success from the beginning and were a wonderful outlet during some very stressful times.

Table of Contents

1. Introduction.....	1
1.1 Recent Climate Trends	1
1.2 Resulting Forest Dieback	2
1.3 White Spruce Autecology	6
1.4 Climate Change Adaptation Strategies	7
1.5 Adaptations of Tree Populations	8
1.6 Methods for Evaluating Drought Tolerance.....	10
1.7 Objectives.....	15
2. Methods.....	16
2.1 Seed sources	16
2.2 Growing conditions	16
2.3 Long Term Growth.....	17
2.4 Drought Experiment.....	17
2.5 Fluorescence.....	18
2.6 Isotopes.....	19
2.7 Bud Break.....	19
2.8 Statistics	19
3. Results.....	22
3.1 Adult and Seedling Growth.....	25
3.2 Seedling Drought Response	28
3.3 Influence of Climate of Origin on Growth and Drought Traits	34
4. Discussion.....	38
4.1 Genetic variation of height growth in seedlings and adult trees	38
4.2 Variability of drought tolerance traits between provenances.....	40
4.3 Trade-offs between growth potential and drought-tolerance	43
4.4 Potential management implications	45
4.5 Conclusions	46
5. References.....	47
6. Appendices.....	55

List of Tables

Table 1. Average source climate variables from the years 1961 to 1990 are shown for the 8 regions. 21

Table 2. Pearson correlation coefficients for mean values of provenances for adult and seedling height as growth variables, 20% Fv/Fm and increase in stable carbon isotope ratios ($\delta^{13}\text{C}$) as drought tolerance variables. 20% Fv/Fm was the volumetric water content (VWC) when plants reached 20% of their healthy Fv/Fm rate. Increase in $\delta^{13}\text{C}$ was the difference between drought and control $\delta^{13}\text{C}$. Statistically significant correlations at $p < .05$ are highlighted in bold. 37

List of Figures

Figure 1. Map of white spruce (*Picea glauca*) species distribution across North America. The seed source provenances are represented by circles and colored by their given region. In total 218 seedlings were used. Seedlings originated from nineteen provenance sites within eight geographic regions. The inset map represents mean annual temperature (MAT, °C) and mean annual precipitation (MAP, mm) at the site of origin for all provenances, colored by region. 23

Figure 2. Soil volumetric water content (VWC) was measured using an EC-5 soil moisture probe (METER, Pullman, USA) over the course of a 87 day drought for white spruce seedlings. Although plants were not watered throughout the entire drought period, VWC fluctuated to some extent presumably due to moisture entering through the roof of the greenhouse. 24

Figure 3. Plot of soil water potential measured with a WP4 water potential meter (METER Pullman, USA) and soil water content measured with an EC-5 soil moisture probe (METER, Pullman, USA). Data points were then fitted to an exponential curve with function $y = 56.47 - 56.24 (1 - e^{-51.6x})$ 25

Figure 4. Comparison of regional height averages for adult white spruce (A) from a long term provenance trial and (B) white spruce seedlings from greenhouse provenance trials. Colors represent regional height averages from all provenances within the region. Letters indicate significant ($p < 0.05$) differences between regions. Error bars represent the standard error of the mean. 27

Figure 5. Budburst was measured in white spruce seedlings after a simulated winter dormancy. Budburst of regions over time as measured on a visual scale of bud burst was determined

with ranks from 0 to 5 (0-dormant, 1-Buds swelling, 2-Bud broken, 3-Needles twice bud length, 4- Needles spreading, 5- Stem elongation)..... 28

Figure 6. Soil water content (VWC) when needles reached 20% of healthy F_v/F_m (maximum quantum efficiency of PSII photochemistry) during the drought treatment. Healthy F_v/F_m was considered 0.8, and therefore 20% of the healthy level was 0.16. Values for each provenance at 20% F_v/F_m were calculated using a sigmoidal curve fit to F_v/F_m vs VWC measurements collected over the course of the drought. Error bars represent standard error. Letters represent significant ($p < 0.05$) differences between regions..... 30

Figure 7. Maximal PS II photochemical efficiency (F_v/F_m) as a function of volumetric soil water content (VWC) for all provenances within each region. F_v/F_m and VWC were measured over the course of the 87 day drought period. Circles represent individual data points colored by region, while curves represent provenance averages. Curves were fit using a sigmoidal equation to each provenance in order to derive the parameter for 20% max F_v/F_m . Sigmoidal functions were generated using the R package *sicegar*. The general sigmoidal equation used was $y_{max}/(1+\exp(\text{slope}*(x-x_{mid})))$, where y_{max} was the maximum F_v/F_m value, slope was the slope at the y midpoint (50% of y_{max}), and x_{mid} was the x value at 50% of y_{max} 31

Figure 8. Box plot of needle $\delta^{13}C$ of drought and control treatments from each region. Lower and upper limits of boxes represent the 25th and 75th percentiles, respectively. The line within the box represents the median of the data. Whiskers represent the largest value no further than $1.5 * IQR$ (interquartile range, or the distance between the 25th and 75th percentiles). Data outside the range of the whiskers are considered outliers and are plotted as circles above their respective boxes. 33

Figure 9. Drought-induced increase in $\delta^{13}C$ was measured as the difference in $\delta^{13}C$ between seedlings in the drought and control treatments. The inset represents the pooled means of Alberta regions, and pooled means of all Ontario regions, compared to Maritime and Yukon provenances to illustrate the significant difference between Ontario and other regions. Error bars represent standard error. Letters represent significant ($p < 0.05$) differences between regions. 34

Figure 10. Drought-induced increase in stable carbon isotope ratio ($\delta^{13}C$, ‰) and the relationship to mean summer precipitation (MSP, mm) and mean annual temperature (MAT, °C). $\delta^{13}C$ and MSP exhibited a nonlinear relationship with equation $y = -9E05x^2 + 0.0678x - 10.602$ ($R^2 = 0.522$). $\delta^{13}C$ and MAT had a positive linear relationship explained by equations $y = 0.2545x + 1.1572$ ($R^2 = 0.4387$). Circles represent provenance means colored by region... 36

1. Introduction

1.1 Recent Climate Trends

The last century has seen global mean surface temperature increases of nearly 1°C. This temperature change has been accompanied by an increase in extreme heat events and a decrease in extreme cold events (Hartmann et al. 2014). These trends are expected to continue for the upcoming century and rates of warming are likely to increase. These changing temperatures have also been accompanied by changes in water resources due to changing precipitation or melting snow and ice (Jefferson 2015).

There are expected to be strong regional differences within our changing global climate, with more northern latitudes being at increased risk of warming and drought. It is estimated that 1983-2012 was the warmest 30-year period in 1400 years in the Northern Hemisphere (Hartmann et al. 2014). This warming has additionally shifted the amount of water stored as snow runoff and snowpack, thus decreasing the amount of water available as spring runoff and altering the timing of this runoff (IPCC 2014). Increased warming also increases the annual precipitation at northern latitudes. This change is accompanied by large seasonal differences in precipitation, which cause increased runoff and flood risks in early spring yet increased likelihood of drought during summer months (Trenberth 2011).

Within the northern hemisphere Canadian boreal ecosystems in particular have already seen a temperature increase of 1.5°C since 1900 and are expected to warm 2°C by 2050 (relative to 2000). These temperature increases will almost certainly be accompanied by more frequent

and intense drought events with the potential to seriously impact boreal ecosystems (Price et al. 2013).

Drought may be caused by decreased precipitation, increased evapotranspiration, or a combination of the two. Because increases in evapotranspiration are often a result of higher temperatures it is important to identify the potential increase in expected drought that is caused by a changing temperature and precipitation combined. Increases in potential evapotranspiration are expected to cause further increases in the occurrence of drought even in areas with little to no change in predicted precipitation, with particularly strong increases in potential evapotranspiration expected at northern latitudes (Cook et al. 2014). Expected increases in global temperatures are likely to further exacerbate changes in precipitation. Although annual precipitation is unlikely to decrease in all regions, low-precipitation years are more likely to cause drought events when accompanied by higher temperatures, thereby increasing the likelihood of drought events as temperatures increase (Diffenbaugh et al. 2015). Warming temperatures are likely to exacerbate drought events despite predicted increases in annual precipitation at northern latitudes. Warming in snow-dominated regions is expected to decrease winter snowfall and cause seasonal snow melt to occur earlier in the spring. Regions unable to hold this early runoff will be at increased risk of summer drought events (Barnett et al. 2005).

1.2 Resulting Forest Dieback

Forest dieback as a result of drought has been documented globally, with no forest type or climate zone being immune to the effects of climate change. Although water is not a limiting resource in all environments, future warming is likely to push more environments into drought conditions, with some forests already exhibiting shifts due to climate (Fensham and Holman 1999, Phillips et al. 2009, Allen et al. 2010, Peng et al. 2011). Predicted increases in warming are likely to exacerbate die-off events by shortening the length of drought able to cause drought-induced mortality by as much as a third (Adams et al. 2009). These drought events are also likely to push further into wetter areas of the species distribution than previous drought events, creating larger scale dieback than previously seen (Breshears et al. 2005). This pervasive increase in forest dieback is likely to affect tree species differently and is likely to cause rapid shifts in ecosystem structures (Anderegg et al. 2013).

Forest sensitivity to climate of northern tree species is likely to be greater than that of other regions with higher latitudes being disproportionately impacted by drought (Charney et al. 2016). Drought in the northern hemisphere has progressively become more disruptive, with long-term effects of temperature, water stress, and tree density increasing defoliation and related tree mortality (Carnicer et al. 2011). Within the northern hemisphere the Canadian boreal forests are likely to exhibit particularly strong responses to future climate change. Boreal forests are of particular importance as they make up 30% of the world's forest area and 75% of Canada's forested area (Bonan 2008), but are vulnerable to both mortality and range shifts as a result of climate change-related permafrost thaw and increase in disturbance by fungi and insects (IPCC 2014). Tree mortality rates within Canada's boreal forest have increased considerably since 1963, regardless of species. However, these increasing mortality rates are variable, with western

regions exhibiting mortality rates twice as large as those in eastern Canada (Peng et al. 2011). This variability between eastern and western regions may have further implications for management under climate change. In eastern Canada the positive effects for tree growth of a longer growing season and higher growth rates due to a warming climate likely outweigh the potential negative effects of low water availability, creating a potential refuge for the more western regions that are negatively impacted by pervasive increases in drought (D'Orangeville et al. 2016). However, this beneficial impact of climate warming in eastern Canada could also potentially be temporary and could result in substantial growth declines if future temperatures rise more than 2°C (D'Orangeville et al. 2018). Such declines have already been observed in a number of important boreal tree species, likely with lasting impacts on ecosystem health.

Recent research suggests that some boreal species, such as lodgepole pine (*Pinus contorta*), may be physiologically maladapted to cope with increasingly dry conditions prevalent in western Canada, and northern die-off may be expected (Isaac-Renton et al. 2018). Drought has also caused large-scale mortality of trembling aspen (*Populus tremuloides*) near the southern boundary of the Canadian boreal, with an estimated 13% increase in dead biomass of broadleaf forests following a severe drought event (Michaelian et al. 2011). In addition to increased mortality rates, decreased growth rates are also expected in response to multi-year decreases in soil moisture following drought (Hogg et al. 2013). These growth reductions and increased mortality rates are also evident in white spruce (*Picea glauca*) populations across Canada (Hogg et al. 2017). Mixed aspen and white spruce stands are less susceptible to drought than separate stands but may be impacted if southern range limits of the two species begin to shift (Chhin and Wang 2016).

In addition to the direct impacts of climate change on tree populations, drought is also a catalyst for other disturbances. Forest fire is a natural occurrence in many regions of the Canadian boreal but is becoming increasingly frequent and severe (de Groot et al. 2013, Abatzoglou and Williams 2016). The increased severity of these fires is made worse by the fact that hotter and drier weather makes regeneration after a fire more difficult for the boreal plant communities (Hansen and Turner 2019). Fire may also be crossing to areas that are traditionally wetter as they become more drought stressed and consume species that are not adapted to fire regimes. In addition to the destruction caused by fire, mass mortality of forest species by insects and fungal pathogens is also being aided by increasingly warm temperatures (Berg et al. 2006). White spruce populations that are sensitive to warmer and drier conditions are also most susceptible to beetle kill (Csank et al. 2016).

Although methods of drought tolerance have been of research interest for decades, there is still little consensus on the mechanisms that drive tree mortality (Sala et al. 2010). Currently the two main hypotheses proposed are hydraulic failure and carbon starvation. Hydraulic failure occurs when plants become irreversibly desiccated, creating extremely high xylem tension and resulting in lethal levels of cavitation. This cavitation eventually impedes the transport of water from the roots to the canopy, leading to loss of conductivity, desiccation, and death (Sperry et al. 1998, McDowell et al. 2008). The alternative hypothesis, carbon starvation, is somewhat related, relying on the tree's ability to avoid hydraulic failure. Carbon starvation is thought to occur as a result of stomatal closure at the onset of drought to prevent desiccation. This stomatal closure, if maintained long enough, eventually causes the tree to run out of carbon stores while being unable to create more (McDowell et al. 2008). More recently, it has been

suggested that tree mortality is likely a combination of these two mechanisms rather than strictly one or the other (McDowell and Sevanto 2010, Sala et al. 2010, McDowell 2011). Hydraulic stress limits turgor in the phloem, slowing access to carbon reserves, indicating the importance of hydraulic failure, yet loss of tissue carbohydrates, due to carbon starvation, decreases the integrity of hydraulic structures, making them more prone to damage and failure (Sevanto et al. 2014). However, examination of tree mortality across species indicates that hydraulic failure always accompanies mortality but carbon starvation, while commonly associated with mortality, is not universal (Adams et al. 2017). These mechanisms resulting in tree mortality can be difficult to tease apart and as such require further research. Although the experiments performed in this thesis do not contribute to further research in this area, tree mortality mechanisms are inherently important to drought tolerance studies.

1.3 White Spruce Autecology

White spruce (*Picea glauca* [Moench] Voss) is a wide-ranging boreal forest species of great importance ecological and economically, representing 20% of Canada's total forest inventory. The distribution of white spruce ranges from the northern tree line of Alaska to the east coast of Canada and the northern United States. This large species covers a correspondingly large range of temperature and precipitation. Across the wide species range, white spruce exhibits highly variable growth potential and strong local adaptation making it ideal for studies of climate adaptation (Rweyongeza 2011). The southern range limit of white spruce commonly occurs in the aspen parkland in the transition between prairie and boreal. This southern

distribution limit is constrained by summer moisture stress induced by high temperatures rather than low precipitation(Chhin and Wang 2008). Growth of white spruce is additionally affected by climatic variables related to drought, implying that white spruce may be particularly sensitive to future climate change, particularly at the edges of the species distribution (Chhin et al. 2004).

1.4 Climate Change Adaptation Strategies

The expected forest dieback due to climate change is likely to continue as temperatures continue to rise, creating more complex and challenging situations for forest management. This indicates the need for adjustment of current management strategies. Such adjustments should focus on reducing the vulnerability of forests without sacrificing productivity. Although climate change has strong negative impacts on northern forests, warmer temperatures may also have positive outcomes for forest productivity if managed efficiently. Currently, much of the increased vulnerability of northern forests is the result of maladaptation of tree populations. Maladaptation of northern forest trees is well-documented and will likely continue to worsen as warming continues to remove forest trees from their optimal climate zones (Isaac-Renton et al. 2018). However, assisted migration of genotypes adapted to future climate scenarios in areas expected to experience maladaptation may result in less negative impacts associated with warming. By translocating local but maladapted populations from their suboptimal environment to more favorable environments within their species range, their survival and growth in the long-term is greatly improved. This is particularly important for white spruce populations as thousands of hectares are planted each year for forestry and conservation purposes. By simply

replacing locally-sourced planting stock with planting stock adapted to cope with the long term climate outlook, the likelihood of future maladaptation is greatly reduced. Due to its large species range, white spruce is an excellent candidate for assisted migration, and may be in particular need as well. Suitable habitat for boreal white spruce populations has already shifted 207 km north (relative to 1961-1990) and is expected to continue this northward movement (Gray and Hamann 2013). Western Canada in particular has experienced greater drying and warming than eastern Canada in past decades and this trend is predicted to continue, indicating the need for assisted migration of maladapted western populations. This assisted migration could mean moving the maladapted western populations to eastern areas, which may be a potential climate refuge in coming years (D'Orangeville et al. 2016).

1.5 Adaptations of Tree Populations

Due to the complexity of genetic research it is not always feasible to use direct genetic assays in the screening for genetic adaptation of populations. Instead, provenance studies, or common garden studies, are used to screen species and populations for genotypic variation. These trials use seed sources from across the species range and are planted at field test sites to identify genetic variation linked to environmental factors and to then identify the well-adapted provenances for the management objective (Morgenstern 1996). Provenances trials are particularly useful for identifying populations well-suited for use in assisted migration programs and have been widely used as a tool to identify potentially drought and cold tolerant populations necessary for seed transfers. Although provenances trials were originally devised to optimize

productivity of forest products, they can now be utilized for climate change research by moving seeds to more southern planting sites as a proxy for future climate conditions. Generally, locally adapted provenances should perform best, but in some cases they may be outperformed by other provenances, an indication of potential maladaptation to current or future climate (Isaac-Renton et al. 2018). Provenance trials of trembling aspen and hybrid poplar identified substantial genetic differences between species and populations in hydraulic traits related to drought tolerance (Schreiber et al. 2013). Long term lodgepole pine field trials in Alberta identified possible seed transfers representative of annual temperature increases of 0.5°C (Gray et al. 2016b). An additional consideration in assisted migration in northern climates is frost-tolerance of potential northward seed transfers. Although northward transfers have been identified as beneficial for white spruce populations, it is also necessary to identify frost-tolerant genotypes that can withstand the colder temperatures associated with more northern sites (Gray et al. 2016a). Further provenance studies of white spruce cold hardiness were able to confirm that moderate northward transfers are possible without significant trade-offs in growth or survival (Sebastian-Azcona et al. 2019). The use of these provenance trials is reflective of the adaptive strategies of populations to their local climate. This information may give greater insight into the climatic drivers behind trait adaptations.

Adaptation to drought is particularly important to the current study. Conifers have adapted to survive in some of the world's harshest conditions and have developed a wide array of strategies for surviving. Conifers have adapted two strategies of avoiding hydraulic failure due to water stress. Isohydric trees close stomata in response to high levels of abscisic acid (ABA) and

thus maintain internal water potentials where as anisohydric trees allow water potential to drop and eventually low leaf water potential causes stomatal closure (Brodribb et al. 2014). These strategies are accompanied by differences in xylem architecture. Because anisohydric trees allow water potential to drop, they tend to have xylem that is more cavitation resistant (McDowell et al. 2008). Additional structural components have been identified as adaptations of drought tolerant species including xylem conduit lumen width, tracheid/vessel cell wall thickness, rooting depth, and xylem refilling (Moran et al. 2017). This large suite of adaptations may vary between species and populations as well as within distinct populations due to local or species-level adaptation.

1.6 Methods for Evaluating Drought Tolerance

Drought tolerance in plants can generally be defined as the ability of plants to survive and grow during periods of drought (Moran et al. 2017). In this context, drought may be defined as water shortage great enough to cause reduced growth or yield in plants (Passioura 1996). The many strategies plants have adapted in order to tolerate such drought conditions fall into the following general categories: escaping water deficit, perhaps by finishing growth before seasonal drought sets in; avoiding water deficit by reducing water loss or increasing water uptake; maintaining growth despite water deficit; resisting water deficit (Tardieu 2005). Drought tolerance differences at both the species and populations level are important to note for future adaptation and are generally comprised of both genetic and environmental traits. Although drought tolerance has been widely studied, the importance of various drought tolerance traits as well as their genetic basis is complicated and still poorly understood (Moran et al. 2017). For this

reason, provenance studies using a select number of drought tolerance indicators are useful for determining the importance of impacts of such traits. In the present study, I chose to use chlorophyll fluorescence and stable carbon isotope ratios to examine how drought may affect photosynthesis and stomatal response in white spruce seedlings. While this approach has not been widely applied to common garden studies, these approaches are widely used in tree physiology. Although this study does not examine potential differences in other important drought traits, previous studies have found fairly weak population differences in wood anatomical and hydraulic traits in white spruce, suggesting that other physiological traits may be of greater importance for heritability of drought tolerance (Sebastian-Azcona et al. 2018).

Chlorophyll fluorescence is a widely used and non-destructive measurement of photosystem II (PSII) function, which provides a glimpse of plant health without damage. The sensitivity of photosynthesis to stress makes fluorescence a relatively simple but important technology for plant physiology (Maxwell and Johnson 2000, Murchie and Lawson 2013). Although chlorophyll fluorescence is not a direct measure of photosynthesis it measures the re-emitted light from PSII, or light that has not been used for photosynthesis, allowing us to see changes in the photosynthesis via changes in fluorescence. One of the most common measures of chlorophyll fluorescence is pulse-amplitude modulated fluorometry (Schreiber 2004). This method uses pulses of measuring light of known wavelength to induce fluorescence yields, also of known, longer, wavelength. Three measuring lights are used: a very weak initial light to detect minimum fluorescence yield (F_0) when all reaction centers within PSII are “open,” a moderate intensity actinic light used to induce photosynthesis, and a saturating pulse of high intensity light

used to “close” reaction centers and give a measure of maximum fluorescence yield (F_m) (Krause 2003). These measurements are taken using dark-adapted leaves to avoid variation due to ambient light interference (Murchie and Lawson 2013). The difference between F_o and F_m is a measure of variable fluorescence (F_v). The ratio of F_v/F_m is considered a strong indicator of the maximum quantum yield of PSII chemistry, or the maximum quantum yield of photosynthesis, and in unstressed leaves gives a value of approximately 0.83 (Demmig and Bjorkman 1987). F_v/F_m is a useful indicator of plant drought stress due to the significant effect water stress has on photosynthesis. As leaf water stress increases, photosynthesis declines due to stomatal closure and decreased activity of the biochemical photosynthetic machinery (Chaves et al. 2009).

The use of chlorophyll fluorescence as a measure of plant stress is not a particularly new tool, yet there have been relatively few studies of the relationship between fluorescence and drought in tree species, and in particular conifers (Mohammed et al. 1995). The relatively few species studied, which include *Picea abies* (Ditmarova et al. 2010), *Picea glauca* (Bigras 2005), and *Pinus radiata* (Conroy 1986) have all demonstrated the capacity to maintain photosynthesis until water stress becomes particularly severe. *Picea abies* seedlings under drought conditions were able to maintain photosynthesis until leaf water potentials were below -2 MPa, at which point F_v/F_m sharply declined by nearly half (Ditmarova et al. 2010). This common ability to maintain photosynthesis under water stress makes chlorophyll fluorescence a useful tool for selection of particularly drought tolerant populations in forest trees. Although chlorophyll fluorescence is commonly used as a screening tool for drought tolerant genotypes in agricultural crops, it is comparatively rare for comparison of forest tree populations (Sayed 2003, Murchie

and Lawson 2013). Fluorescence measurements, used to assess health of white spruce seedlings under water stress, revealed a trade-off between growth rate and susceptibility of photosynthetic processes to drought. Trees with superior growth rates showed sharper decline at higher water potentials than did those trees with intermediate or inferior growth rates (Bigras 2005). This information suggests that fluorescence may be useful in selection of genotypes adapted to dry periods or environment for use in future planting endeavors.

Stable isotopes are a particularly useful tool for evaluating plant response to drought. Gas exchange in the leaves or needles of a plant is regulated through pores, known as stomata, located on the leaf surface. Stomata allow carbon dioxide to enter the leaves, but are able to open and close based on environmental factors such as wind, humidity, and soil water, so as to maintain balance between water uptake and water loss. The opening and closing of the stomata, facilitated by cells located at the opening called guard cells, alters stomatal conductance. When stomata are open, and conductance is high, carbon dioxide passes into the leaf with relative ease. Carbon dioxide contains carbon atoms that may take two forms, known as stable isotopes. An isotope of an element is simply a variation in the number of neutrons compared to protons. Carbon-13, the rarer isotope of carbon, has 7 neutrons and 6 protons, as compared to the more common carbon-12 which has 6 neutrons and 6 protons. Although these isotopes have nearly identical chemical processes, carbon-13's extra neutron makes it slightly heavier than carbon-12, which allows for discrimination against it during important biological processes (McCarroll and Loader 2004). The ratio of ^{13}C to ^{12}C is expressed as compared to the Vienna-PDB standard in parts per thousand (‰) using the following equation:

$$\delta^{13}\text{C} = [(R_{\text{sample}} / R_{\text{standard}}) - 1] * 1000$$

The carbon isotopic ratio of CO₂ in air is approximately -8‰, with plants having lower δ¹³C than air due to the aforementioned discrimination. In plants, this discrimination against ¹³CO₂ occurs during diffusion through stomata and during use of carbon dioxide during carboxylation (Farquhar and Sharkey 1982). The discrimination of both processes may result in ¹³C enrichment of the assimilated sugars. Changes in these physiological processes may therefore be reflected in δ¹³C of plant tissues such as leaves and tree rings. When water stress occurs, plants will close stomata, decreasing CO₂ inside the leaf. This decrease in CO₂ also lowers the ability of the enzyme RuBisCo to discriminate against the carbon-13 isotope during carboxylation, which then results in increased δ¹³C (less negative) in the sugars produced (Farquhar et al. 1989). The subsequent increase in δ¹³C of plant tissues is therefore indicative of plants' stomatal response to drier conditions. Although it is possible that increased δ¹³C may result from either stomatal closure or increased carbon assimilation via carboxylation, the carbon isotopic composition of plant tissues, particularly in moisture-limited environments, is chiefly due to stomatal closure and water availability (Chaves et al. 2003, McCarroll and Loader 2004, Brien et al. 2011, Knutzen et al. 2015).

Stable carbon isotope ratios have been used for a variety of studies on the effects of drought stress on plant physiology and to compare the drought response of different species and populations (Chaves et al. 2003, Condon et al. 2004, McCarroll and Loader 2004). Previous work using δ¹³C in genome screening reveals a genetic basis for δ¹³C response to drought, likely

via stomatal behavior, useful in potential breeding and selection programs (Dhanapal et al. 2015, Avramova et al. 2019). The stomatal response of trees in previous provenance trials, as indicated by changes in $\delta^{13}\text{C}$, shows more correlation to provenance of origin than water availability, suggesting that there are heritable differences in this drought response trait (Knutzen et al. 2015). Particularly, trees from drier climates exhibited more negative $\delta^{13}\text{C}$ during moderate drought, indicating less stomatal limitation than in trees originating from moister climates (Rose et al. 2009). Carbon isotope ratios have been established as a useful indicator of drought stress in multiple species of conifers, exhibiting differences between species as well as between populations of the same species (Guy and Holowachuk 2001, Warren et al. 2001). Provenance trials of *Pinus contorta* seedlings and saplings revealed strong population differences in $\delta^{13}\text{C}$ at both seedling and sapling level (Guy and Holowachuk 2001). Additional studies using seedlings of white spruce (*Picea glauca*) have similar results and confirm that the $\delta^{13}\text{C}$ may be a useful tool for the selection of drought tolerant white spruce genotypes (Sun et al. 1996).

1.7 Objectives

This thesis aims to investigate the physiology of drought tolerance of white spruce populations and identify genotypes capable of coping with current and future drought events without sacrificing productivity. Greenhouse trials coupled with 40-year-old field trials will be used to investigate growth rates in seedlings under favorable conditions as well as long term field performance. Greenhouse seedling provenance trials also allow me to determine if drought tolerance in white spruce seedlings is a heritable trait and, if such heritability exists, what the

relationship between drought tolerance and climate at seed origin is. These physiological measures, coupled with growth trials, will give insight into trade-offs between drought tolerance and growth among white spruce populations and the potential for future management of white spruce stands.

2. Methods

2.1 Seed sources

I used white spruce (*Picea glauca*) seeds from 19 provenances across Canada. Seeds were collected from parent trees of white spruce between the years 1968 and 1976. They were then stored in a cooled seed vault at the Alberta Tree Improvement and Seed Centre, Smoky Lake, AB, until planting in summer of 2017.

2.2 Growing conditions

In order to break dormancy, seeds were stratified before being planted. Seeds were placed over a tray of water overnight for humidification, after which time they were soaked in distilled water for a second night. They were then placed on moistened paper towel inside plastic bags and refrigerated for 3 weeks at 4°C. After stratification, seeds were planted in styroblocks filled with peat-based soil (Sunshine Mix #4, Sun Gro Horticulture, Agawam, USA) in June 2017 and September 2017. Seeds were germinated and grown in a growth chamber at 24°C with 16 hours

of daylight until November 2017. At this time, temperature, light, and humidity were gradually lowered over 6 weeks, to ready them for simulated winter dormancy in a cold room at 4°C. The dormancy period lasted 6 weeks, after which time they were returned to growth chambers to gradually return to ideal growing conditions. Plants were watered with water of pH 5.5 and fertilized using 20-10-20 fertilizer plus iron chelate added to water every two weeks. pH was adjusted from pH 7.8 to pH 5.5 using 1mL phosphoric acid added to 6L water. In April 2018, plants were repotted into square pots 5” in diameter filled with peat-based soil (Sunshine Mix #4, Sun Gro Horticulture, Agawam, USA) and moved into a greenhouse. Pots were arranged in a randomized block design of 13 total blocks with individuals from each provenance distributed randomly among the blocks. The soil was kept at ~35% water content.

2.3 Long Term Growth

Long term data was collected from previously established provenance trials in Calling Lake, Alberta. These trials were established in 1982 using four-year-old seedlings. Height and survival data were collected after 32 growing seasons.

2.4 Drought Experiment

Beginning in July 2018, seedlings were divided between control and dry treatments. One-third of total seedlings from each provenance were placed in the control group, two-thirds in the dry group. The control group was held at ~30% soil water content for the duration of the

experiment. During dry down, dry treatment seedlings were not watered for the duration of the experiment. During drought treatment, soil water content was measured every 4-7 days using an EC-5 soil moisture sensor (METER, Pullman, USA) attached to a ProCheck handheld data readout (METER, Pullman, USA) which was calibrated for use in sunshine mix soil.

A soil water potential curve was measured for reference using a WP4 psychrometer (METER, Pullman, USA) and known water potential standards. Standards were calculated using KCl solutions of known osmolarity. Soil samples were oven dried and rehydrated to known water contents for water potential measurements.

2.5 Fluorescence

Chlorophyll fluorescence was measured using a fluorometer (Mini-PAM, Heinz Walz GmbH, Effeltrich, Germany). Measurements of maximal photosystem II (PSII) efficiency, or F_v/F_m , were taken using dark adapted leaves. Dark adapted fluorescence values were measured using leaf clips (Heinz Walz GmbH, Effeltrich, Germany) placed on groups of needles for 15 minutes. All measurements were conducted between 5 am and 12pm every 4-7 days.

Fluorescence was measured until plants reached F_v/F_m values <0.1 , or when the needles began falling off when a leaf clip was applied. At this time, plants were considered dead and harvested for later use.

2.6 Isotopes

Approximately 70 mg of needles were collected from dead, dry treatment trees or oven-dried controls and placed in glass vials. Trees were then submitted to the Natural Resources Analytical Laboratory (NRAL) at the University of Alberta for analysis of ^{13}C isotope ratio ($\delta^{13}\text{C}$). A ThermoFinnigan Delta⁺ Advantage Continuous Flow Isotope Ratio Mass Spectrometer by Thermo Finnigan Corp was used to conduct isotope analysis. Samples were ground in a ball mill and weighed before analysis.

2.7 Bud Break

Bud break was measured every two to four days after trees broke their simulated winter dormancy beginning in March 2018. A visual scale of bud burst was determined with ranks from 0 to 5 (0-dormant, 1-Buds swelling, 2-Bud broken, 3-Needles twice bud length, 4- Needles spreading, 5- Stem elongation).

2.8 Statistics

Seedlings were grown in a randomized block design with 13 blocks and 0 to 7 seedlings per provenance and block due to variable germination rates (the median number of seedlings per provenance and block was 3). Drought treatments were applied to random seedlings within blocks, where two thirds of seedlings of the same provenance within a block received the drought treatment and on third served as a well-watered control. Data analysis was completed

using R statistical software. Significant differences between regions for growth, fluorescence, and $\delta^{13}\text{C}$ were assessed with analysis of variance (ANOVA) performed using a linear mixed model with region of origin specified as a fixed effect, and provenance within region and block as random affect.

Volumetric water content at 20% fluorescence values were used for regional comparisons as they reflected sufficiently strong drought condition to reveal differences among regions and provenances. Volumetric water content values at 20% fluorescence were estimated from curves fitted to each provenance using a sigmoidal equation. Sigmoidal functions were generated using the R package *sicegar*. The general sigmoidal equation used was $y_{\text{max}}/(1+\exp(\text{slope}*(x-x_{\text{mid}}))$, where y_{max} was the maximum F_v/F_m value, slope was the slope at the y midpoint (50% of y_{max}), and x_{mid} was the x value at 50% of y_{max} . To assess how the climate of provenance origins were associated with physiological and growth characteristics, Pearson correlations were calculated between mean provenance values for physiological and growth and the following climate variables (Table 1). Climate variables used were mean annual temperature (MAT) in °C; degree days above 5°C (DD>5), or growing degree days; mean warmest month temperature (MWMT) in °C; frost free period (FFP) in number of days; mean coldest month temperature (MCMT) in °C; degree days below 0°C (DD<0), or chilling degree days; temperature difference (TD) between MWMT and MCMT is a measure of continentality and is given in °C; mean annual precipitation (MAP) is given in mm; mean summer precipitation (MSP) is given in mm; precipitation as snow (PAS) between August in previous year and July in current year is given in mm; climatic moisture deficit (CMD) is given in mm; annual heat moisture index (AHM); summer heat moisture index (SHM). Climate data for provenance origins were based on a 1961-1990 climate

normal period that was generated for point locations using the ClimateNA v5.10 software package, available at <http://tinyurl.com/ClimateNA>, based on methodology described by Wang et al. (2016).

Table 1. Average source climate variables from the years 1961 to 1990 are shown for the 8 regions.

Climate Variable	Yukon	Northern Alberta	Central Alberta	Foothills Alberta	Southern Alberta	Western Ontario	Southern Ontario	Maritime
MAT	-4.25	-0.25	0.27	1.3	1.6	0.3	5.3	3.17
DD>5	786	1351	1233.33	857	971.33	1275	1961	1292.67
MWMT	13.65	16.95	16.07	12.75	13.77	17.15	20.2	16.4
FFP	67.50	108	102	90	82.67	95.5	135	112
MCMT	-23.65	-22	-19.33	-10.35	-10.63	-19.45	-11.6	-9.43
DD<0	3055	2291.5	2015.67	1289	1281	2042	1090.5	1111.33
TD	37.3	38.85	35.4	23.1	24.47	36.55	31.8	25.83
MAP	330	411	453.67	636	593.67	746	830.5	1152
MSP	209	263	306.67	404.5	319	417.5	388	460.67
PAS	134	120.5	123.33	203	237.67	267.5	241	392.33
CMD	174	226.5	177.67	31	158.33	122.5	193.5	34
AHM	17.9	23.75	22.8	17.7	20.9	13.75	18.4	11.43
SHM	66.2	64.45	52.43	31.45	45.7	41	52.1	35.67

Climate variables include mean annual temperature (Berg et al.) in °C; degree days above 5°C (DD>5), or growing degree days; mean warmest month temperature (MWMT) in °C; frost free period (FFP) in number of days; mean coldest month temperature (MCMT) in °C; degree days below 0°C (DD<0), or chilling degree days; temperature difference (TD) between MWMT and MCMT is a measure of continentality and is given in °C; mean annual precipitation (MAP) is given in mm; mean summer precipitation (MSP) is given in mm; precipitation as snow (PAS) between August in previous year and July in current year is given in mm; climatic moisture deficit (CMD) is given in mm; annual heat moisture index (AHM); summer heat moisture index (SHM).

3. Results

The 19 provenances were widely distributed across the white spruce (*Picea glauca*) species range (Fig.1). Provenances varied greatly in both mean annual temperature (MAT, °C) and mean annual precipitation (MAP, mm). Yukon and Maritime provenances fell at opposite ends of the temperature-precipitation gradient, with Yukon provenances being at the cool and dry extreme and Maritime provenances in the warm and wet end (Fig.1 inset). Southern Ontario provenances were also warmer and wetter than most other provenances.

Seedlings were exposed to drought for 87 days with water contents at the end of the drought ranging from 0.2% to 1% (Fig. 2). Volumetric soil water content was converted to water potential with an exponential curve that shows the tight threshold at which soil water potential begins a sharp decline (Fig. 3). Although water contents were intended to be kept in a consistent decline, greenhouse leaks caused some irregularities.

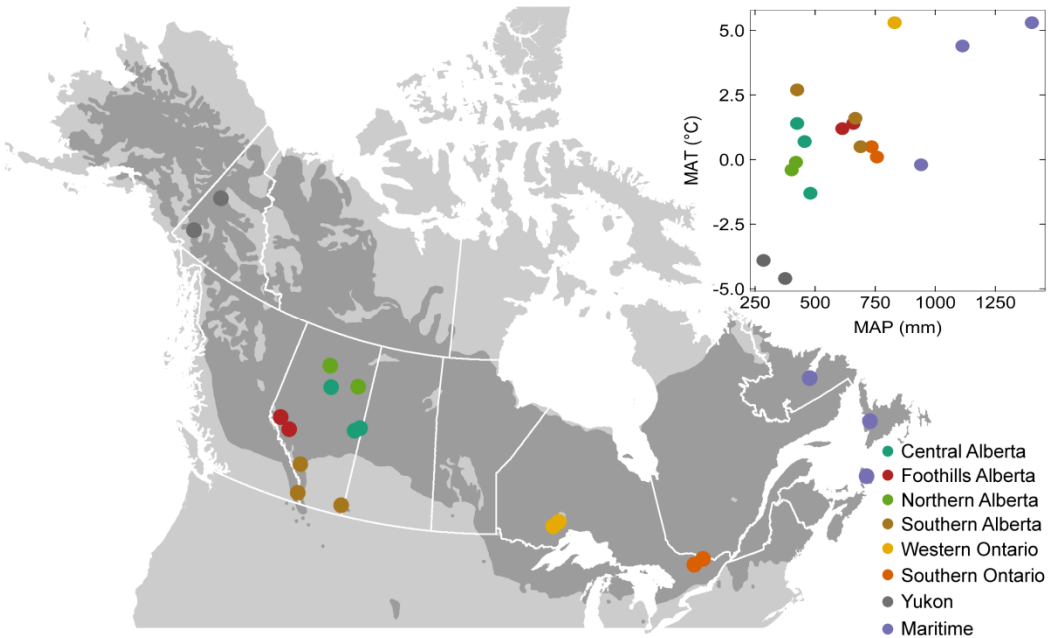


Figure 1. Map of white spruce (*Picea glauca*) species distribution across North America. The seed source provenances are represented by circles and colored by their given region. In total 218 seedlings were used. Seedlings originated from nineteen provenance sites within eight geographic regions. The inset map represents mean annual temperature (MAT, °C) and mean annual precipitation (MAP, mm) at the site of origin for all provenances, colored by region.

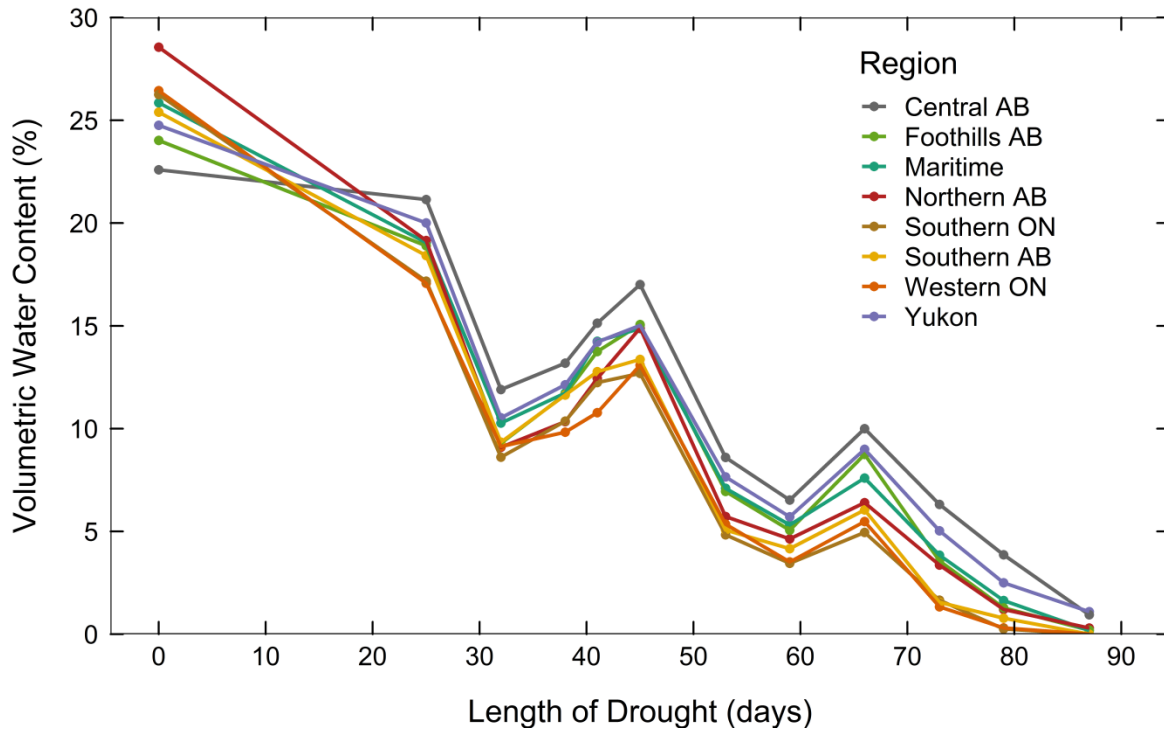


Figure 2. Soil volumetric water content (VWC) was measured using an EC-5 soil moisture probe (METER, Pullman, USA) over the course of a 87 day drought for white spruce seedlings. Although plants were not watered throughout the entire drought period, VWC fluctuated to some extent presumably due to moisture entering through the roof of the greenhouse.

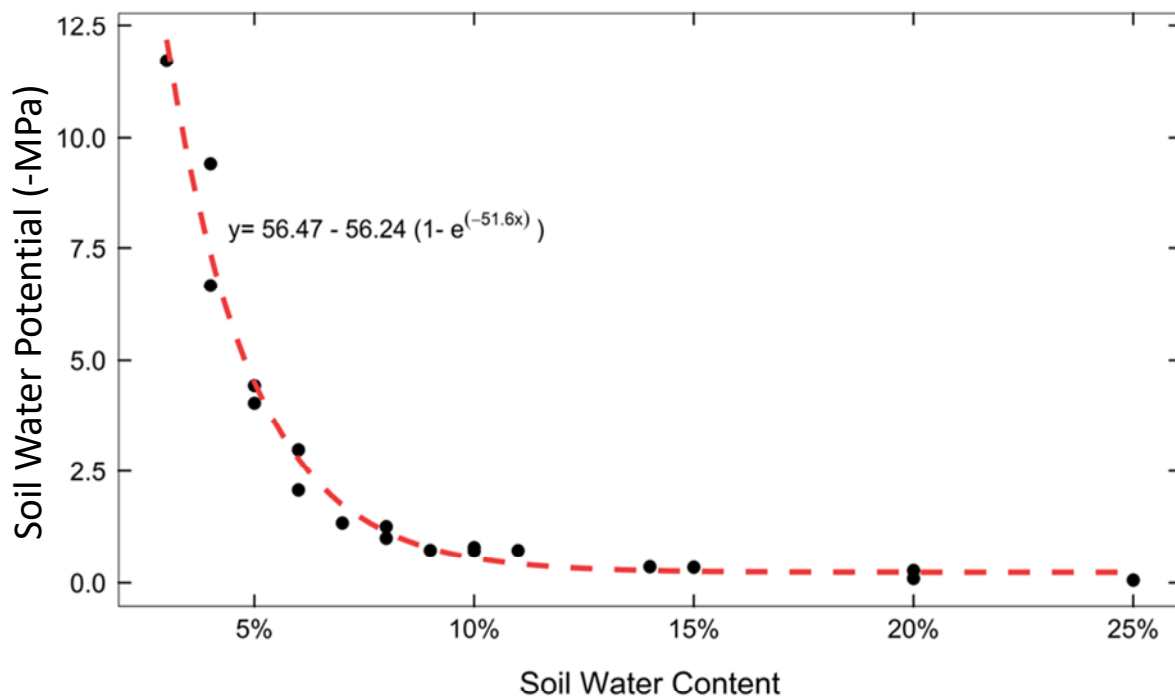


Figure 3. Plot of soil water potential measured with a WP4 water potential meter (METER Pullman, USA) and soil water content measured with an EC-5 soil moisture probe (METER, Pullman, USA). Data points were then fitted to an exponential curve with function $y = 56.47 - 56.24 (1 - e^{-51.6x})$.

3.1 Adult and Seedling Growth

Height was significantly different between regions in the long-term provenance trial and seedling provenance trial (Fig.4). Adult height was clearly greatest in local provenances, with Central Alberta, the nearest provenance to the common garden site, having the overall greatest height. Northern Alberta provenances, also near the common garden site, performed similarly to Central Alberta. In contrast, both Southern and Western Ontario showed similar growth performance to Central and Northern Alberta and showed clearly greater heights than both the

Foothills and Southern Alberta provenances despite being non-local. Southern Alberta had relatively low growth, especially compared to other Alberta provenances and was more similar to the Maritimes performance. Yukon provenances, which are far more northwestern than all other provenances, had the poorest growth performance in both adult and seedling provenance trials. Under ideal growth conditions, seedlings from Southern Ontario exhibited significantly greater height than all Alberta provenances despite similar heights in adult field trials. In additional contrast, Maritime provenances performed quite well and significantly greater than both Northern and Southern Alberta. Although Yukon seedlings exhibited the overall lowest growth among seedlings, they had large variability and were quite similar to all Alberta provenances, unlike their far lower height in adult trials. Budbreak in seedlings did not show clear geographic trends. However, Alberta Foothills was surprisingly most similar to Ontario provenance and these were significantly slower to break bud than Northern Alberta provenances (Fig. 5).

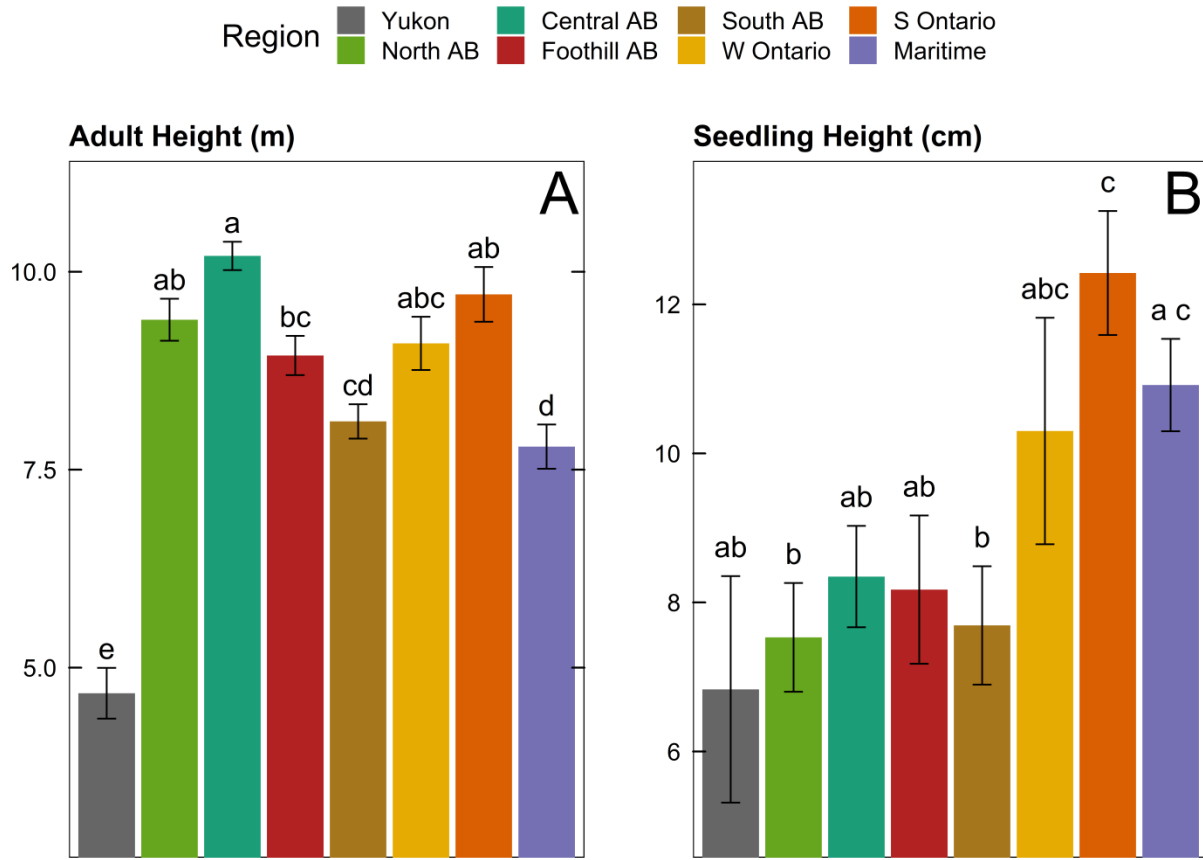


Figure 4. Comparison of regional height averages for adult white spruce (A) from a long term provenance trial and (B) white spruce seedlings from greenhouse provenance trials. Colors represent regional height averages from all provenances within the region. Letters indicate significant ($p < 0.05$) differences between regions. Error bars represent the standard error of the mean.

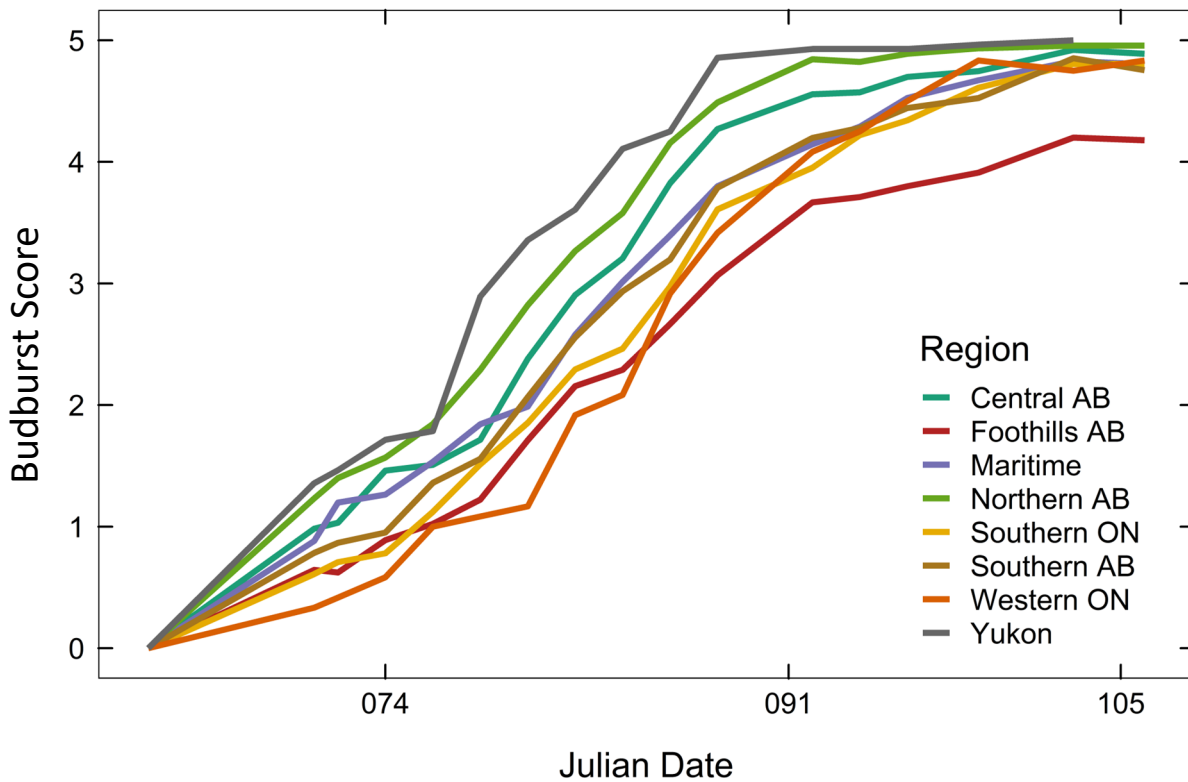


Figure 5. Budburst was measured in white spruce seedlings after a simulated winter dormancy. Budburst of regions over time as measured on a visual scale of bud burst was determined with ranks from 0 to 5 (0-dormant, 1-Buds swelling, 2-Bud broken, 3-Needles twice bud length, 4-Needles spreading, 5- Stem elongation).

3.2 Seedling Drought Response

The soil water content (VWC, %) at which white spruce seedlings reached 20% of their healthy maximum photochemical efficiency (Fv/Fm), or an Fv/Fm reading of 0.16, varied

between regions. The eastern and northwestern edges of the species range, Maritime and Yukon provenances, showed the drop to 20% Fv/Fm at higher soil water contents than other regions, reaching these low Fv/Fm rates at above 6% soil water content (Fig. 6). Provenances from both regions experienced sharp drops in Fv/Fm relatively early on in the drought, with one Maritime provenance beginning its decline at nearly 7% water content. Two of three Central Alberta provenances also declined early on, similar to the Maritime and Yukon provenances (Fig. 7). The two Northern Alberta provenances were quite different from each other, with one dropping to low Fv/Fm levels at water contents more similar to Western Ontario, while the other was similar to Yukon and Central Alberta provenances (Fig 7). In contrast, the Foothills and Southern Alberta maintained Fv/Fm levels above 20% to significantly lower contents than Maritime provenances. Western Ontario had the lowest water content at 20% Fv/Fm and notably lower than Southern Ontario and Central Alberta.

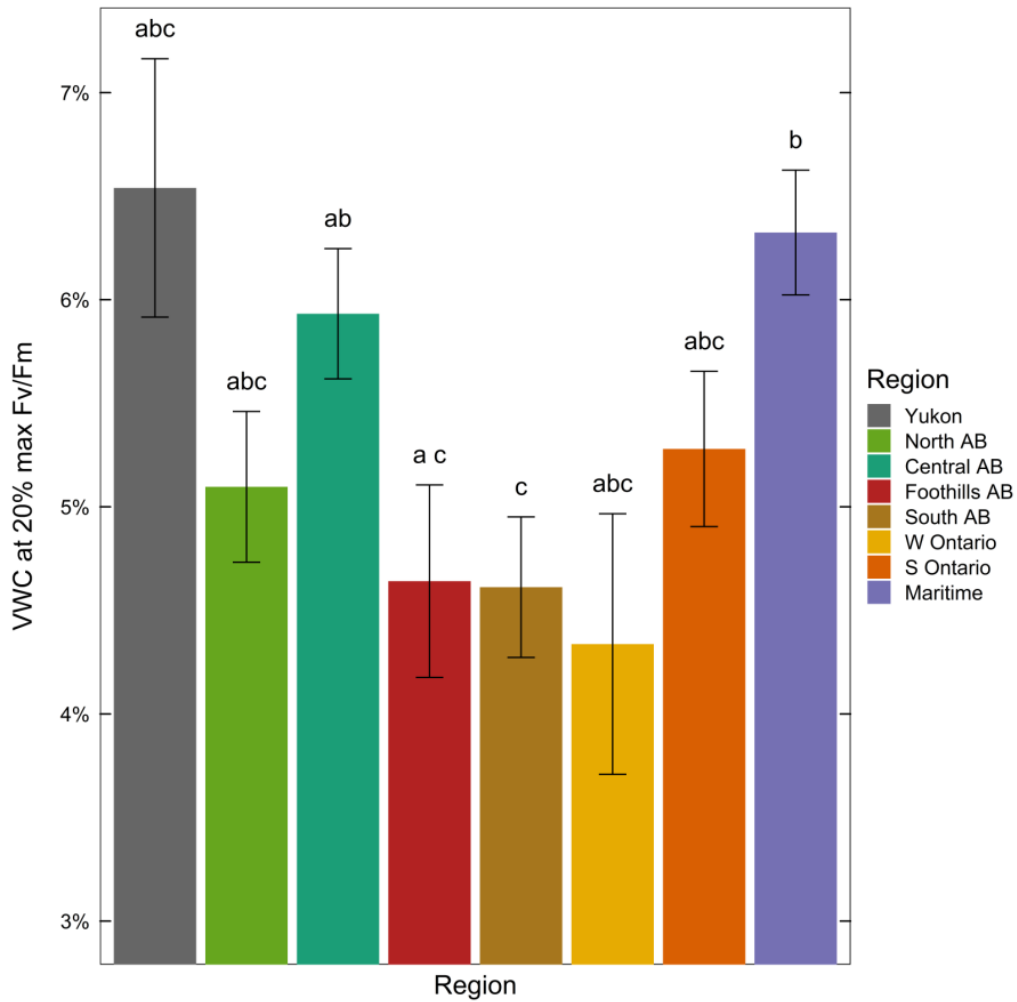


Figure 6. Soil water content (VWC) when needles reached 20% of healthy Fv/Fm (maximum quantum efficiency of PSII photochemistry) during the drought treatment. Healthy Fv/Fm was considered 0.8, and therefore 20% of the healthy level was 0.16. Values for each provenance at 20% Fv/Fm were calculated using a sigmoidal curve fit to Fv/Fm vs VWC measurements collected over the course of the drought. Error bars represent standard error. Letters represent significant ($p < 0.05$) differences between regions.

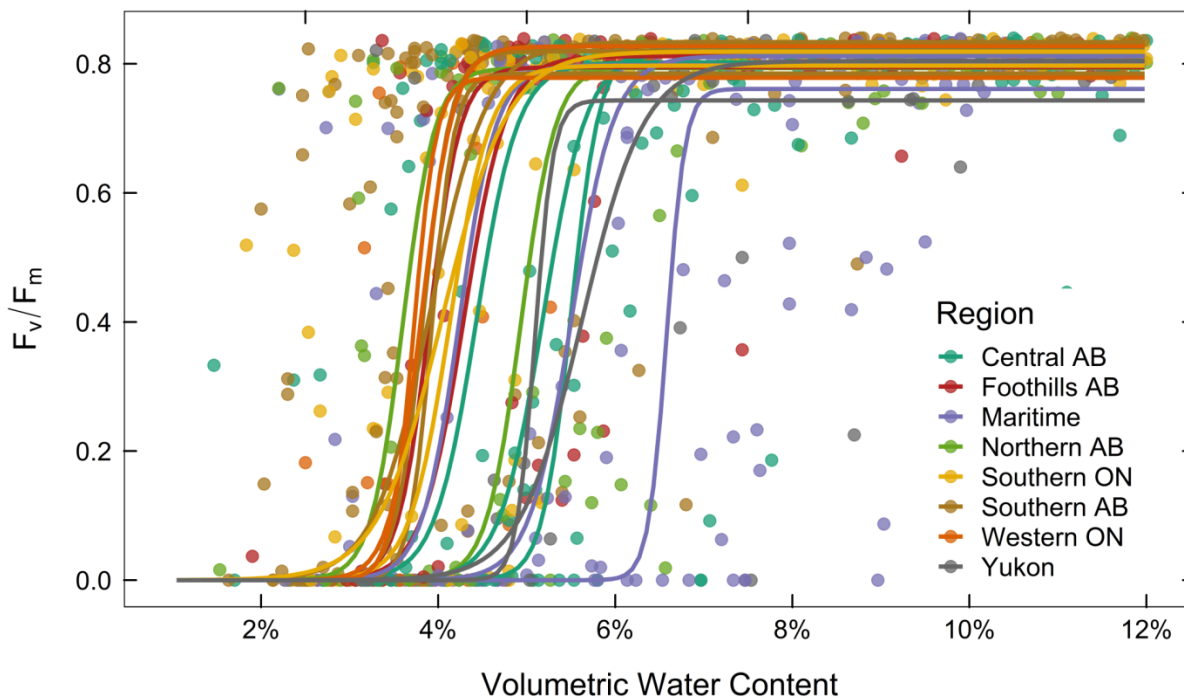


Figure 7. Maximal PS II photochemical efficiency (F_v/F_m) as a function of volumetric soil water content (VWC) for all provenances within each region. F_v/F_m and VWC were measured over the course of the 87 day drought period. Circles represent individual data points colored by region, while curves represent provenance averages. Curves were fit using a sigmoidal equation to each provenance in order to derive the parameter for 20% max F_v/F_m . Sigmoidal functions were generated using the R package *sicegar*. The general sigmoidal equation used was $y_{max}/(1+\exp(\text{slope}*(x-x_{mid})))$, where y_{max} was the maximum F_v/F_m value, slope was the slope at the y midpoint (50% of y_{max}), and x_{mid} was the x value at 50% of y_{max} .

Under control conditions, stable carbon isotope ratios ($\delta^{13}\text{C}$, ‰) were similar between most regions. However, Southern and Western Ontario provenances had the most negative ratios under control conditions. Carbon isotope ratios under drought conditions were higher than under control conditions for most provenances, but $\delta^{13}\text{C}$ under drought varied between regions (Fig.8).

Southern Ontario exhibited the highest carbon isotope ratio under drought and had the largest difference between drought and control $\delta^{13}\text{C}$. Plants from Western Ontario had similar change in isotope ratios to plants from Southern Ontario (Fig. 9). When Ontario provenances were pooled and compared to pooled Alberta provenances, Yukon, and Maritime, plants from Ontario had significantly larger differences between control and drought carbon isotope ratios (Fig.9 inset). Under drought conditions, Yukon provenances had both the lowest isotope ratio and the smallest difference between treatments. All Alberta and Maritime provenances were similar and showed similar differences between drought and control conditions.

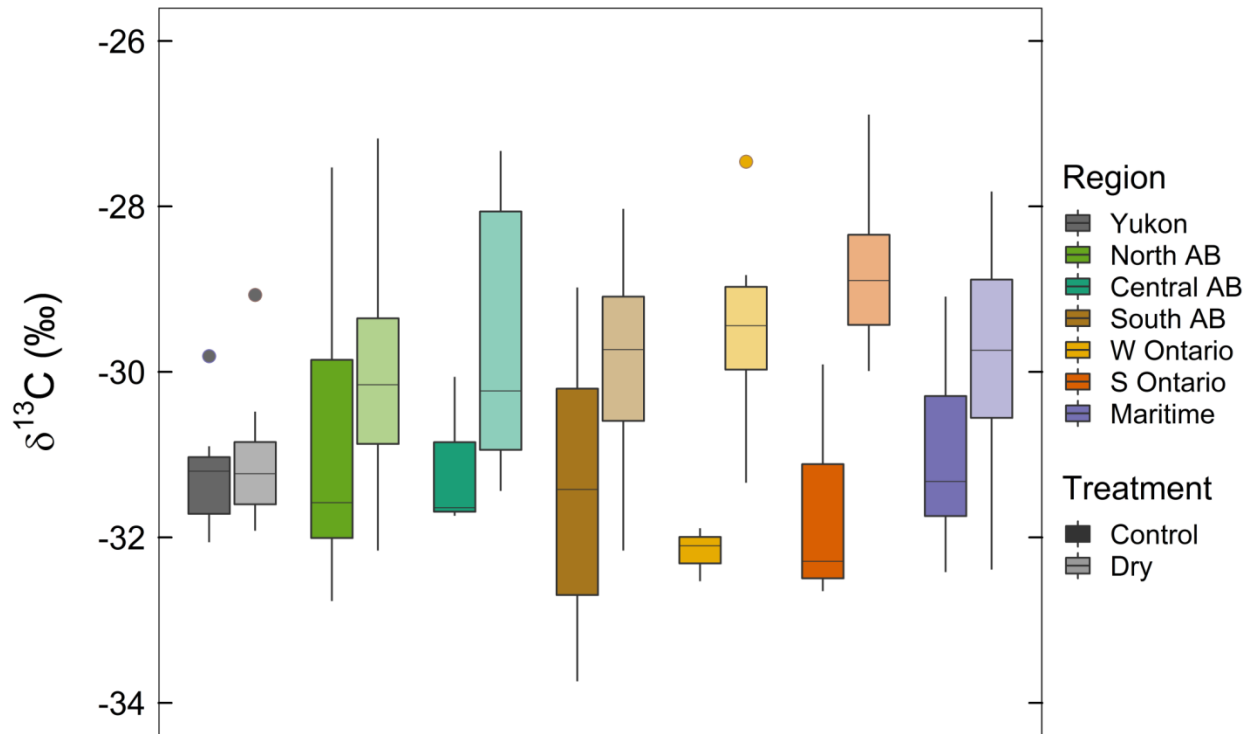


Figure 8. Box plot of needle $\delta^{13}\text{C}$ of drought and control treatments from each region. Lower and upper limits of boxes represent the 25th and 75th percentiles, respectively. The line within the box represents the median of the data. Whiskers represent the largest value no further than $1.5 \times \text{IQR}$ (interquartile range, or the distance between the 25th and 75th percentiles). Data outside the range of the whiskers are considered outliers and are plotted as circles above their respective boxes.

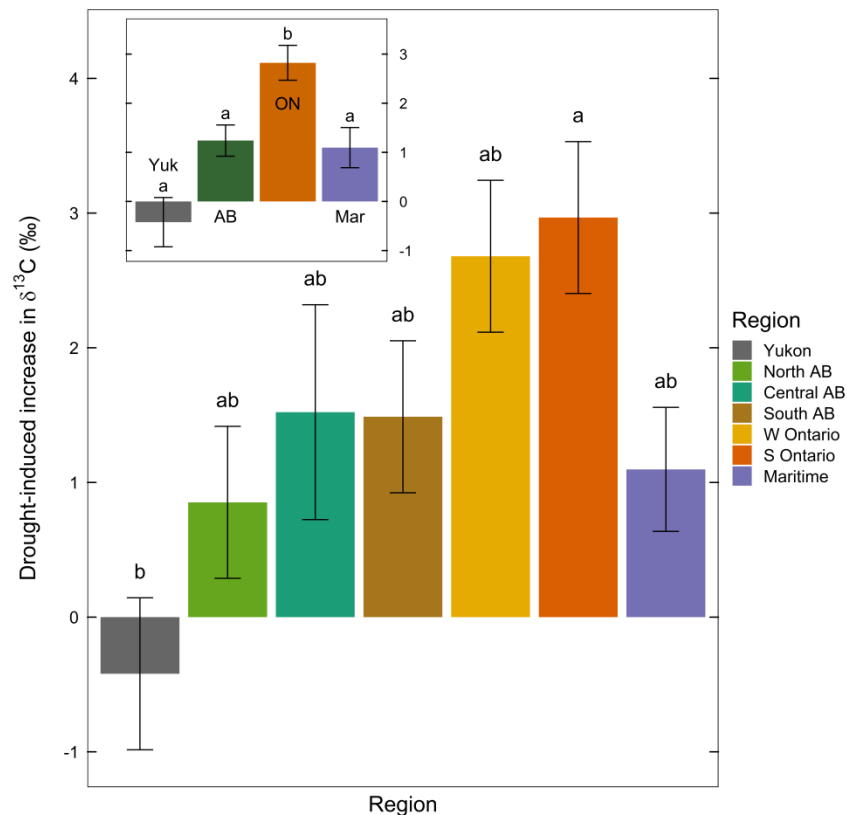


Figure 9. Drought-induced increase in $\delta^{13}\text{C}$ was measured as the difference in $\delta^{13}\text{C}$ between seedlings in the drought and control treatments. The inset represents the pooled means of Alberta regions, and pooled means of all Ontario regions, compared to Maritime and Yukon provenances to illustrate the significant difference between Ontario and other regions. Error bars represent standard error. Letters represent significant ($p < 0.05$) differences between regions.

3.3 Influence of Climate of Origin on Growth and Drought Traits

The observed drought-induced change in $\delta^{13}\text{C}$ was greatest in provenances representing intermediate to high levels of summer precipitation (Fig. 10A). Provenances representing lower (e.g., Yukon) and higher (Maritime) levels of summer precipitation exhibited smaller changes in

stomatal regulation. A positive linear relationship existed between the drought-induced change in $\delta^{13}\text{C}$ and the mean annual temperature of the provenances. Warmer temperatures were associated with a greater degree of stomatal regulation; i.e., plants from warmer regions tended to regulate water loss more strongly than plants originating from cooler provenances.

Climate of origin was also correlated with height growth in adult and seedling provenances trials (Table 2). For both adult trees and seedlings, warmer temperatures at the site of origin were strongly related to greater tree height. Unlike adult trees, seedling height strongly correlated with precipitation. For seedlings, both greater precipitation and higher temperatures were related to greater height. Similar to growth traits, temperature also correlated significantly with drought-induced change in $\delta^{13}\text{C}$. There were no correlations with 20% Fv/Fm.

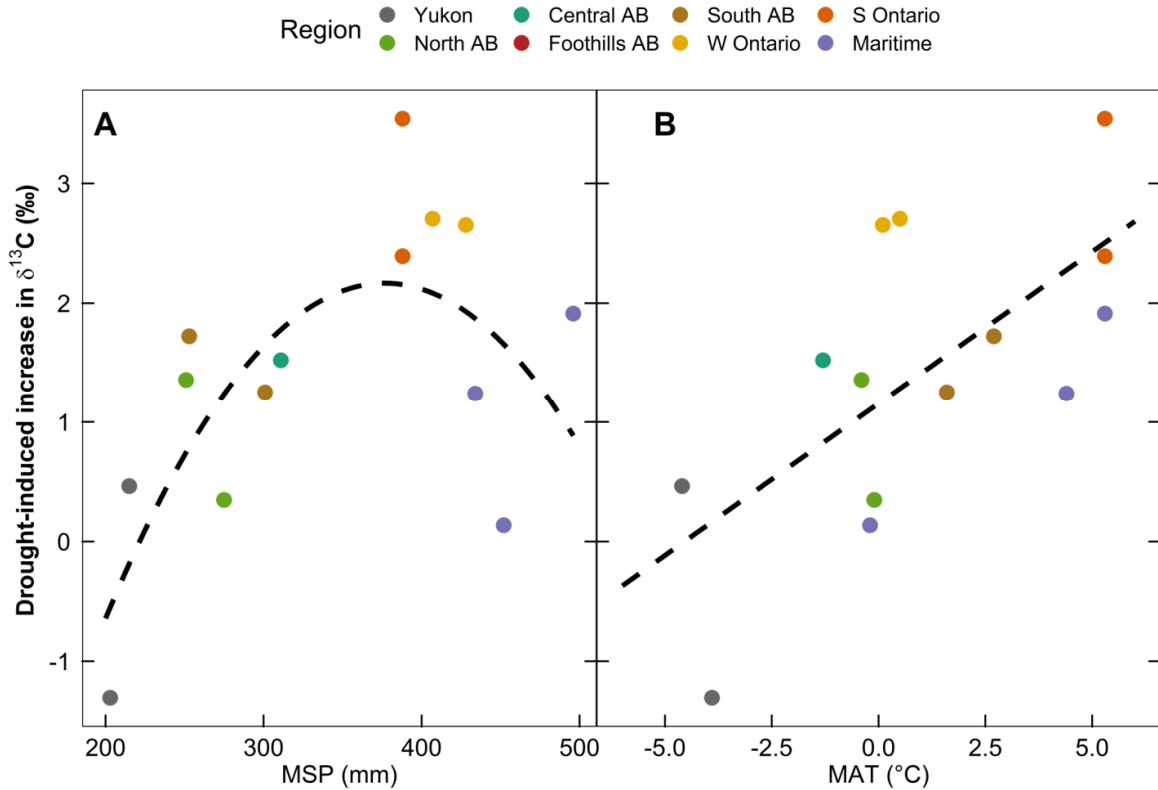


Figure 10. Drought-induced increase in stable carbon isotope ratio ($\delta^{13}\text{C}$, ‰) and the relationship to mean summer precipitation (MSP, mm) and mean annual temperature (MAT, °C). $\delta^{13}\text{C}$ and MSP exhibited a nonlinear relationship with equation $y = -9\text{E}05x^2 + 0.0678x - 10.602$ ($R^2 = 0.522$). $\delta^{13}\text{C}$ and MAT had a positive linear relationship explained by equations $y = 0.2545x + 1.1572$ ($R^2 = 0.4387$). Circles represent provenance means colored by region.

Table 2. Pearson correlation coefficients for mean values of provenances for adult and seedling height as growth variables, 20% Fv/Fm and increase in stable carbon isotope ratios ($\delta^{13}\text{C}$) as drought tolerance variables. 20% Fv/Fm was the volumetric water content (VWC) when plants reached 20% of their healthy Fv/Fm rate. Increase in $\delta^{13}\text{C}$ was the difference between drought and control $\delta^{13}\text{C}$. Statistically significant correlations at $p < .05$ are highlighted in bold.

Climate Variable	Adult Height	Seedling Control Height	20% Fv/Fm	Increase in $\delta^{13}\text{C}$
MAT	0.46	0.72	-0.16	0.66
DD>5	0.57	0.64	-0.20	0.75
MWMT	0.58	0.60	-0.19	0.77
FFP	0.53	0.68	-0.07	0.69
MCMT	0.11	0.56	-0.02	0.38
DD<0	-0.36	-0.64	0.13	-0.53
TD	0.08	-0.39	-0.05	-0.14
MAP	0.09	0.75	0.13	0.41
MSP	0.32	0.78	0.02	0.52
PAS	0.01	0.56	0.19	0.23
CMD	0.20	-0.46	-0.33	0.07
AHM	0.24	-0.49	-0.30	-0.12
SHM	-0.22	-0.62	-0.03	-0.31

Climate variables include mean annual temperature (MAT) in $^{\circ}\text{C}$; degree days above 5°C (DD>5), or growing degree days; mean warmest month temperature (MWMT) in $^{\circ}\text{C}$; frost free period (FFP) in number of days; mean coldest month temperature (MCMT) in $^{\circ}\text{C}$; degree days below 0°C (DD<0), or chilling degree days; temperature difference (TD) between MWMT and MCMT is a measure of continentality and is given in $^{\circ}\text{C}$; mean annual precipitation (MAP) is given in mm; mean summer precipitation (MSP) is given in mm; precipitation as snow (PAS) between August in previous year and July in current year is given in mm; climatic moisture deficit (CMD) is given in mm; annual heat moisture index (AHM); summer heat moisture index (SHM).

4. Discussion

4.1 Genetic variation of height growth in seedlings and adult trees

My first objective was to determine whether seedlings showed variation in growth under favorable conditions and whether this variation can be linked with source climate. Indeed, I found variation in the seedlings' growth potential; seedling height was positively correlated with temperature and precipitation at the seed origin. The superior growth potential of Ontario and Maritime provenances allows these seedlings to compete for sunlight and other resources under favorable conditions. By contrast, the slower growth of seedlings from colder and drier regions (Alberta and Yukon) may not be a disadvantage in habitats where long-term survival and growth is more constrained by abiotic stress than competition for light (Hartmann 2011).

In order to further investigate variations in growth rate, I investigated the performance of adult trees in long term provenance trials in north central Alberta. Regional growth differences in adult field trials of white spruce generally support previous suggestions that local provenances are best adapted to the home environment or test site (Gray et al. 2016b). Central Alberta, the closest region to the test site, had the highest growth, nearly doubling that of Yukon provenances. However, Ontario provenances were quite similar to the more local provenances despite the potential setbacks of a drier and colder climate. The faster growth of Ontario provenances at the seedling stage did not result in a height advantage in adult trees, although the height of adult trees originating from Ontario was comparable to the best performers from

Alberta. This indicates some maladaptation to central Alberta that is holding back growth of southern Ontario provenances. More exposure to cold or drought may be limiting Ontario provenances from outperforming local populations (Sebastian-Azcona et al. 2019).

Trees from the Yukon had the slowest growth of all the provenances, both at the seedling stage and in field trials. This is likely due to a number of factors, including an inherently short growing season. In their native environment, Yukon trees are constrained by a very early end of the growing season. This early termination of growth is likely a prerequisite for developing cold-tolerance in late summer (Sebastian-Azcona et al. 2019). There are likely other factors that constrain growth potential in Yukon trees. This notion is supported by the slow growth of seedlings in the greenhouse. In addition, the fact that adult trees from the Yukon only reached approximately half the height of trees originating from Alberta and Ontario suggests that Yukon trees invariably invest into features that confer resistance to abiotic stress, and that this comes at the cost of height growth.

Adult trees originating from Maritime provenances also exhibited sub-optimal growth, albeit to a smaller degree than Yukon trees. The growth reduction of Maritime trees in field trials can probably be explained by a lack of drought tolerance and cold hardiness. In their native environment, Maritime trees experience much warmer winter temperatures and more than double the MAP than in the central Alberta field trial.

Across all provenances, adult tree growth was positively correlated with source temperatures, but the correlation was weaker than in seedlings. Moreover, adult height was not correlated with precipitation metrics. Again, this can be partially explained by the putative lack of drought tolerance of Maritime provenances.

The performance of seedlings growing under favorable greenhouse conditions reflects the growth *potential* of genotypes and this turned out to be linked with climate parameters at the seed sources. However, forest managers are primarily concerned with the *actual* performance of adult trees in field trials and trends in actual field performance are much more complex than patterns of growth potential observed under ideal greenhouse conditions. Height growth in the field was not only influenced by the genetically determined growth potential of genotypes, but was constrained by the harsh climate of central Alberta, namely very cold winter temperatures, a short growing season, and limited moisture availability. It is therefore not surprising that the *actual* growth of adult trees was not a simple function of temperature and precipitation at the seed source. For instance, some of the Maritime provenances are characterized by high mean annual temperatures, but this did not translate into higher growth, for reasons outlined above.

4.2 Variability of drought tolerance traits between provenances

I next studied drought tolerance and asked the following questions: (1) Is drought tolerance a heritable trait in white spruce seedlings? (2) If so, how is genetic differentiation in drought tolerance linked to the climate at the seed origin? Previous research on white spruce provenances identified the onset of cold hardiness as an important adaptive trait that was associated with cold winter temperatures and latitude at the site of seed origin (Sebastian-Azcona et al. 2018, Sebastian-Azcona et al. 2019). I expected a weaker degree of heritability for drought tolerance than what has been reported for cold hardiness, for two reasons. First, cold hardiness in white spruce is a highly influential trait. It was negatively related to growth and positively to

survival (Sebastian-Azcona et al. 2019). This indicates a strong selection pressure to optimize the onset of cold hardiness in late summer/early fall. While drought tolerance could also be an important trait for white spruce (Gray et al. 2016a), the link with growth and survival may be less direct. Moreover, the “right” degree of drought tolerance may not be the subject of natural selection year after year (if droughts occur infrequently), contrary to cold hardiness. Second, drought tolerance is one of the most complex traits in plant biology (Moran et al. 2017) and is determined by multiple genes. Much remains to be learned about the genes controlling drought tolerance traits such as stomatal behavior, rooting patterns, aquaporin regulation, and xylem cavitation resistance.

Although many traits can be measured to assess drought tolerance, previous studies of white spruce have identified both $\delta^{13}\text{C}$ and Fv/Fm as potential areas of genetic differentiation, while wood anatomical and hydraulic traits showed very little populations differences (Sun et al. 1996, Bigras 2005, Sebastian-Azcona et al. 2018). For this reason, I chose to use carbon isotope ratios ($\delta^{13}\text{C}$) as a proxy for stomatal response, and the decline in maximal PSII photochemical efficiency (Fv/Fm), as a measure of the effects of drought on photosynthetic processes.

Under well-watered conditions, $\delta^{13}\text{C}$ was similar across provenances with the exception of Ontario provenances, which had slightly lower levels, indicative of greater discrimination. Variation in $\delta^{13}\text{C}$ is used as a proxy for stomatal behavior. Although it is possible that increased $\delta^{13}\text{C}$ may be the result of increased assimilation rates, in moisture-limited environments stomatal closure is likely the limiting factor and main contributor to $\delta^{13}\text{C}$ (Farquhar and Sharkey 1982, Chaves et al. 2003, McCarroll and Loader 2004). Higher (less negative) values of $\delta^{13}\text{C}$ indicate less discrimination against ^{13}C (McCarroll and Loader 2004), which can be primarily attributed

to stomatal closure. Therefore the lower $\delta^{13}\text{C}$ observed in Ontario provenances compared to other regions in the control group indicate more open stomata when under ideal growth conditions. The lower $\delta^{13}\text{C}$ values of trees with higher growth potential are similar to findings in *Pinus halepensis* (Voltas et al. 2015). This increase in stomatal conductance likely allows trees from warmer and wetter climates to take advantage of available resources by allowing increased gas exchange and photosynthesis. Importantly, Ontario provenances also exhibited the greatest increase in $\delta^{13}\text{C}$ under drought conditions. This drought-induced increase in $\delta^{13}\text{C}$ is consistent with isohydric behavior and a sensitive stomatal control of water loss. Moreover, provenances from Ontario and parts of Alberta were able to maintain PS II photochemistry (Fv/Fm) at low water content levels.

I therefore conclude that Ontario provenances are well suited to drought-prone environments despite the fact that they may experience less frequent and lower intensity droughts in their native environment than populations from Alberta. However, it should be noted that although inferred stomatal response and water-use efficiency seem to indicate high drought tolerance, they do not necessarily indicate increased drought survival (Zhang et al. 1997).

Seedlings from Yukon seed sources exhibited no increase in $\delta^{13}\text{C}$ under drought conditions, suggesting poor stomatal control of water loss. These seedlings also reached 20% of Fv/Fm at a relatively high soil water content; i.e. the decline in Fv/Fm occurred at a relatively early stage of drought. Despite previous research suggesting that cold tolerance may indicate increased drought tolerance (Bansal et al. 2016), Yukon provenances showed very little ability to withstand drought. In addition to being unable to cope with drought, northern populations may also be unable recover after drought (Isaac-Renton et al. 2018). The apparent inability of

northern populations to regulate water loss may lead to future maladaptation of these populations under predicted warming.

4.3 Trade-offs between growth potential and drought-tolerance

Many conifer species exhibit trade-offs between climate adaptation and growth traits. Trade-offs have been found between drought tolerance and growth in multiple conifer species (Bigras 2005, Montwe et al. 2015). Trees from dry environments may sacrifice high growth potential in favor of greater investment in traits that may provide hydraulic safety such as increased wood density and tracheid wall reinforcement (Hacke et al. 2001). In this study, I found no evidence for a trade-off between growth and drought tolerance across regions. The lack of expected trade-offs may indicate different adaptive strategies between white spruce populations. Provenances from sites with greater summer drought, or more extreme temperatures are generally less productive but may not show greater resistance to drought, they instead exhibit faster recovery from drought events (Montwe et al. 2015). Similarly, *Pinus pinaster* forest trees show variable adaptive strategies to drought, with those from wet climates being more resistant to short drought, but dry forests exhibiting faster growth recovery after extensive drought (Sanchez-Salguero et al. 2018). Fluorescence response may be used as an indicator of survival under drought, and an important indicator of drought tolerance. Those trees that lose the ability to photosynthesize earlier are also likely at risk of earlier mortality.

Seedlings from the Yukon had the slowest growth while also exhibiting poor stomatal control of water loss and a relatively early decline in F_v/F_m . This poor performance in growth

and drought response goes against the expected trade-off and may indicate that these provenances are not adapted to drought during their growing season. It is possible that they don't respond to drought conditions due to the extremely short growing season during which they grow quickly for a short amount of time and can't afford to close stomata.

A similar trend is seen in Alberta provenances which show little trade-off between drought and stomatal response. The stomatal response in these provenances would be expected to be greater because of their lower growth potential in greenhouse trials. In contrast, they were generally able to maintain photosynthesis levels at lower water contents, indicating some response to drought that may not be related to stomatal control. This may indicate an adaptation to drought that is less dependent on stomatal response and more dependent on their ability of their hydraulic structures to withstand extreme water potentials and recover from long droughts.

Seedlings from southern Ontario had a strong stomatal response to drought as indicated by their pronounced increase in $\delta^{13}\text{C}$ under drought conditions; yet they also had high growth rates. In addition to relatively strong stomatal response they also maintained photosynthesis at low water content, indicating the ability to survive during drought as well. Ontario provenances experience summer droughts that are shorter and less intense than the slower-growing provenances in Alberta, indicating that they may perform well in drought conditions but may recover slower than less productive provenances.

Maritime provenances have high growth performance in greenhouse trials but both adult field trials and seedling drought experiments indicate an inability to cope with water stress. They are likely not adapted to drought and show little ability to respond to water limitation. However, unlike the relatively low stomatal control exhibited in Alberta provenances the Maritimes

displayed less ability to continue photosynthesis at low water contents indicating they may not be adapted to longer droughts and could exhibit poor recovery and increased hydraulic damage.

4.4 Potential management implications

Future climate predictions suggest that more northern populations of white spruce are likely to see substantial declines in both survival and growth as heat and drought events continue to increase (Rweyongeza et al. 2007). Our experiments also indicate that Yukon provenances show maladaptation to warmer climates in central Alberta, indicating future warming in the north may induce similar maladaptation. Many forest populations are expected to be outside their optimal climate range by more than 300km latitudinally in the next decade, likely more in northern boreal populations (Gray and Hamann 2013). These risks posed by future climate changes indicate a need for seeds and planting stock better adapted to these climate situations. Provenances originating from southern Ontario, near the southern edge of the species range, show similar growth to some of these northern populations and better drought tolerance, making them a potentially suitable choice for seed transfers. Lu et al. (2014) found that provenances originating from similar regions also demonstrated superior growth, outperforming populations from western Canada and Alaska, suggesting they have the potential to be useful now for northern planting. Although these provenances seem to be well adapted for transfer to more northern sites there is still a risk of lower survival and growth due to less adaptation to frost events, and as such caution should be used (Sebastian-Azcona et al. 2019). Future research is needed to identify physiological mechanisms sensitive to drought in adult white spruce as adult trees and seedlings tend to exhibit different vulnerabilities.

4.5 Conclusions

The variation between populations at the seedling and adult stage indicates significant genetic differences that may be helpful for future management of white spruce stands. The experiments of drought tolerance in seedlings should be coupled with similar studies of adult trees. Carbon isotope ratios in the long-term field trials may give insight into the performance of different populations in variable climatic conditions. These studies would be highly valuable as seedlings are often more vulnerable and have potentially different reactions to drought than adult trees. Future research should aim at understanding the effects of drought on adult trees and future management of these adult stands.

5. References

- Abatzoglou, J. T., and A. P. Williams. 2016. Impact of anthropogenic climate change on wildfire across western US forests. *Proceedings of the National Academy of Sciences of the United States of America* **113**:11770-11775.
- Adams, H. D., M. Guardiola-Claramonte, G. A. Barron-Gafford, J. C. Villegas, D. D. Breshears, C. B. Zou, P. A. Troch, and T. E. Huxman. 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 of the United States of America* **106**:7063-7066.
- Adams, H. D., M. J. B. Zeppel, W. R. L. Anderegg, H. Hartmann, S. M. Landhausser, D. T. Tissue, T. E. Huxman, P. J. Hudson, T. E. Franz, C. D. Allen, L. D. L. Anderegg, G. A. Barron-Gafford, D. J. Beerling, D. D. Breshears, T. J. Brodribb, H. Bugmann, R. C. Cobb, A. D. Collins, L. T. Dickman, H. L. Duan, B. E. Ewers, L. Galiano, D. A. Galvez, N. Garcia-Forner, M. L. Gaylord, M. J. Germino, A. Gessler, U. G. Hacke, R. Hakamada, A. Hector, M. W. Jenkins, J. M. Kane, T. E. Kolb, D. J. Law, J. D. Lewis, J. M. Limousin, D. M. Love, A. K. Macalady, J. Martinez-Vilalta, M. Mencuccini, P. J. Mitchell, J. D. Muss, M. J. O'Brien, A. P. O'Grady, R. E. Pangle, E. A. Pinkard, F. I. Piper, J. A. Plaut, W. T. Pockman, J. Quirk, K. Reinhardt, F. Ripullone, M. G. Ryan, A. Sala, S. Sevanto, J. S. Sperry, R. Vargas, M. Vennetier, D. A. Way, C. G. Xu, E. A. Yezpez, and N. G. McDowell. 2017. A multi-species synthesis of physiological mechanisms in drought-induced tree mortality. *Nature Ecology & Evolution* **1**:1285-1291.
- Allen, C. D., A. K. Macalady, H. Chenchouni, D. Bachelet, N. McDowell, M. Vennetier, T. Kitzberger, A. Rigling, D. D. Breshears, E. H. Hogg, P. Gonzalez, R. Fensham, Z. Zhang, J. Castro, N. Demidova, J. H. Lim, G. Allard, S. W. Running, A. Semerci, and N. Cobb. 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and Management* **259**:660-684.
- Anderegg, W. R. L., J. M. Kane, and L. D. L. Anderegg. 2013. Consequences of widespread tree mortality triggered by drought and temperature stress. *Nature Climate Change* **3**:30-36.
- Avramova, V., A. Meziane, E. Bauer, S. Blankenagel, S. Eggels, S. Gresset, E. Grill, C. Niculaes, M. Ouzunova, B. Poppenberger, T. Presterl, W. Rozhon, C. Welcker, Z. Y. Yang, F. Tardieu, and C. C. Schon. 2019. Carbon isotope composition, water use efficiency, and drought sensitivity are controlled by a common genomic segment in maize. *Theoretical and Applied Genetics* **132**:53-63.
- Bansal, S., C. A. Harrington, and J. B. St Clair. 2016. Tolerance to multiple climate stressors: a case study of Douglas-fir drought and cold hardiness. *Ecology and Evolution* **6**:2074-2083.

- Barnett, T. P., J. C. Adam, and D. P. Lettenmaier. 2005. Potential impacts of a warming climate on water availability in snow-dominated regions. *Nature* **438**:303-309.
- Berg, E. E., J. D. Henry, C. L. Fastie, A. D. De Volder, and S. M. Matsuoka. 2006. Spruce beetle outbreaks on the Kenai Peninsula, Alaska, and Kluane National Park and Reserve, Yukon Territory: Relationship to summer temperatures and regional differences in disturbance regimes. *Forest Ecology and Management* **227**:219-232.
- Bigras, F. J. 2005. Photosynthetic response of white spruce families to drought stress. *New Forests* **29**:135-148.
- Bonan, G. B. 2008. Forests and climate change: Forcings, feedbacks, and the climate benefits of forests. *Science* **320**:1444-1449.
- Breshears, D. D., N. S. Cobb, P. M. Rich, K. P. Price, C. D. Allen, R. G. Balice, W. H. Romme, J. H. Kastens, M. L. Floyd, J. Belnap, J. J. Anderson, O. B. Myers, and C. W. Meyer. 2005. Regional vegetation die-off in response to global-change-type drought. *Proceedings of the National Academy of Sciences of the United States of America* **102**:15144-15148.
- Brienen, R. J. W., W. Wanek, and P. Hietz. 2011. Stable carbon isotopes in tree rings indicate improved water use efficiency and drought responses of a tropical dry forest tree species. *Trees-Structure and Function* **25**:103-113.
- Brodribb, T. J., S. A. M. McAdam, G. J. Jordan, and S. C. V. Martins. 2014. Conifer species adapt to low-rainfall climates by following one of two divergent pathways. *Proceedings of the National Academy of Sciences of the United States of America* **111**:14489-14493.
- Carnicer, J., M. Coll, M. Ninyerola, X. Pons, G. Sanchez, and J. Penuelas. 2011. Widespread crown condition decline, food web disruption, and amplified tree mortality with increased climate change-type drought. *Proceedings of the National Academy of Sciences of the United States of America* **108**:1474-1478.
- Charney, N. D., F. Babst, B. Poulter, S. Record, V. M. Trouet, D. Frank, B. J. Enquist, and M. E. K. Evans. 2016. Observed forest sensitivity to climate implies large changes in 21st century North American forest growth. *Ecology Letters* **19**:1119-1128.
- Chaves, M. M., J. Flexas, and C. Pinheiro. 2009. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. *Annals of Botany* **103**:551-560.
- Chaves, M. M., J. P. Maroco, and J. S. Pereira. 2003. Understanding plant responses to drought - from genes to the whole plant. *Functional Plant Biology* **30**:239-264.
- Chhin, S., and G. G. Wang. 2008. Climatic response of *Picea glauca* seedlings in a forest-prairie ecotone of western Canada. *Annals of Forest Science* **65**:Artn 207, 210.1051/Forest:2007090.

- Chhin, S., and G. G. Wang. 2016. Climatic Sensitivity of a Mixed Forest Association of White Spruce and Trembling Aspen at Their Southern Range Limit. *Forests* **7**:Artn 235, 210.3390/F7100235.
- Chhin, S., G. G. Wang, and J. Tardif. 2004. Dendroclimatic analysis of white spruce at its southern limit of distribution in the Spruce Woods Provincial Park, Manitoba, Canada. *Tree-Ring Research* **60**:31-43.
- Condon, A. G., R. A. Richards, G. J. Rebetzke, and G. D. Farquhar. 2004. Breeding for high water-use efficiency. *Journal of Experimental Botany* **55**:2447-2460.
- Conroy, J. S., RM; Koppers, M; Bevege, DI; Barlow, EW. 1986. Chlorophyll a Fluorescence and Photosynthetic and Growth Responses of *Pinus radiata* to Phosphorus Deficiency, Drought Stress, and High CO₂. *Plant Physiology* **81**:423-429.
- Cook, B. I., J. E. Smerdon, R. Seager, and S. Coats. 2014. Global warming and 21st century drying. *Climate Dynamics* **43**:2607-2627.
- Csank, A. Z., A. E. Miller, R. L. Sherriff, E. E. Berg, and J. M. Welker. 2016. Tree-ring isotopes reveal drought sensitivity in trees killed by spruce beetle outbreaks in south-central Alaska. *Ecological Applications* **26**:2001-2020.
- D'Orangeville, L., L. Duchesne, D. Houle, D. Kneeshaw, B. Cote, and N. Pederson. 2016. Northeastern North America as a potential refugium for boreal forests in a warming climate. *Science* **352**:1452-1455.
- D'Orangeville, L., D. Houle, L. Duchesne, R. P. Phillips, Y. Bergeron, and D. Kneeshaw. 2018. Beneficial effects of climate warming on boreal tree growth may be transitory. *Nature Communications* **9**:Artn 3213, 3210.1038/S41467-41018-05705-41464.
- de Groot, W. J., M. D. Flannigan, and A. S. Cantin. 2013. Climate change impacts on future boreal fire regimes. *Forest Ecology and Management* **294**:35-44.
- Demmig, B., and O. Bjorkman. 1987. Comparison of the Effect of Excessive Light on Chlorophyll Fluorescence (77k) and Photon Yield of O₂ Evolution in Leaves of Higher-Plants. *Planta* **171**:171-184.
- Dhanapal, A. P., J. D. Ray, S. K. Singh, V. Hoyos-Villegas, J. R. Smith, L. C. Purcell, C. A. King, P. B. Cregan, Q. J. Song, and F. B. Fritschi. 2015. Genome-wide association study (GWAS) of carbon isotope ratio (delta C-13) in diverse soybean [*Glycine max* (L.) Merr.] genotypes (vol 128, pg 73, 2015). *Theoretical and Applied Genetics* **128**:375-376.
- Diffenbaugh, N. S., D. L. Swain, and D. Touma. 2015. Anthropogenic warming has increased drought risk in California. *Proceedings of the National Academy of Sciences of the United States of America* **112**:3931-3936.

- Ditmarova, L., D. Kurjak, S. Palmroth, J. Kmet, and K. Strelcova. 2010. Physiological responses of Norway spruce (*Picea abies*) seedlings to drought stress. *Tree Physiology* **30**:205-213.
- Farquhar, G. D., J. R. Ehleringer, and K. T. Hubick. 1989. Carbon Isotope Discrimination and Photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* **40**:503-537.
- Farquhar, G. D., and T. D. Sharkey. 1982. Stomatal Conductance and Photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* **33**:317-345.
- Fensham, R. J., and J. E. Holman. 1999. Temporal and spatial patterns in drought-related tree dieback in Australian savanna. *Journal of Applied Ecology* **36**:1035-1050.
- Gray, L. K., and A. Hamann. 2013. Tracking suitable habitat for tree populations under climate change in western North America. *Climatic Change* **117**:289-303.
- Gray, L. K., A. Hamann, S. John, D. Rweyongeza, L. Barnhardt, and B. R. Thomas. 2016a. Climate change risk management in tree improvement programs: selection and movement of genotypes. *Tree Genetics & Genomes* **12**:ARTN 23, 10.1007/s11295-11016-10983-11291.
- Gray, L. K., D. Rweyongeza, A. Hamann, S. John, and B. R. Thomas. 2016b. Developing management strategies for tree improvement programs under climate change: Insights gained from long-term field trials with lodgepole pine. *Forest Ecology and Management* **377**:128-138.
- Guy, R. D., and D. L. Holowachuk. 2001. Population differences in stable carbon isotope ratio of *Pinus contorta* Dougl. ex Loud.: relationship to environment, climate of origin, and growth potential. *Canadian Journal of Botany-Revue Canadienne De Botanique* **79**:274-283.
- Hacke, U. G., J. S. Sperry, W. T. Pockman, S. D. Davis, and K. A. McCulloch. 2001. Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. *Oecologia* **126**:457-461.
- Hansen, W. D., and M. G. Turner. 2019. Origins of abrupt change? Postfire subalpine conifer regeneration declines nonlinearly with warming and drying. *Ecological Monographs* **89**:e01340.
- Hartmann, D. L., A. M. G. K. Tank, M. Rusticucci, L. V. Alexander, S. Bronnimann, Y. A. R. Charabi, F. J. Dentener, E. J. Dlugokencky, D. R. Easterling, A. Kaplan, B. J. Soden, P. W. Thorne, M. Wild, P. M. Zhai, R. Adler, R. Allan, R. Allan, D. Blake, O. Cooper, A. G. Dai, R. Davis, S. Davis, M. Donat, V. Fioletov, E. Fischer, L. Haimberger, B. Ho, J. Kennedy, E. Kent, S. Kinne, J. Kossin, N. Loeb, C. Mears, C. Merchant, S. Montzka, C. Morice, C. L. Myhre, J. Norris, D. Parker, B. Randel, A. Richter, M. Rigby, B. Santer, D. Seidel, T. Smith, D. Stephenson, R. Teuling, J. H. Wang, X. L. Wang, R. Weiss, K.

- Willett, and S. Wood. 2014. Observations: Atmosphere and Surface. *Climate Change 2013: The Physical Science Basis*:159-254.
- Hartmann, H. 2011. Will a 385 million year-struggle for light become a struggle for water and for carbon? - How trees may cope with more frequent climate change-type drought events. *Global Change Biology* **17**:642-655.
- Hogg, E. H., A. G. Barr, and T. A. Black. 2013. A simple soil moisture index for representing multi-year drought impacts on aspen productivity in the western Canadian interior. *Agricultural and Forest Meteorology* **178**:173-182.
- Hogg, E. H., M. Michaelian, T. I. Hook, and M. E. Undershultz. 2017. Recent climatic drying leads to age-independent growth reductions of white spruce stands in western Canada. *Global Change Biology* **23**:5297-5308.
- IPCC. 2014. *Climate Change 2014: Impacts, Adaptation, and Vulnerability, Pt A: Global and Sectoral Aspects*. *Climate Change 2014: Impacts, Adaptation, and Vulnerability, Pt A: Global and Sectoral Aspects*:1-1131.
- Isaac-Renton, M., D. Montwe, A. Hamann, H. Spiecker, P. Cherubini, and K. Treydte. 2018. Northern forest tree populations are physiologically maladapted to drought. *Nature Communications* **9**:Artn 5254, 5210.1038/S41467-41018-07701-41460.
- Jefferson, M. 2015. IPCC fifth assessment synthesis report: "Climate change 2014: Longer report": Critical analysis. *Technological Forecasting and Social Change* **92**:362-363.
- Knutzen, F., I. C. Meier, and C. Leuschner. 2015. Does reduced precipitation trigger physiological and morphological drought adaptations in European beech (*Fagus sylvatica* L.)? Comparing provenances across a precipitation gradient. *Tree Physiology* **35**:949-963.
- Krause, G., Jahns, P. 2003. Pulse Amplitude Modulated Chlorophyll Fluorometry and its Application in Plant Science. . Pages 373-399 in P. W. Green BR, editor. *Light-Harvesting Antennas in Photosynthesis*. Springer.
- Lu, P. X., W. H. Parker, M. Cherry, S. Colombo, W. C. Parker, R. Z. Man, and N. Roubal. 2014. Survival and growth patterns of white spruce (*Picea glauca* [Moench] Voss) rangewide provenances and their implications for climate change adaptation. *Ecology and Evolution* **4**:2360-2374.
- Maxwell, K., and G. N. Johnson. 2000. Chlorophyll fluorescence - a practical guide. *Journal of Experimental Botany* **51**:659-668.
- McCarroll, D., and N. J. Loader. 2004. Stable isotopes in tree rings. *Quaternary Science Reviews* **23**:771-801.

- McDowell, N., W. T. Pockman, C. D. Allen, D. D. Breshears, N. Cobb, T. Kolb, J. Plaut, J. Sperry, A. West, D. G. Williams, and E. A. Yezpez. 2008. Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytologist* **178**:719-739.
- McDowell, N. G. 2011. Mechanisms Linking Drought, Hydraulics, Carbon Metabolism, and Vegetation Mortality. *Plant Physiology* **155**:1051-1059.
- McDowell, N. G., and S. Sevanto. 2010. The mechanisms of carbon starvation: how, when, or does it even occur at all? *New Phytologist* **186**:264-266.
- Michaelian, M., E. H. Hogg, R. J. Hall, and E. Arsenault. 2011. Massive mortality of aspen following severe drought along the southern edge of the Canadian boreal forest. *Global Change Biology* **17**:2084-2094.
- Mohammed, G. H., W. D. Binder, and S. L. Gillies. 1995. Chlorophyll Fluorescence - a Review of Its Practical Forestry Applications and Instrumentation. *Scandinavian Journal of Forest Research* **10**:383-410.
- Montwe, D., H. Spiecker, and A. Hamann. 2015. Five decades of growth in a genetic field trial of Douglas-fir reveal trade-offs between productivity and drought tolerance. *Tree Genetics & Genomes* **11**:ARTN 29, 10.1007/s11295-11015-10854-11291.
- Moran, E., J. Lauder, C. Musser, A. Stathos, and M. Shu. 2017. The genetics of drought tolerance in conifers. *New Phytologist* **216**:1034-1048.
- Morgenstern, E. K. 1996. Geographic variation in forest trees : genetic basis and application of knowledge in silviculture. UBC Press, Vancouver.
- Murchie, E. H., and T. Lawson. 2013. Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. *Journal of Experimental Botany* **64**:3983-3998.
- Passioura, J. B. 1996. Drought and drought tolerance. *Plant Growth Regulation* **20**:79-83.
- Peng, C. H., Z. H. Ma, X. D. Lei, Q. Zhu, H. Chen, W. F. Wang, S. R. Liu, W. Z. Li, X. Q. Fang, and X. L. Zhou. 2011. A drought-induced pervasive increase in tree mortality across Canada's boreal forests. *Nature Climate Change* **1**:467-471.
- Phillips, O. L., L. E. O. C. Aragao, S. L. Lewis, J. B. Fisher, J. Lloyd, G. Lopez-Gonzalez, Y. Malhi, A. Monteagudo, J. Peacock, C. A. Quesada, G. van der Heijden, S. Almeida, I. Amaral, L. Arroyo, G. Aymard, T. R. Baker, O. Banki, L. Blanc, D. Bonal, P. Brando, J. Chave, A. C. A. de Oliveira, N. D. Cardozo, C. I. Czimczik, T. R. Feldpausch, M. A. Freitas, E. Gloor, N. Higuchi, E. Jimenez, G. Lloyd, P. Meir, C. Mendoza, A. Morel, D. A. Neill, D. Nepstad, S. Patino, M. C. Penuela, A. Prieto, F. Ramirez, M. Schwarz, J. Silva, M. Silveira, A. S. Thomas, H. ter Steege, J. Stropp, R. Vasquez, P. Zelazowski, E.

- A. Davila, S. Andelman, A. Andrade, K. J. Chao, T. Erwin, A. Di Fiore, E. Honorio, H. Keeling, T. J. Killeen, W. F. Laurance, A. P. Cruz, N. C. A. Pitman, P. N. Vargas, H. Ramirez-Angulo, A. Rudas, R. Salamao, N. Silva, J. Terborgh, and A. Torres-Lezama. 2009. Drought Sensitivity of the Amazon Rainforest. *Science* **323**:1344-1347.
- Price, D. T., R. I. Alfaro, K. J. Brown, M. D. Flannigan, R. A. Fleming, E. H. Hogg, M. P. Girardin, T. Lakusta, M. Johnston, D. W. McKenney, J. H. Pedlar, T. Stratton, R. N. Sturrock, I. D. Thompson, J. A. Trofymow, and L. A. Venier. 2013. Anticipating the consequences of climate change for Canada's boreal forest ecosystems. *Environmental Reviews* **21**:322-365.
- Rose, L., C. Leuschner, B. Kockemann, and H. Buschmann. 2009. Are marginal beech (*Fagus sylvatica* L.) provenances a source for drought tolerant ecotypes? *European Journal of Forest Research* **128**:335-343.
- Rweyongeza, D. M. 2011. Pattern of genotype-environment interaction in *Picea glauca* (Moench) Voss in Alberta, Canada. *Annals of Forest Science* **68**:245-253.
- Rweyongeza, D. M., R. C. Yang, N. K. Dhir, L. K. Barnhardt, and C. Hansen. 2007. Genetic variation and climatic impacts on survival and growth of white spruce in Alberta, Canada. *Silvae Genetica* **56**:117-127.
- Sala, A., F. Piper, and G. Hoch. 2010. Physiological mechanisms of drought-induced tree mortality are far from being resolved. *New Phytologist* **186**:274-281.
- Sanchez-Salguero, R., J. J. Camarero, V. Rozas, M. Genova, J. M. Olano, A. Arzac, A. Gazol, L. Caminero, E. Tejedor, M. de Luis, and J. C. Linares. 2018. Resist, recover or both? Growth plasticity in response to drought is geographically structured and linked to intraspecific variability in *Pinus pinaster*. *Journal of Biogeography* **45**:1126-1139.
- Sayed, O. H. 2003. Chlorophyll fluorescence as a tool in cereal crop research. *Photosynthetica* **41**:321-330.
- Schreiber, S. G., A. Hamann, U. G. Hacke, and B. R. Thomas. 2013. Sixteen years of winter stress: an assessment of cold hardiness, growth performance and survival of hybrid poplar clones at a boreal planting site. *Plant Cell and Environment* **36**:419-428.
- Schreiber, U. 2004. Pulse-Amplitude-Modulated (PAM) fluorometry and saturation pulse method: An Overview. Pages 279-319 in G. Papageorgiou GC, editor. *Chlorophyll a Fluorescence: A Signature of Photosynthesis*. Springer, Dordrecht, Netherlands.
- Sebastian-Azcona, J., U. G. Hacke, and A. Hamann. 2018. Adaptations of white spruce to climate: strong intraspecific differences in cold hardiness linked to survival. *Ecology and Evolution* **8**:1758-1768.

- Sebastian-Azcona, J., A. Hamann, U. G. Hacke, and D. Rweyongeza. 2019. Survival, growth and cold hardiness tradeoffs in white spruce populations: Implications for assisted migration. *Forest Ecology and Management* **433**:544-552.
- Sevanto, S., N. G. McDowell, L. T. Dickman, R. Pangle, and W. T. Pockman. 2014. How do trees die? A test of the hydraulic failure and carbon starvation hypotheses. *Plant Cell and Environment* **37**:153-161.
- Sperry, J. S., F. R. Adler, G. S. Campbell, and J. P. Comstock. 1998. Limitation of plant water use by rhizosphere and xylem conductance: results from a model. *Plant Cell and Environment* **21**:347-359.
- Sun, Z. J., N. J. Livingston, R. D. Guy, and G. J. Ethier. 1996. Stable carbon isotopes as indicators of increased water use efficiency and productivity in white spruce (*Picea glauca* (Moench) Voss) seedlings. *Plant Cell and Environment* **19**:887-894.
- Tardieu, F. 2005. Plant tolerance to water deficit: physical limits and possibilities for progress. *Comptes Rendus Geoscience* **337**:57-67.
- Trenberth, K. E. 2011. Changes in precipitation with climate change. *Climate Research* **47**:123-138.
- Voltas, J., D. Lucabaugh, M. R. Chambel, and J. P. Ferrio. 2015. Intraspecific variation in the use of water sources by the circum-Mediterranean conifer *Pinus halepensis*. *New Phytologist* **208**:1031-1041.
- Wang, T. L., A. Hamann, D. Spittlehouse, and C. Carroll. 2016. Locally Downscaled and Spatially Customizable Climate Data for Historical and Future Periods for North America. *Plos One* **11**:ARTN e0156720, 0156710.0151371/journal.pone.0156720.
- Warren, C. R., J. F. McGrath, and M. A. Adams. 2001. Water availability and carbon isotope discrimination in conifers. *Oecologia* **127**:476-486.
- Zhang, J. W., Z. Feng, B. M. Cregg, and C. M. Schumann. 1997. Carbon isotopic composition, gas exchange, and growth of three populations of ponderosa pine differing in drought tolerance. *Tree Physiology* **17**:461-466.

6. Appendices

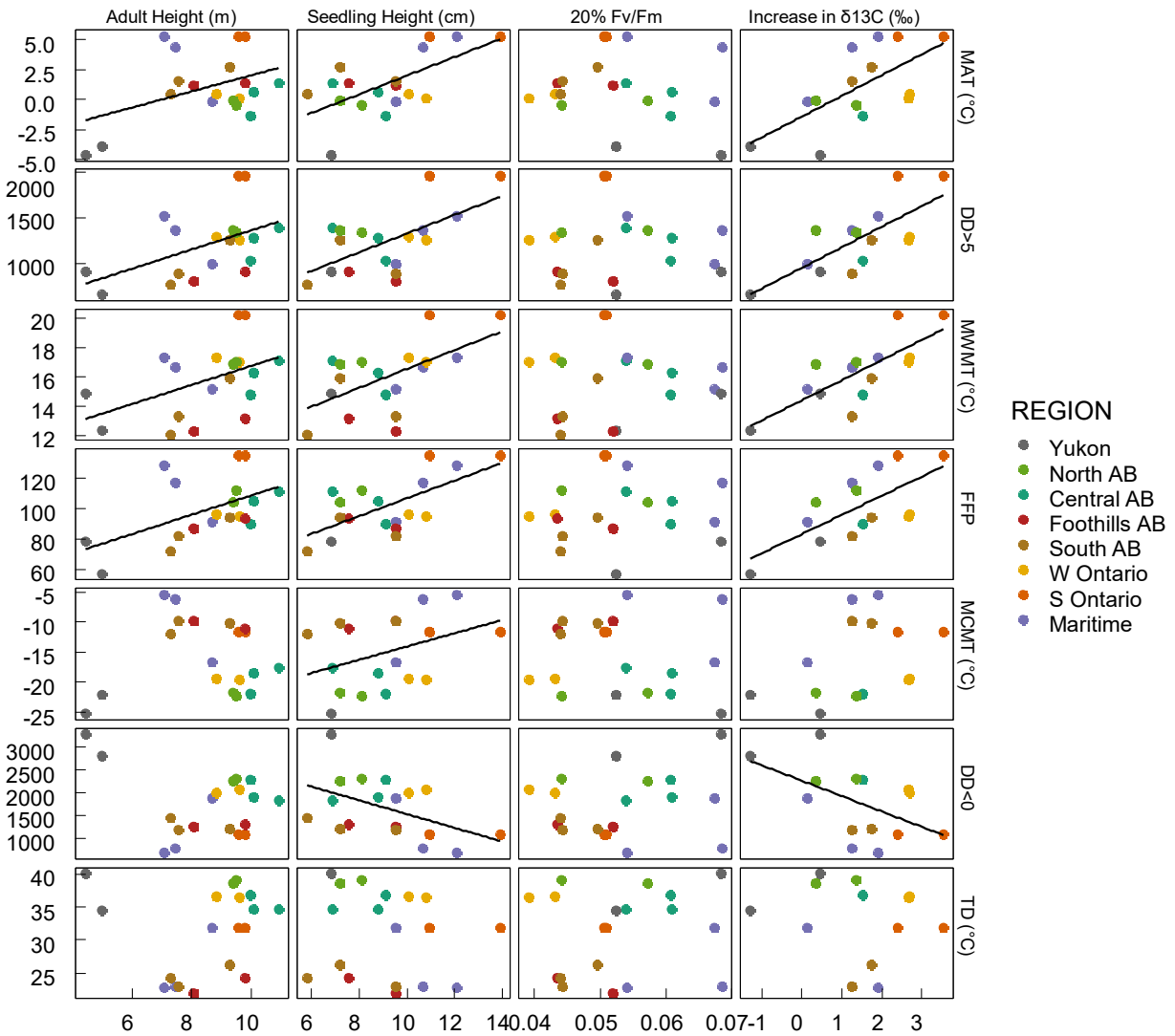


Figure S1. Scatterplots of correlations with significant relationships given lines. Climate variables include mean annual temperature (MAT) in °C; degree days above 5°C (DD>5), or growing degree days; mean warmest month temperature (MMWT) in °C; frost free period (FFP) in number of days; mean coldest month temperature (MCMT) in °C; degree days below 0°C (DD<0), or chilling degree days; temperature difference (TD) between MMWT and MCMT is a measure of continentality and is given in °C.

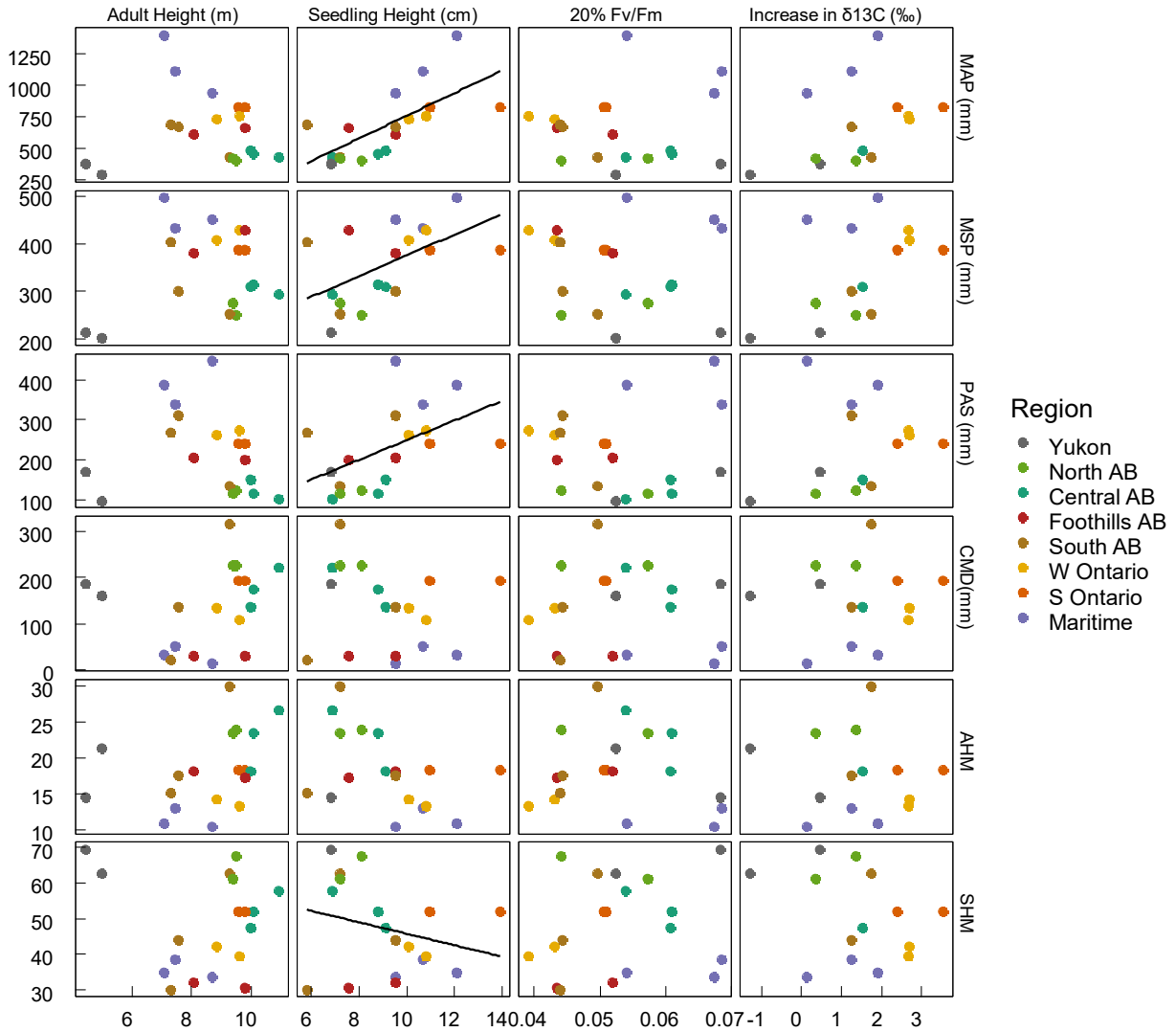


Figure S2. Scatterplots of correlations with significant relationships given lines. Climate variables include mean annual precipitation (MAP) is given in mm; mean summer precipitation (MSP) is given in mm; precipitation as snow (PAS) between August in previous year and July in current year is given in mm; climatic moisture deficit (CMD) is given in mm; annual heat moisture index (AHM); summer heat moisture index (SHM).