

Tree population responses to extreme climate events to guide
reforestation under climate change

by

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Abstract

As climates warm and extreme climatic events occur with more frequency and severity, maintaining forest health and productivity may involve planting seed sources from warmer, drier areas. To guide such reforestation strategies, this work analyzes the risks of both climate change and assisted migration seed transfers using methods from dendrochronology and ecological genetics. Inter-annual growth, drought and frost signatures are studied in tree-ring records from a large provenance trial for lodgepole pine (*Pinus contorta* Dougl. ex Loud.) in western North America.

Results show differences in growth among tree populations to warming, drought and cold under a variety of seed transfer distances. Genotypes from the far south of the range show high drought tolerance, which appears linked to an anisohydric strategy relying on cavitation-resistant xylem. These populations, however, are sensitive to cold and may therefore not be suitable candidates for large northward seed transfers under assisted migration; transfers should be limited to shorter distances. Populations from the central part of the species range are productive, which may be explained by an isohydric strategy related to large xylem conduits. Central populations nevertheless achieve moderate drought tolerance due to lowered inferred stomatal conductance under dry conditions and may therefore show some continued resilience.

When grown in southern planting sites, northern genotypes show low drought tolerance as well as susceptibility to spring cold events. Their responses to extreme events indicate that growth benefits from warming may be limited, and that northern boreal forests may be at risk due to increasing climatic variability. Northern areas may therefore benefit from judicious seed

transfer from central interior areas. When central interior seed sources are grown in northern environments, they show consistently competitive annual growth relative to local sources, indicating suitability of this possible solution. Generally, however, the intra-specific variation in multiple growth and physiological traits suggest that risks of both climate change and assisted migration vary across species distributions. A cautious and population-oriented approach is recommended for climate-based seed transfer to maintain forest growth and resilience under global change.

Preface

Intellectual Contributions to Publications and Chapters

A version of Chapter 3 has been published as: Montwé D, **Isaac-Renton M**, Hamann A, Spiecker H (2016) Drought tolerance and growth in populations of a wide-ranging tree species indicate climate change risks for the boreal north. *Global Change Biology*, **22**(2):806-815. The idea was conceived by AH with contributions from DM and HS. Study objectives were developed by DM and MIR. Experimental design developed by DM and MIR with input from AH. Statistical analyses were led by DM with input from MIR. The manuscript was prepared equally by DM and MIR and edited by AH and HS.

A version of Chapter 4 has been published as: **Isaac-Renton M**, Treydte K, Schneider L (2016) Contamination risk of stable isotope samples during milling. *Rapid Communications in Mass Spectrometry*, **30**(13):1513-1522. KT conceived the idea. With input from KT and LS, MIR led the study design, performed the analyses and wrote the manuscript.

Chapter 5 has been published as: Montwé D, **Isaac-Renton M**, Hamann A, Spiecker H (2015) Using steam to reduce artifacts in micro sections prepared with corn starch. *Dendrochronologia*, **35**:87-90. The study was conceived and designed by DM with input from MIR. DM prepared the manuscript with input from MIR. AH and HS edited the manuscript.

A version of Chapter 6 will be submitted to a peer-reviewed journal as: **Isaac-Renton M**, Montwé D, Hamann A, Spiecker H, Cherubini P, Treydte K (2017) North American pine populations are limited in their physiological adaptation to drought. AH outlined an idea to use isotope and wood anatomy methodologies in a grant. The scientific aim behind the approach as well as specific study objectives were developed by MIR and DM. The study was led by MIR,

and designed by MIR and DM with guidance by KT and input from all co-authors. The manuscript was prepared by MIR with input from DM and KT and edits from all co-authors.

A version of Chapter 7 has been submitted as: Montwé D & **Isaac-Renton M**, Hamann A, Spiecker H (2017) Minimizing risk of assisted migration to address climate change: cold adaptation recorded in tree rings. DM and MIR are co-first authors who developed the idea together. DM and MIR then jointly designed and led the project, conducted statistical analyses and prepared the manuscript. AH provided input into statistical aspects and edited the manuscript. HS edited the manuscript.

A version of Chapter 8 will be submitted, possibly as: Assisted migration and status quo reforestation in a wide-ranging North American pine: population-specific strategies needed for climate change. Inspired in part from a grant to AH, MIR developed the study with input from DM. To date, statistical analyses and manuscript preparation was conducted by MIR with input from DM. Future work and other possible contributions and collaborations may follow.

Other Research Contributions

Successful research requires non-academic work that complements intellectual contributions. While it was not feasible to outsource some basic work, hiring student assistants made the research more efficient, facilitating academic work and student training in research. MIR hired, trained, managed and supervised 2 student field assistants, with input and co-supervision by DM in 2014. DM hired, trained, managed and supervised 7 laboratory assistants, with input and co-supervision by MIR.

The first 6-month field season in 2013 was organized by MIR with input from DM; the second 6-month field season in 2014 was organized jointly by MIR and DM. Field equipment

was provided by AH, HS and MIR. Felling, tree measurements and sampling were conducted equally by MIR and DM, with 1-week contributions by HS in both years. With input from DM, MIR arranged logistics of 3 major sample shipments to collaborating laboratories.

Laboratory facilities, equipment and advice were provided by HS at the Chair of Forest Growth and Dendroecology at the University of Freiburg. Laboratory work in Freiburg by MIR involved sample preparation for tree-ring width analyses intermittently from 2014 to 2016. MIR established collaborations with KT and PC at the Swiss Federal Institute for Forest, Snow and Landscape Research (WSL). With input from these partners and working in their laboratory facilities in 2015, MIR: performed quality improvements tests and processed tree-rings for stable isotope analyses. MIR compared mass spectrometry laboratories to obtain best value and arranged related logistics and administration.

Project funding from NSERC covered research costs. MIR managed the research budget from 2013 to 2015 (~\$90,000). AH developed another NSERC grant from the idea of MIR and DM (Chapter 7), which was used to fund other laboratory members. MIR acquired scholarships for self-financing. Research grant funding for field and laboratory costs was also provided by a grant from the Deutsche Forschungsgemeinschaft (DFG) to HS and DM.

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Collaborators

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Table of Contents

Abstract	ii
Preface	iv
Intellectual Contributions to Publications and Chapters.....	iv
Other Research Contributions.....	v
Acknowledgements	vii
Collaborators.....	vii
Academic, Field and Laboratory Assistance	ix
Technical and Logistical Support.....	x
Administrative Support.....	xi
Funders	xii
Personal Support and Camaraderie.....	xii
Table of Contents.....	xiv
List of Tables	xxii
List of Figures	xxiv
List of Symbols.....	xxvii
Glossary of Terms	xxviii
1 Introduction	1
1.1 Thesis Themes	1
1.2 Thesis Objective and Motivation.....	3
1.3 Research Approach.....	5

Provenance Trials.....	5
Tree Rings: Growth and Inferred Physiology.....	6
1.4 Thesis Organization.....	8
1.5 References.....	10
2 Background Information.....	13
2.1 Magnitude and Pace of Global Change.....	13
General Warming.....	13
Increasing Probability of Extremes.....	14
Greenhouse Effect and Atmospheric Carbon.....	15
2.2 Tree Growth and Climate Change.....	17
Measures of Forest Productivity.....	17
Forest and Lodgepole Pine Growth under Climate Change.....	18
2.3 Forest Tree Populations in a Warmer Climate.....	19
Genetic Adaptation of Forests to Local Environments.....	19
Genetic Maladaptation under Climate Warming.....	20
2.4 Forests and Extreme Climatic Events.....	22
Maladaptation to Extreme Climatic Events: Discussing Drought.....	22
Maladaptation to Extreme Climatic Events: Cold Considerations.....	24
2.5 Interacting Stressors: Fire, Pests and Pathogens.....	25
Changing Fire Regimes.....	25
Lodgepole Pine Forest Pests and Pathogens.....	26
Cumulative Stresses.....	27
2.6 Human-Assisted Adaptation of Forests to Future Climates.....	27
Human Intervention: Matching Populations to Future Climates.....	27
Risks, Objections and Social Acceptability of Assisted Gene Flow in a Forestry Context..	29
2.7 Provenance Trials for Studying Climate Adaptation.....	32
2.8 Tree Rings as Paleoarchives.....	34

2.9	Wood Tissue Structure and Adaptive Functions	35
	Wood Tissues and Function.....	35
	Drought Tolerance through the Lens of Functional Wood Anatomy.....	36
	Tracheid Structure and Cold Tolerance	39
2.10	Stable Isotopes in Tree Rings.....	41
	The Route to Carbohydrate: Stomata and the C3 Carbon Assimilation Pathway	41
	Chemical Components of Wood.....	42
	Stable Carbon Isotopes in Tree Rings: A Stomatal Closure Signal.....	42
	Water Use Efficiency: A Fertilization Effect under Higher Atmospheric Carbon?	44
	Stable Oxygen Isotopes in Tree Rings: A Proxy for Transpiration	47
2.11	Combining Growth, Functional Wood Anatomy and Dual-Isotopes in a Provenance Trial Setting.....	48
2.12	Physiological Behaviors.....	49
	Physiological Risks and Trade-offs: Isohydry and Anisohydry	49
2.13	References.....	51
3	Growth and Drought Tolerance of Lodgepole Pine	70
3.1	Publication	70
3.2	Abstract.....	70
3.3	Introduction.....	71
3.4	Materials and Methods.....	74
	Experimental Design.....	74
	Field and Laboratory Measurements	75
	Calculation of Drought Indices.....	75
	Statistical Analyses	76
3.5	Results	77
	Source Climate and Growth of Populations.....	77
	Population Differences in Drought Tolerance	78

Drought Tolerance and Source Climates	79
Relationship between Growth and Drought Tolerance.....	80
3.6 Discussion.....	80
Source Temperature Predicts Productivity	80
Trade-offs Indicate Differing Adaptive Strategies	81
Boreal Provenances Appear At-Risk	82
Implications for Assisted Migration	83
3.7 References.....	85
3.8 Figures and Tables.....	89
4 Reducing Contamination Risk in Wood Samples for Isotope Analysis.....	98
4.1 Publication	98
4.2 Abstract.....	98
4.3 Introduction.....	99
4.4 Materials and Methods.....	101
Tree-Ring Selection and Separation	101
Experimental Design.....	102
Cellulose Extraction.....	104
Measurements of Isotope Ratios.....	105
Statistical Analysis.....	105
4.5 Results	106
Sample Tube Abrasion and Isotope Signatures of Potential Contaminants	106
Effect of Cellulose Extraction on the Amount of Material.....	107
Carbon and Oxygen Isotope Signatures in Contaminants, Milled Wood and Cellulose	107
4.6 Discussion.....	109
Sample Tube Abrasion and Heterogeneity of Particle Size in Milled Wood	109
Contamination Concentration due to Cellulose Extraction	109

Recommendations and Possible Alternatives	110
4.7 References	113
4.8 Figures and Tables	117
4.9 Supplementary Information	125
5 Reducing Image Artifacts for Faster Analysis of Wood Micro Sections	126
5.1 Publication	126
5.2 Abstract	126
5.3 Introduction	127
5.4 Materials and Methods	129
5.5 Results and Discussion	132
5.6 References	134
5.7 Figures and Tables	136
6 Physiology of Lodgepole Pine Drought Tolerance	139
6.1 Abstract	139
6.2 Introduction	140
6.3 Methods	142
Experimental Design and Field Measurements	142
Field and Tree-Ring Measurements	143
Functional Wood Anatomy	143
Stable Isotope Analyses	144
Statistical Analyses	144
6.4 Results	145
6.5 Discussion	147
6.6 Conclusion	149
6.7 References	150

6.8	Figures	153
6.9	Supplementary Information: Methods	156
	Experimental Design and Field Measurements	156
	Field and Tree Ring Measurements	158
	Drought Indicators and Period of Study	158
	Sample Preparation for Wood Anatomical Analysis	159
	Pilot Study: Sample Preparation for Stable Isotope Analysis.....	160
	Full Design: Sample Preparation for Stable Isotope Analysis.....	163
	Atmospheric Carbon Data and Calculation of Intrinsic Water Use Efficiency	164
	Climate Data	165
	Statistical Analyses	165
6.10	Supplementary Information: Results	167
6.11	Supplementary Information: References	175
7	Cold Tolerance Considerations for Assisted Gene Flow	179
7.1	Abstract	179
7.2	Introduction	179
7.3	Methods	182
	Experimental Design and Field Methods.....	182
	Preparation of Micro Sections for Wood Anatomical Analyses.....	183
	Measurements of Blue and Frost Ring Occurrence and Intensity	183
	Climate Data	184
	Statistical Analyses	185
7.4	Results	186
7.5	Discussion	188
7.6	References	190
7.7	Figures and Tables	195
7.8	Supplementary Information	199

8	Limits to Lodgepole Pine Seed Transfer	201
8.1	Abstract.....	201
8.2	Introduction.....	202
8.3	Methods.....	206
	Experimental Design.....	206
	Field and Tree Ring Measurements	206
	Statistical Analyses	207
8.4	Results	208
	Local Performance: Testing Historic Seed Transfer Guidelines as a Baseline	208
	Southward Transfers: Responses under Simulated Warming.....	209
	Northward Transfers: Growth under Simulated Assisted Gene Flow	211
	Climate-Growth Correlations: Planting Sites Draw Out Adaptations	212
8.5	Discussion.....	213
	Leading Edge of the Lodgepole Pine Range: Risks of Inaction Outweigh Risks of Assisted Gene Flow	213
	Central Interior of BC: Judicious Assisted Gene Flow Implementation Possible	215
8.6	Conclusions.....	215
8.7	References.....	217
8.8	Figures.....	222
8.9	Tables	225
8.10	Supplementary Information	229
9	Conclusions	232
9.1	Northern Forests at Risk: Possible Candidates for Assisted Gene Flow	232
9.2	Central Interior Areas: Resilient and Unsuitable for Assisted Gene Flow	236
9.3	Southern Interior Areas: Maladaptation to Cold Limits Assisted Gene Flow.....	237
9.4	Uncertainties for Trailing-Edge Lodgepole Pine Populations	241
9.5	Proactive or Reactive: Population-Specific Risks in Assisted Gene Flow	242

9.6	Refined Methodologies to Support Sound Tree-Ring Research.....	244
9.7	Thesis Contributions to Science and Management.....	245
9.8	Concluding Statement	246
9.9	References.....	247
	Cumulative Bibliography	251

List of Tables

Chapter 3: Growth and Drought Tolerance of Lodgepole Pine

Tab. 3.1. Average source climate variables (1961-1990 normal) for provenances	94
Tab. 3.2. Planting site climate variables (1961-1990 normal) for field tests	95
Tab. 3.3. Height and diameter at 1.3 m of four lodgepole pine populations after 32 years of growth	96
Tab. 3.4. Drought indicator values (larger values are better) for resistance, recovery, resilience, and relative resilience for four regions ordered by latitude	97

Chapter 4: Reducing Contamination Risk in Wood Samples for Isotope Analysis

Tab. 4.1. Mean $\delta^{18}\text{O}$ values and standard error for polytetrafluoroethylene plastic (PTFE), polypropylene plastic, and wood that underwent different ball milling treatments	120
Tab. 4.2. Mean $\delta^{13}\text{C}$ values and standard error for polytetrafluoroethylene plastic (PTFE), polypropylene plastic, and wood that underwent different ball milling treatments	121
Tab. 4.3. Analysis of variance for the cellulose model	122
Tab. 4.4. Analysis of variance for the whole wood model.....	123
Tab. 4.5. Options for homogenizing wood and cellulose prior to stable- and radio-carbon isotope analyses	124
Tab. 4.S1. Cellulose extraction data.....	125

Chapter 6: Physiology of Lodgepole Pine Drought Tolerance

Tab. 6.S1. Geography and average source climate variables (1961-1990 long-term average) are shown for provenances	167
Tab. 6.S2. Geography and average annual climate variables (1981-2010 long-term average) of the three planting sites	168
Tab. 6.S3. Pearson's correlation coefficients of annual site climate to growth and physiology of four populations representing distinct climatic regions in the distribution of lodgepole pine.....	169

Tab. 6.S4. Pearson’s correlation coefficients of provenance growth, drought tolerance and physiology to climate of seed origin (1961-1990 climate normal).....	170
Tab. 6.S5. Least square means of growth, drought and isotope values over the 10 year study period	171
Tab. 6.S6. Differences among the four populations tested here in terms of growth, drought and isotope responses over the 10 year study period.....	172

Chapter 7: Cold Tolerance Considerations for Assisted Gene Flow

Tab. 7.1. Spearman correlation coefficients of blue and frost ring intensities to annual climate variation	197
Tab. 7.2. Spearman correlation coefficients of blue and frost ring intensities to provenance source climate (1961-1990 climate normal)	198
Tab. 7.S1. Climate of provenance origin	199
Tab. 7.S2. Climate of the three planting sites	200

Chapter 8: Limits to Lodgepole Pine Seed Transfer

Tab. 8.1. Transfer distances in terms of geographic distance in kilometers (km) from all sites to all provenances.....	225
Tab. 8.2. Climate transfer distances in terms of mean annual temperature (°C) from all sites to all provenances.....	226
Tab. 8.3. Least square means of height and diameter growth in year 2005 (age 33) of four lodgepole pine regional populations tested across three major climatic zones in the lodgepole pine distribution	227
Tab. 8.4. Climate warming expected by 2085 on all seed-collection and planting sites under two IPCC emissions’ scenarios.....	228
Tab. 8.S1. Average source climate variables from the 1961-1990 normal period are shown for provenances.....	229
Tab. 8.S2. Average source climate variables from the 1961-1990 normal are shown for all planting sites	230
Tab. 8.S3. Multiple comparisons among planting regions and tested populations.....	231

List of Figures

Chapter 1: Introduction

- Fig. 1.1.** Western North America showing the lodgepole pine species distribution5
- Fig. 1.2.** Stem disks allow the accurate estimation of basal area increment.....6
- Fig. 1.3.** A micro section of a transverse cut of a tree ring, with earlywood on the left and latewood on the right6
- Fig. 1.4.** A micro cross-section of a pine needle after a double staining procedure.....6

Chapter 2: Background Information

- Fig. 2.1.** A schematic displaying historic variability and expected changes14
- Fig. 2.2.** Height increments can be estimated by measuring distance between branch whorls17
- Fig. 2.3.** Annual growth rings (tree rings) can be used to measure basal area increment17
- Fig. 2.4.** An illustration of the four drought indicators as defined by Lloret et al. (2011)23
- Fig. 2.5.** Sketch of a response function.....32
- Fig. 2.6.** Sketch of a transfer function33
- Fig. 2.7.** A micro section of a tree ring boundary with latewood on the left and earlywood on the right.....36
- Fig. 2.8.** A cartoon of tree growth showing annual variations in tracheid size represented by shades of grey40

Chapter 3: Growth and Drought Tolerance of Lodgepole Pine

- Fig. 3.1.** Map of western North America showing the lodgepole pine species distribution.....89
- Fig. 3.2.** Height of lodgepole pine provenances at three test sites in southern British Columbia and the relationship to their climate of origin.....90
- Fig. 3.3.** Climate anomalies and basal area increments91

Fig. 3.4. Relative drought resilience of four lodgepole pine populations compared to source climate.....92

Fig. 3.5. The trade-off relationship height and relative resilience to drought in lodgepole pine ..93

Chapter 4: Reducing Contamination Risk in Wood Samples for Isotope Analysis

Fig. 4.1. Results of the plastic abrasion test by milling empty sample tubes by time and number of balls added117

Fig. 4.2. Boxplots of the percent of sample mass remaining after the first round of cellulose extraction.....118

Fig. 4.3. Boxplots of the distribution of $\delta^{13}\text{C}$ values of treatments, represented as ‰ deviations from VPDB119

Chapter 5: Reducing Image Artifacts for Faster Analysis of Wood Micro Sections

Fig. 5.1. Three examples of micro sections of stained xylem cells made from lodgepole pine samples.....136

Fig. 5.2. Schematic view of steaming a micro section137

Fig. 5.3. Transverse sections of tree-rings from the same wood sample close to the border between latewood and earlywood photographed at 200x magnification.....138

Chapter 6: Physiology of Lodgepole Pine Drought Tolerance

Fig. 6.1. Variations in growth and physiology of lodgepole pine in relation to climate and provenance153

Fig. 6.2. Genetic adaptations revealed from growth, drought tolerance and physiology154

Fig. 6.3. Plasticity among populations of lodgepole pine in terms of stable isotope and functional wood anatomical values155

Fig. 6.S1. Study design and climates across the species distribution of lodgepole pine157

Fig. 6.S2. Pilot study results comparing materials and methodologies for stable carbon and oxygen isotope analyses in tree rings from lodgepole pine162

Fig. 6.S3. Change in average tracheid number per tree ring by regional population over the study period173

Fig. 6.S4. Relationship between stable carbon and oxygen isotope ratios in four large populations of lodgepole pine.....174

Chapter 7: Cold Tolerance Considerations for Assisted Gene Flow

Fig. 7.1. Blue and frost rings as a function of growing degree days195

Fig. 7.2. Blue and frost ring intensities by population and growth196

Chapter 8: Limits to Lodgepole Pine Seed Transfer

Fig. 8.1. Experimental design and transfer distances222

Fig. 8.2. Growth of four lodgepole pine regional populations on planting sites in three areas in the lodgepole pine distribution.....223

Fig. 8.3. Heatmaps showing climate-growth correlations224

List of Symbols

δ : Proportion of the heavy isotope (rare) to the light isotope (common) relative to a standard

Δ : Fractionation, or changes in isotopic composition relative to a natural source due to chemical or physical processes

A : Assimilation rate (accumulation of carbon or cumulative photosynthesis)

C_a : Carbon dioxide concentration in the atmosphere

C_i : Carbon dioxide concentration internally in the leaf

g_s : stomatal conductance

BAI : Basal Area Increment (cm^2/year)

DBH : Diameter at Breast Height (1.3 m)

WUE or $iWUE$: (intrinsic) Water Use Efficiency (A/g_s)

Glossary of Terms

Acclimation: Adjustments in response to an event. Tree acclimation involves adapting to new climates through physiological means and by modifying morphology.

Adaptation: A suite of characteristics that result in higher fitness of the species or population to external conditions, e.g. the environment.

Adaptive Trait: A characteristic of the phenotype that confers higher fitness, e.g. phenology and physiology of water use efficiency (Kremer et al., 2012).

Anisohydry: One of two behaviours evolved by some plants to cope with water limitation. Anisohydric plants allow more variable water potentials due to changing dryness (Sade et al., 2012). These plants keep stomata open under drier conditions to maintain photosynthesis and are often associated with xylem adaptations. This strategy is acceptable under moderately stressful conditions but has increased risk under intense water shortage (Sade et al., 2012). See also: *Isohydric*.

Assisted Migration: An umbrella term that involves the intentional translocation of seed sources (seeds, planting stock or individuals) to realign species or populations with their adapted optimal climates. The intention is to provide human-assistance to ecosystems to adapt to rapid climate change. This could help reduce problems of maladaptation due to climate spaces which are shifting poleward and higher in elevation faster than natural migration can occur.

Assisted Gene Flow (Assisted Population Migration): A type of assisted migration scenario involving transfers of seed sources (seeds or planting stock) of the same species within the existing species range to realign populations with optimal climates.

Basal Area Increment: The area represented by new secondary growth, usually referring to a height on the stem at 1.3 m (Diameter at Breast Height or DBH). Although derived from tree-ring widths, basal area increment is more representative of overall growth and is often preferred in forest ecology to understand tree growth.

Bordered Pit: An opening between two tracheid cell walls that allows water to flow from tracheid to tracheid under normal water-flow conditions through permeable support structures (margo) that suspend an impermeable structure (torus) in the middle. If one tracheid becomes cavitated, the change in pressure causes the torus to be pushed to one side, reducing air expansion into neighbouring cells.

Cambium: A layer of meristemic tissue around the tree stem underneath the bark; this area of cellular division creates xylem on the inner side of the stem and phloem cells on the inside of the bark. See also: *Meristemic Tissue*.

Cavitation (Embolism): The presence of air in a xylem conduit, which reduces flow through the stem.

Cline: Genetic adaptation that varies across a gradient, often reflecting the gradients of an environmental gradient, e.g. cold.

Cross-dating: A dendrochronological technique that aligns different pieces of wood based on similar tree ring patterns to assign an exact date to each tree ring.

Dendrochronology: Systematic study of exactly-dated tree rings to enable analysis of physical or cultural patterns across space and time.

Dendroclimatology: A sub-specialty of dendrochronology relating to climatological reconstructions using tree ring proxies, while dendrometeorology refers to tree-ring anomalies that can be used to infer intra-annual variability.

Dendroecology: A sub-specialty of dendrochronology that uses tree rings to reconstruct ecological events of interest: e.g. stand or species initiation, fire history or return intervals, or tree response across space to events of interest (e.g. drought).

De-trending: The practice of mathematically removing the longer-term age trend in tree-ring width or height data to emphasize annual variations. Common methods include spline functions and negative exponential functions.

Dormancy: The winter state of temperate and boreal tree species; dormancy increases resistance to cold and is followed by de-acclimation or “de-hardening” in spring.

Drought: An extended period of aridity, often having biological impacts. Can be identified with the Standardized Precipitation Evapotranspiration Index (SPEI), which incorporates monthly precipitation and potential evapotranspiration. Another common method for longer time scales (>12 months) is the Palmer Drought Severity Index (PDSI) which expresses relative dryness with a standardized index. To study response of tree growth to drought, the four indicators coined by Lloret et al. (2011) are particularly useful (see section 2.4).

Forcing – i.e., Climate Forcing: Natural or human-caused factors affecting climate – they ‘force’ change through physical mechanisms. Human-caused forcings to climate include increased atmospheric concentrations of greenhouse gases.

Flushing: Bud burst in spring after chilling and heat-summer requirements have been met, also associated with “de-hardening” of a tree’s cold tolerance in winter.

Fractionation: The change in isotope ratios in a material relative to a natural source due to chemical or physical processes.

Frost: This thesis defines frost as a cold event below 0 °C.

Genotype: The complete inherited genetic make-up which contributes to the outward expression of an organism (see also *phenotype*).

Hectare (ha): A unit to measure areas measuring 100 m by 100 m.

Height Increment: The annual contribution height growth of a tree. These can be estimated by measuring the distance between branch whorls.

Hydraulic Conductivity (Hydraulic Efficiency): A measure of the flow of water to the crown through the xylem conduits – the hydraulic pipeline.

Hydraulic Safety Margin: The range of water potentials (xylem pressure) a plant can cope with before succumbing to hydraulic failure (major loss of hydraulic conductivity) caused by aridity.

Isohydric: One of two behaviours employed by plants to cope with water limitation (see also *anisohydric*). Isohydric plants are able to maintain stable mid-day water potentials under water-abundant and water-shortage situations by reducing transpiration through lower stomatal conductance (Sade et al., 2012). This strategy is successful under moderate and intense drought, but could risk slow starvation of carbohydrates under prolonged drought (McDowell et al., 2008).

Isotope: An atom of an element (which is defined by the number of protons in the nucleus) that has a different number of neutrons in the nucleus. The relative numbers of neutrons does not alter the atom chemically, but changes physical properties (i.e., higher mass).

Isotope Ratio Mass Spectrometry: A method of mass spectrometry (measures masses within a sample) used to quantify a sample's relative abundances of isotopes.

Leading Edge: The most pole-ward areas in a species distribution that are expected to expand into higher latitudes to keep pace with climate change. These areas are the front of the changing species distribution; they “lead” the distribution. See also: *Trailing Edge*.

Maladaptation: An adaptation that has become inadequate or harmful in an individual organism or population, potentially leading to lower fitness and survival in the long-term.

Meristemic Tissue: Specialized tissues in the plant that are areas of cell division during growth. These occur at the tree leader (apical meristem), at buds, and surrounding the tree underneath the bark (cambium).

Phenotype: The observed characteristics of an organisms (morphology, physiological properties, etc.) resulting from interactions between the environment and genotype.

Phenotypic Plasticity: the ability to respond to a range of environments (Morgenstern, 1996).

Phenology: The study of the timing of cyclic events and their contributing factors, namely, climate variations. A portion of this thesis focuses specifically on plant life cycles with regard to cold adaptation, e.g. flushing, bud set and dormancy.

Pluvial: A period of relatively high moisture.

Population: Generally, it is a group of individuals of a same species living in a particular area and capable of interbreeding. In forest genetics, it is further clarified that these

interbreeding organisms exchange genes, transmitting them to the next generation (Morgenstern, 1996).

Primary Growth: Vertical growth controlled by the apical meristems (the region of cell division at the tip of shoots and roots).

Provenance Trial: A reciprocal transplant experiment that collects seeds (provenances) from across a species range and grows them over a range of conditions at multiple test sites.

Risk: The interaction between probability and consequence of an event. If consequence is very high, an event may still be high-risk even if probability is low.

Secondary Growth: Horizontal growth controlled by the lateral meristems or cambium (the region of cell division at the circumference)

Stomata (singular: Stoma): Small pores in the leaves or needles of plants that regulate water loss from the plant in exchange for carbon dioxide uptake from the atmosphere to the plant.

Stomatal Control: The assumption that stomata instantaneously regulate the trade-off between water-loss and carbon gain. The marginal gain versus water “cost” is optimized for “profit maximization” (Sperry et al., 2017).

Stomatal Aperture: The width of the stomata; this is regulated by two guard cells that can close or open depending on moisture stress.

Stomatal Conductance (g_s): The rate of water loss and carbon dioxide uptake through the stomata (in $\text{mmols m}^{-2} \text{s}^{-1}$).

Stomatal Responsiveness: The rapidity of change in stomatal aperture to changes in environment.

Super Epoch Analysis (SEA): An analysis that standardizes annual response data for a certain time period (epoch) prior to a certain event (e.g. drought) to understand post-event anomalies scaled to this period of normalization.

Tracheid: Tube-like cells forming the xylem of woody vascular plants. They die after formation, allowing transport of water through the plant stem to the crown.

Tracheid Lumen: The cavity or inside space of the tube-shaped tracheid cell.

Trailing Edge (also known as the Rear-Edge): Areas of a species distribution that are closest to the equator, expected to shrink and pull poleward under climate change. Currently-existing species in that area are expected to become extirpated as their adapted climates shift northward. Hence, they “trail” or are at the “rear edge” of the distribution change. See also: *Leading Edge*.

Tree Ring Widths: The width of a tree’s annual growth increment along one radial file to the pith.

Water Potential (ψ): The ability of water to do work; in the case of plants, it causes a gradient that allows water to flow through the plant stem.

Water Use Efficiency (WUE): The definition of water use efficiency depends on the context, but the basic definition relates to the ratio of the rate of biomass production to the rate of transpiration (Bacon, 2004). Instantaneous water use efficiency is ratio of net carbon assimilation to water loss through transpiration (E), measuring the instantaneous exchange of water and carbon dioxide through gas exchange studies. Intrinsic water use efficiency is the ratio of carbon uptake (A) versus stomatal conductance (g_s). Integrated water use efficiency is total carbon gain to total water loss of a leaf, plant or ecosystem, measured over a longer time period (time integral) (Seibt et al., 2008). Using isotopic discrimination methods provides a measure of intrinsic water use efficiency (Jones, 2004), as used in this dissertation.

Xylem: Plant tissue made of wood cells that perform transport, storage and structural functions.

1 Introduction

1.1 Thesis Themes

The ecological, economic and social functions of forests may be compromised by current relatively rapid, high-magnitude climate change (Parmesan & Yohe, 2003; Williamson et al., 2009; IPCC, 2014; Sanford et al., 2014). As warming occurs, long-lived trees will become increasingly unsuited to new climates given historic adaptation: Over generations, tree populations become genetically adapted to the environment in which they grow. The climates to which they are adapted, however, are shifting northward and higher in elevation faster than tree populations can migrate (Aitken et al., 2008; Gray & Hamann, 2013; Williams & Dumroese, 2013). In addition to general warming, drought is expected to become more frequent and severe (Christidis et al., 2014, see also section 2.1), while trees may become less able to cope with cold events. Thus, both climate *warming* and *variability* are a cause for concern in forest ecosystems (see sections 2.2 and 2.3).

The mismatch between tree population adaptive traits and new climates could lead to *maladaptation*, which means that a genetic adaptation has become inadequate or harmful to an individual or population. Forest maladaptation to climate change is expected to reduce forest health, productivity and survival (Allen et al., 2010, 2015; Peng et al., 2011; McDowell et al., 2015; Anderegg et al., 2016). Since tree populations are adapted to narrower climate envelopes within an overall species distribution, maladaptation will likely occur throughout a species' range; not just at the most southern, low elevation areas of a species range – also known as the trailing edge (Davis & Shaw, 2001; Aitken et al., 2008).

Assisted migration has been proposed as a method of adapting the forests to climate change. This concept involves moving pre-adapted seed sources from southern, warmer and drier areas to locations that are more poleward or higher in elevation (Aitken & Bemmels, 2016). When seeds are moved within a species range, this is known more specifically as *assisted gene flow* or *assisted population migration* since it requires no species introductions to new areas. The aim of assisted gene flow is to reduce maladaptation and increase drought tolerance by re-aligning tree populations with their historic climatic optimums. After harvesting, for example, forest practitioners could replant with seed sources from slightly lower latitudes or elevations in anticipation of future warming. The implementation of assisted migration may therefore require a re-evaluation of historic seed planning zones or floating seed transfer policies.

Seed transfer regulations were initially designed to prevent inappropriately large translocations of seeds, which could result in reduced productivity and survival through maladaptation. Seed transfer guidelines were based on geography on the assumption of a static climate, but this assumption is no longer valid under changing climates. If warm-adapted populations are moved northward in the anticipation of a future warmer climate, however, the vulnerable seedlings may be exposed to damaging cold to which they are not adapted. There is therefore also a need to estimate cold adaptation to determine the acceptable limits to seed transfer for a warmer climate. Accurate conceptual models of forest ecosystems are important for predicting forest response to climate warming and climate variability; better predictions provide better foundations for management.

1.2 Thesis Objective and Motivation

Ecological health is linked to economic health, which in turn helps support healthy communities (Parkins & MacKendrick, 2007). Generally, my research goals are to understand how forest ecosystems respond to climate warming and disturbance, and to contribute to sound management. Filling these research needs in Canada is of particular value because, at over 310 million hectares, Canada represents more than 10% of the global forest area. In western Canada, forests in British Columbia (BC) cover approximately 58 million ha, over 27 million ha in Alberta (AB) and 7.8 million ha in the Yukon Territory (YT). These forests sustain a forest industry that contributes to \$20.9 billion to Canada's gross domestic product and employs over 200,000 people (Canadian Council of Forest Ministers, 2015). More than half of Canada's annual forest harvested area is in BC and AB (Statistics Canada, 2011). These forests further act as a key source of quality air; are a critical element of the global carbon cycle; play an important role in the hydrological cycle; filter water and reduce flooding; reduce erosion; and provide ecosystems that support wildlife and biodiversity. Forest ecosystems upon which these ecological services and economic values depend are sensitive to climate, and will therefore be affected by climate warming combined with an expected increase in climatic extremes.

The aim here is to inform the reforestation strategy for a changing climate by evaluating population drought response, climate maladaptation and seed transfer possibilities. Specifically, this work quantifies the *risk* for different tree populations to simulated climate warming and drought, where risk is defined as the combined effect of probability and consequence. By analyzing response to cold, this thesis further assesses the risk of the proposed solution, assisted migration. Because tree population responses may not remain constant under rapid environmental change, this work evaluates the physiological behaviours underlying these

responses: The relative influence of water-controlling mechanisms at the leaf-level and within the tree's wood can hint at adaptive physiology that may or may not be suitable for a warmer, drier future. This could indicate reduced suitability for reforestation in areas that may otherwise appear suitable while expanding other areas for consideration, or vice versa.

This research focuses on lodgepole pine (*Pinus contorta* Dougl. ex Loud.), which is one of the most widespread tree species in western North America. This species shows high ecological amplitude, covering an area estimated to be between 1.3 million km² (Schroeder et al., 2010) and 1.7 million km² (Little, 1971). The species distribution stretches over 4000 km along a north-south transect covering diverse climates. The more predominant interior variety (*P. contorta* var. *latifolia*) is particularly important to BC, AB and the YT as an ecological foundation species. As a timber species, it further represents billions of dollars of economic value, government tax revenue, and employment in forestry-dependent communities (Hajjar et al., 2014). In recent years, however, the forest industry in western Canada has been hard-hit by lost value from an unprecedented epidemic of mountain pine beetle (*Dendroctonus ponderosae*) (Williamson et al., 2009) that caused high mortality in over 17.5 million hectares of lodgepole pine forests. This epidemic impacted carbon emissions (Kurz et al., 2008) as well as communities (Parkins & MacKendrick, 2007).

After mountain pine beetle killed forests are salvage-logged, however, policy requires that ecologically suitable trees be re-planted. Lodgepole pine may remain suitable in these areas with some reconsideration of seed zones (O'Neill et al., 2008, 2017). There now exists an opportunity to re-plant with the most suitable lodgepole pine populations for a warming climate. As western Canada's most common tree, millions of lodgepole pine seedlings are planted annually. Each year in BC's interior, for example, approximately 250 million seedlings are

requested for reforestation of crown land and about 50% of these are lodgepole pine (Province of British Columbia, 2016). By quantifying risks associated with seed transfer against the risks of reforestation status quo, this research aimed to identify lodgepole pine genotypes that are resilient to climate extreme events and yet remain productive under projected climate change. Given the annual reforestation effort, results may be immediately implemented, possibly yielding significant benefits. Therefore, one of the province's largest forestry problems can help solve one of the next most pressing challenges; maladaptation due to climate change.

1.3 Research Approach

Provenance Trials

This work relies on a combined methodology from quantitative forest genetics and tree-ring research. Samples are derived from trees grown in perhaps the largest and most valuable genetic field experiment, the Illingworth lodgepole pine provenance trial, established in 1974 (Illingworth, 1978). With 153 seed sources tested at 60 planting sites (Fig. 1.1), this experiment speaks to the value of lodgepole pine to western North America. *Provenance trials* provide the experimental structure to study adaptation among tree populations. They can also be used as a climate change laboratory since they can simulate either climate warming or assisted migration through positive or negative transfer distances. More detail is provided in section 2.7.

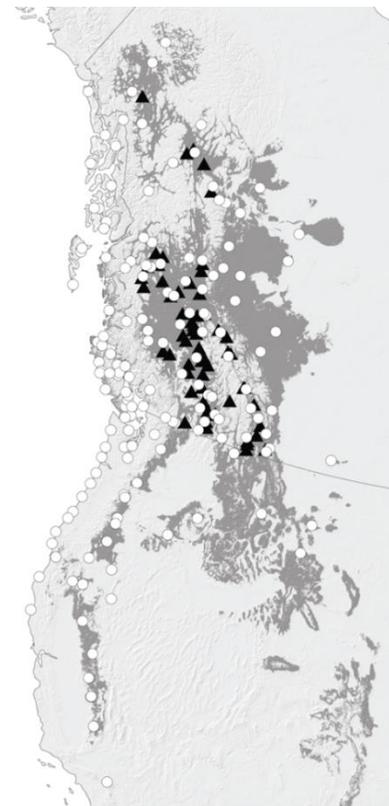


Fig. 1.1. Western North America showing lodgepole pine range (grey). White circles are seed sources; black triangles are planting sites. This is the full design of the Illingworth lodgepole pine trial.

Tree Rings: Growth and Inferred Physiology

The mountain pine beetle caused high mortality at many of the provenance trial test sites, allowing us to fell dead trees to obtain accurate field measurements and stem disks (Fig. 1.2). Tree rings act as archives of tree responses to past climatic events. In addition to facilitating precise annual estimates of growth (Fig. 1.2, see section 2.2), there are subtle signals within tree rings that can be used to infer physiological responses to different stressors, including drought and cold. *Functional wood anatomy* enables quantification of properties of the hydraulic pathway that carries water from tree roots to the photosynthesizing crown along the soil-plant-atmosphere continuum. Adaptations of the xylem represent one avenue of adaptation to climate, with species and populations from more drought-prone regions generally having narrower lumen with thick cell walls against high water tension. The degree to which plant xylem acclimates to changing environments also depends in part on species and genotype. These overall cellular differences, as well as the ability to modify these cells under stress, can be studied with thin cross-sections of tree rings (Fig. 1.3). Different traits of these micro sections reveal various adaptations and responses to drought and cold. (See section 2.2 for more information on tree growth and section 2.9 for more information on wood tissue structure and function.)



Fig. 1.2. Stem disks allow the accurate estimation of basal area increment, which is the annual contribution to stem surface area at 1.3 m.

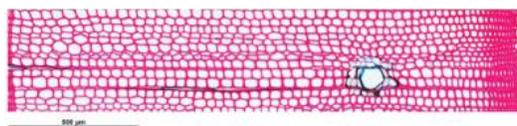


Fig. 1.3. A micro section of a transverse cut of a tree ring, with earlywood on the left and latewood on the right (pith to the left of the image). A double staining procedure highlights cellulose and lignin, facilitating measurements of cell wall thickness and cell lumen diameter.

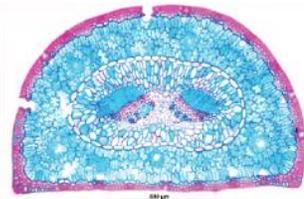


Fig. 1.4. A micro cross-section of a pine needle after a double staining procedure; 2 sunken stomatal openings clearly visible.

Another measure to adapt to changing environmental conditions relies on the stomata (Fig. 1.4). *Stomata* are small pores on plant leaves with specialized guard cells that regulate the trade-off between carbon assimilation and water loss by opening or closing under different conditions. Under stress, this also changes the proportions of carbon and oxygen isotopes taken up by the tree: this stress signal can be retrospectively analyzed from the photosynthate stored in tree rings. (See section 2.10 for more background information on stable isotope analyses from tree rings.) When combining the understanding of such leaf-level processes with functional wood anatomy analyses, the behaviour that a tree uses to cope with water limitation can be assessed.

Under growing conditions that are non-water limited, woody plants can maximize photosynthesis through larger xylem conduits that facilitate water transport to the crown. Under drier conditions, stomata can close to prevent excessive water loss, reducing the risk of long-term damage to the plant's hydraulic system. This may be linked to the ability to maintain stable water potential, known as *isohydry*. If dry conditions continue for too long, however, isohydric plants risk slow mortality due to carbon starvation because the carbohydrate losses through respiration are not replaced through photosynthesis. An alternative behaviour evolved by some plants involves leaving stomata open to allow continued carbon gain; this risks hydraulic failure because negative stem water pressures (tension) can cause cavitation. This behaviour is therefore also often combined with narrower, sturdier stem conduits that help lower the risk of cavitation and hydraulic failure. Such behaviour is known as *anisohydry*. (Please see section 2.12 for additional background information about physiological strategies for coping with water limitation.)

1.4 Thesis Organization

This dissertation includes nine chapters, including six research chapters. For published work, the citation is listed at the beginning of the corresponding chapter. References are provided at the end of each chapter in addition to a cumulative bibliography at the end of the dissertation. Chapter 1 is an introductory chapter providing a general overview of this volume. Chapter 2 is a background information chapter providing further definitions and coverage useful for those of whom this type of work is unfamiliar and specifically to help them interpret subsequent data chapters. It expands on Chapter 1 by including basic concepts and is not intended to be an in-depth discussion of all topics covered. Rather, it provides context to make the subsequent, more detailed data chapters interpretable and accessible to as wide an audience as possible.

Chapter 3 covers the first primary research goal of the thesis, which is to quantify the genetic differences in drought tolerance in terms of impact on growth. All subsequent chapters build on this chapter. Chapter 4 evaluates the suitability of a common methodology used in the preparation of plant material for stable isotope analyses and isotope ratio mass spectrometry. The possibility of isotopic contamination through plastic abrasion during ball milling had to be confirmed (or falsified) prior to approaching the next major thesis goal in an ensuing chapter. Chapter 5 covers a methodology that we developed to more efficiently process the thousands of samples for functional wood anatomy analyses, which facilitated the following chapter.

Chapter 6 seeks to explain the drought tolerances of different lodgepole pine genotypes (found in Chapter 3) by using functional wood anatomy and a dual-isotope approach. It assesses relative contributions of xylem adaptations and stomatal responsiveness to better predict responses under climate change and drought. Chapter 7 assesses genetic differences in risk to

cold damage and the implications to forest growth to assess possible assisted migration scenarios. This builds on earlier chapters (3, 6) that found assisted migration could reduce risks under drought and climate change; this chapter assesses the risks of implementation. Chapter 8 synthesizes the knowledge from previous chapters, further analyzing inter-annual variability to explain genetic differences in cumulative growth under multiple southward and northward transfer distances. This dissertation concludes with Chapter 9, which highlights the value of the knowledge gained and how it advances the field. It further sets this new understanding in the context of forest management for future climates by discussing the risks of action against inaction.

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2 Background Information

2.1 Magnitude and Pace of Global Change

General Warming

Climate change is defined as persistent change in climate means or variability (IPCC, 2007). From 1880 to 2012, global average air and ocean temperatures rose by 0.85 °C (IPCC, 2014). Physical manifestations of this warming include loss of mass of ice sheets; a decrease in sea ice extent of 3.5-4.1% per decade (1979-2012); and rising ocean levels of 19 cm since 1901 (IPCC, 2014). While *absolute temperatures* may have been warmer in the past in some regions, current temperatures (1983-2012) are considered the warmest that have occurred in over 1400 years in the Northern Hemisphere (Chuine et al., 2004; IPCC, 2014). Furthermore, the *rate* of temperature change is unprecedented (Büntgen et al., 2011; IPCC, 2014).

Future projections predict rising surface temperatures under all emission scenarios, known as Representative Concentration Pathways (RCP). These range from the more optimistic RCP 2.6 scenario, which implicates mitigation strategies that curb emissions, to the pessimistic RCP 8.5 scenario, with very high greenhouse gas emissions (IPCC, 2014). Despite an increase in mitigation policies, anthropogenic greenhouse gas emissions are accelerating (IPCC, 2014). Since fossil fuel combustion has continued to increase consistent with the most high-emissions scenario (Zhao & Running, 2010; Sanford et al., 2014), the high-magnitude warming associated with the pessimistic RCP 8.5 scenario appears to be most realistic. The RCP 8.5 scenario is associated with global mean surface air temperature increases of 2.6 to 4.8°C by the end of the century (2081-2100), while continued changes may occur over millennia (IPCC, 2014). Global

average temperature increases will unfold differently across regions; for example, the poles are experiencing higher than average warming (IPCC, 2014). Since climate is a major driver in biological systems, many ecosystems have been, and will continue to be, affected (see also section 2.3).

Increasing Probability of Extremes

In addition to warming, climate change is also associated with increased variability, which translates into a higher probability of extreme weather events (IPCC, 2014). Fig. 2.1 is a schematic showing an expected increase in temperature anomaly probabilities. As with *average* warming, the effects of *extreme events* will be realized differently across the globe. For example, while Pakistan has been experiencing a pluvial (Treydte et al., 2006), drought has been affecting much of North America (e.g. Cook et al., 2004).

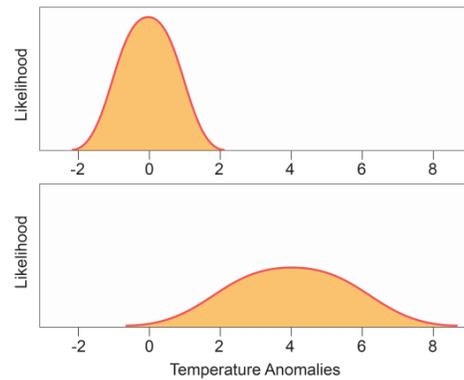


Fig. 2.1. A schematic displaying historic variability (top panel) and expected changes (lower panel): Associated with an increasing average temperature is a flattening of the bell curve, increasing variability and the probability of extreme events. (Modified from Christidis et al. 2014.)

In general, however, global aridity is already increasing (Dai et al., 1998, 2004; Dai, 2011). In many regions (e.g. Europe and North America), drought events are also projected to become more frequent around the world due to moisture demand and changing circulation patterns (Dai et al., 2004; Meehl, 2004; Dai, 2011; Cook et al., 2016). Heat events are also expected to become more frequent and severe (Meehl, 2004; Dai, 2011; Christidis et al., 2014). These could combine with natural drought variability to lead to unprecedented aridity (Cook et al., 2004). This is a concern because projected droughts and climate variability could have significant impacts on ecosystems (e.g., Thuiller et al., 2005; Bellard et al., 2012, see also section

2.4). In turn, this increases the susceptibility of humans relying on them (Funk et al., 2008; Büntgen et al., 2011). Past climate variability, for example, has been linked to mass human migrations as well as the rise and fall of the Roman and Mongolian Empires (Büntgen et al., 2011; Pederson et al., 2014).

This work focuses on a severe spring drought that occurred in 2002. Compared to the 1961-1990 climate normal, this drought represented a drop of one standard deviation in mean annual precipitation, while standard deviations from mean precipitation in June, July, August and September were -0.61, -0.62, -0.90 and -0.75, respectively (according to ClimateWNA, Wang et al., 2016). This drought was problematic across Canada (Wheaton et al., 2005; Williamson et al., 2009), the United States of America and Mexico (Cook et al., 2004). In two Canadian prairies alone, this drought caused multi-sector impacts totalling billions of dollars in lost gross domestic product and job losses exceeding 40,000 (Wheaton et al., 2005).

Greenhouse Effect and Atmospheric Carbon

The mechanism behind climate warming relates to positive ‘forcing’ effects of greenhouse gasses. When thermal radiation absorbed from the sun is re-radiated by the Earth, greenhouse gasses cause thermal radiation to be re-radiated back to Earth. Greenhouse gas molecules include water vapour, carbon dioxide (CO₂), methane (CH₄), nitrous oxide (N₂), ozone (O₃) and other gasses. Adding more of these greenhouse gasses to the atmosphere intensifies the greenhouse effect. Greenhouse gas emissions have increased substantially since pre-industrial times. It is estimated that over half the global average surface temperature increase since 1951 is due to anthropogenic forcings such as greenhouse gas concentrations (IPCC, 2014). Concentrations of carbon dioxide in particular are increasing faster than other greenhouse gasses (IPCC, 2014). Carbon dioxide contributes approximately 78% to the total greenhouse gas

emissions increases and is therefore “the largest single contributor” from 1750 to present (IPCC, 2014). There is a strong and consistent relationship between cumulative carbon dioxide emissions and projected global temperatures (IPCC, 2014). The current *absolute* values of atmospheric carbon concentrations are unprecedented over at least 800,000 years (IPCC, 2014).

The *rate of change* of atmospheric carbon dioxide is also unprecedented over at least the last 20,000 years. This is known through multiple observations or proxies: Values from 1951 to present are derived from precise, accurate and systematic data records from the Mauna Loa Observatory in Hawaii. Earlier estimates of global average abundances of atmospheric carbon dioxide are derived from dated ice cores (Hartmann et al., 2013), or other proxy methods, for example: isotope ratios in peat (White et al., 1994); C4 plant cellulose (Marino & McElroy, 1991); and tooth enamel (Cerling et al., 1997). The 7,000 years prior to the year 1750 had values rising very slowly from 260 ppm to 280 ppm, while values during the past 800,000 years ranged from 180 ppm during glacial periods to 300 ppm during warm periods (Ciais et al., 2013).

The increase in carbon dioxide is attributed primarily to emissions from fossil fuel burning and land use change (IPCC, 2014). Evidence of fossil fuel burning is apparent through changes in carbon isotope ratios of atmospheric carbon dioxide. Fossil fuels are derived from ancient plants which, like many plants today, discriminated against heavier isotope of carbon (carbon-13) relative to more abundant carbon-12 (see section 2.10). Fossil fuels therefore have lower ratio of carbon-13 to carbon-12. Burning of fossil fuels thus changes the ratio of stable carbon isotopes in the atmosphere, known as the Suess effect (Suess, 1955; Treydte *et al.*, 2009; Dean *et al.*, 2014). Earth has seen a large increase in global atmospheric carbon concentration change to current levels above 407 ppm (e.g., July 17, 2017: 407.47 ppm, www.co2.earth/daily-co2). RCP 8.5 projections of atmospheric carbon dioxide exceed 1,000 ppm by the end of the

century; natural processes would take a few hundred thousand years to remove the emitted carbon dioxide, and is thus considered irreversible on a human time scale (Ciais et al., 2013)

2.2 Tree Growth and Climate Change

Measures of Forest Productivity

Forest productivity is of interest economically and also for ecosystem health: Reduced growth can be used as an indication of tree stress, which increases susceptibility to insect attack (Hogg et al., 2002; Aubin et al., 2016). Growth can also be used as a measure of fitness (Wu & Ying, 2001), and to estimate carbon absorption and storage. Modeling of growth often relies on tree height as well as diameter at breast height (1.3 m) at a certain tree age. Diameter can also be used to calculate basal area, which is the surface area represented by the trees across a management unit. Also useful is the ability to reconstruct annual growth increases. Height increment is the additional height gained each year and can be estimated by measuring the distance between branch whorls (Fig. 2.2). Basal area increment (BAI) is the total surface area of annual growth and it can be calculated from tree-ring widths. Although increment borers are most often used to sample living trees, when possible, stem disks enable a more precise estimation of BAI because there are more radii from which to calculate tree ring widths (Fig 2.3).



Fig. 2.2. Height increments can be estimated by measuring the distance between branch whorls.

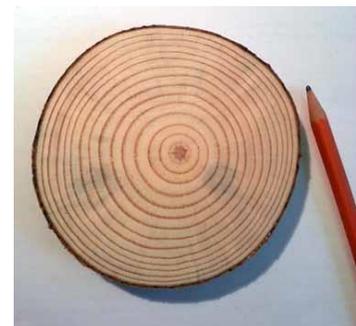


Fig. 2.3. Annual growth rings (tree rings) can be used to measure basal area increment.

Forest and Lodgepole Pine Growth under Climate Change

With rising temperatures and atmospheric carbon, changes to growth are expected for many trees. Earlier reports indicated that net primary productivity was increasing (Nemani et al., 2003), but recent global reports indicate more conservative estimates (Zhao & Running, 2010). As with rising temperatures and probability of extremes, forest responses to these changes may vary by region. For example, rising temperatures and longer growing seasons have been linked to increased productivity in the temperate forests of Europe (e.g., Spiecker et al., 1996; Pretzsch et al., 2014). In the Canadian context, however, projections of forest growth are expected to vary in direction and magnitude according to region (Lemprière et al., 2008). Recent work, for example, shows that forest growth patterns in the boreal have been increasing or decreasing depending on region (Girardin et al., 2016).

For lodgepole pine in western Canada, growth predictions are conflicted, with indications of growth increases or decreases based on region (Wang *et al.*, 2006, 2010; Coops & Waring, 2011; Hember *et al.*, 2018). Importantly, however, many such models look at species-level changes, not at sub-species populations. By accounting for population-level climate genetic adaptation in the case of lodgepole pine, such estimates of increased forest productivity are reduced (O'Neill et al., 2008). The consequence of maladaptation to new climate assemblages, including extreme events, are reduced forest health and productivity (O'Neill et al., 2017), losses in economic value (Hanewinkel et al., 2012) and reduced provision of ecosystem services (Mina et al., 2017). Reduction in net primary productivity could further contribute to feedbacks through carbon dioxide forcing (Bonan, 2008; Kurz et al., 2008; Zhao & Running, 2010).

2.3 Forest Tree Populations in a Warmer Climate

Genetic Adaptation of Forests to Local Environments

Over generations, trees become adapted to their local environments, including climate. For wide-ranging tree species, populations within the species range can exhibit strong differences that reflect varying climate conditions. Despite high levels of gene flow, selection pressures at the environment are more influential (Aitken & Whitlock, 2013; Alberto et al., 2013). Such local adaptation is indicated by higher fitness in nearby planting locations and reduced fitness in other areas of the range (Savolainen et al., 2007). Local adaptation can be reflected in steep clinal variation in phenotypic traits. This means that variation is expressed across a gradient corresponding to an important environmental gradient. For example, height changes gradually across the climate differences caused by latitude (gentle cline) compared to more rapid changes across an elevation gradient (steep cline). Such clines have been well-studied in several conifer species, for example, in Scots pine (Rehfeldt et al., 2002; Savolainen et al., 2004) and lodgepole pine (e.g., Rehfeldt, 1980, 1983, 1987, 1988; Xie & Ying, 1995; Rehfeldt et al., 1999; Wu & Ying, 2001; Wang et al., 2006; Liepe et al., 2016). Many adaptive traits are polygenic in nature, meaning many genes with small additive effects are involved (Yeaman, 2015). For adaptation to cold, for example, 47 genes contribute to the control of expression (Yeaman et al., 2016).

Although the genetic mechanisms were not understood, the geographic nature of local adaptation has been long recognized (Morgenstern, 1996; Aitken & Bemmels, 2016). Recognition of adaptation and the need to match the right trees to appropriate environments led to the regulation of seed procurement through seed transfer limits and breeding zones (O'Neill & Aitken, 2004; Ying & Yanchuk, 2006; O'Neill et al., 2017). In British Columbia, for example,

local seed sources were used for reforestation, but, as the size of reforestation programs grew, seed zones began to be delineated in the 1940s (Ying & Yanchuk, 2006). A geographic transfer system was developed in 1987 (O'Neill et al., 2017). Such regulations were historically based on geography, because increasing risk of maladaptation is associated with distance of seed transfer (O'Neill & Aitken, 2004). These historic seed transfers were based on a system that either relies on fixed zones, which permit transfers within a designated area only, or focal point zones, which were based on the distance from the seed origin (Ukrainetz et al., 2011; O'Neill et al., 2017). Focal point zones were less commonly used (O'Neill et al., 2017), but lend a greater area of use without increasing maladaptation (Ukrainetz et al., 2011). Such “floating seed transfer” distances were determined based on modelled adaptive clines (Ying & Yanchuk, 2006).

Genetic Maladaptation under Climate Warming

Due to the strong adaptation to climate, forest populations may be strongly affected under changing climates. Altered climate patterns could cause maladaptation of forests. Warming causes increasing water deficit and drought stress on trees, for example, which has been linked to increased tree mortality in the western United States (van Mantgem et al., 2009). Since warmer winters are projected for the future, for example, the patterns of chilling requirements affecting cold hardiness and dormancy could be altered (Vitasse et al., 2014). Cold temperatures are required prior to bud flush in spring; plants not meeting chilling requirements may perform poorly (Laube et al., 2014).

It is also well recognized that, under climate warming, the optimal climate spaces to which species are adapted are shifting poleward and higher in elevation (Davis & Shaw, 2001; Walther et al., 2002; Hampe & Petit, 2005; Thuiller et al., 2005, 2008; Loarie et al., 2009). Most plant species across landscapes cannot naturally shift their ranges at the rate required for current

and projected warming (Peters & Darling, 1985; Jump & Peñuelas, 2005; Aitken et al., 2008; Gray & Hamann, 2013; Williams & Dumroese, 2013; IPCC, 2014). These issues are especially challenging for trees, which are long-lived organisms (Aitken et al., 2008; Alberto et al., 2013; Aitken & Bemmels, 2016). Historic tree species migration rates across the landscape have been estimated to be 100 m/year (1 km/decade) (Aitken et al., 2008), while current climate change is shifting suitable habitat by at least 100 km/decade (Wang et al., 2006; Loarie et al., 2009; Gray & Hamann, 2013); leading to a growing ‘adaptation lag’. Genetic change in trees is also slow, lagging behind moving optimums. Despite high gene flow (Kremer et al., 2012), the ability to evolve in situ to climate change within projected 100-year time-frames is limited given the long tree generation times (Rehfeldt et al., 2001; Savolainen et al., 2004; St Clair & Howe, 2007). Phenotypic plasticity may also be exceeded (Duputié et al., 2015). Importantly, such mismatches are expected to occur not just at the trailing edge of a species range, but throughout the distribution due to tree population climate adaptation (Davis & Shaw, 2001; Aitken et al., 2008). For example, O’Neill *et al.* (2008) found substantial additional loss of growth due to maladaptation when lodgepole pine models accounted for differences at the population level.

Due to their long-lived nature, a typical planning horizon for forest management will encompass much change. The limits of phenotypic plasticity to changing conditions, while generally high in trees (Alberto et al., 2013), may eventually be exceeded for some populations of some trees (Duputié et al., 2015). Human attempts at re-aligning tree populations with their optimal climate spaces may be difficult as trees will experience this optimum for only part of their life span. This is a challenge to maintaining healthy, productive forests.

2.4 Forests and Extreme Climatic Events

Maladaptation to Extreme Climatic Events: Discussing Drought

According to Reyer *et al.* (2013), plants are more vulnerable to extreme events, such as drought, than they are to mean climate change. While some net primary production increased globally in the 1980's and 1990's (Nemani *et al.*, 2003), global net primary productivity began declining in the 2000's due to the effects of drought (Zhao & Running, 2010). Drought has also already led to reduced forest productivity and survival in western North America and is expected to continue to do so (van Mantgem *et al.*, 2009; Williamson *et al.*, 2009; Allen *et al.*, 2010, 2015; Michaelian *et al.*, 2011; Peng *et al.*, 2011; Anderegg *et al.*, 2013a; McDowell *et al.*, 2015; Restaino *et al.*, 2016).

Using tree-ring analyses to determine the impacts of drought on growth can be conducted with super-epoch analysis (see e.g., Hartl-Meier *et al.*, 2014). These analyses involve standardizing the data to a certain time period (epoch) to understand changes relative to this period of normalization. Here, the three years prior to the drought year in 2002 are used as pre-drought normal growth conditions, while a three-year post-drought allows the evaluation of population drought tolerances (Lloret *et al.*, 2011).

Specifically, drought *resistance* is the ability of a population to resist growth loss due to drought. In this work, it is calculated by dividing basal area increment in the drought year of 2002 to its average growth during the pre-drought period. Drought *recovery* represents the speed at which post-drought growth levels are regained, and is estimated by dividing average basal area increment during the post-drought period by its growth in the drought year. Drought *resilience* is the ability to achieve pre-drought performance, calculated by division of post-drought growth to pre-drought performance. Similarly, *relative resilience* accounts for the damage that the population incurred during drought, and is calculated by subtracting resistance from resilience (Lloret et al., 2011, see Fig. 2.4).

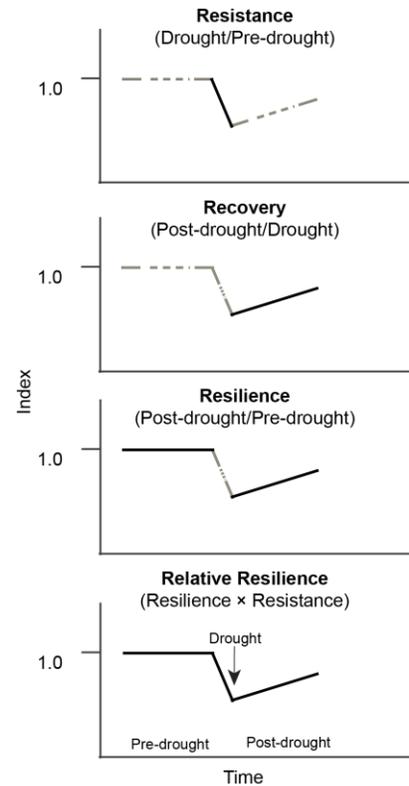


Fig. 2.4. An illustration of the four drought indicators as defined by Lloret *et al.* (2011)

It is also important to look at the mechanisms underlying these growth losses, as the physiological plasticity can influence the expectations surrounding forest tolerance to projected conditions (Anderegg et al., 2013b, 2016; Aubin et al., 2016). For example, a meta-analysis of 475 species recently suggested that drought-induced tree mortality is best explained by a low hydraulic safety margin, defined as “the difference between typical minimum xylem water potential and that causing xylem dysfunction” (Anderegg et al., 2016). In another study, Choat et al. (2012) found that 70% of 226 forest species around the world had narrow hydraulic safety margins; these species have a low ability to tolerate increasing aridity before succumbing to desiccation and mortality. Most trees have evolved to optimize the relationship of water loss and

carbon gain to maximize growth in good conditions in order to gain a competitive advantage (Hartmann, 2011). Through this evolutionary arms race, many plants are operating at lower safety margins to better tolerate competition, although this is a higher-risk strategy under drought conditions. Such drought-induced mortality is therefore expected to become problematic across the globe, not just in drier regions (Choat et al., 2012; Anderegg et al., 2015; McDowell et al., 2015). For example, drought- and temperature-induced conifer mortality in the south-western United States is predicted due to physiological limitations in the coming decades (Anderegg et al., 2015; McDowell et al., 2015). In general, there is a growing need to temporally predict the physiological traits that determine the vulnerability of trees to drought to assess forest adaptive capacity and competitive dynamics under changing climates (Hartmann, 2011; Anderegg et al., 2016; Aubin et al., 2016).

Maladaptation to Extreme Climatic Events: Cold Considerations

Climate change will lead to warmer winters and nights (IPCC, 2014). Although fewer cold temperature extremes are expected (IPCC, 2014), relatively minor cold events may have more biological impact. Warmer winters may increase the degree day warmth accumulation, causing plants to flush earlier in spring. These changes are leading to altered phenological patterns, which have been widely documented (Beaubien & Freeland, 2000; Menzel, 2000; Schwartz & Reiter, 2000; Wolkovich et al., 2012; Reyer et al., 2013). Longer growing seasons also do not necessarily translate into better growth: while plants may flush (leaf-out) earlier, the increasing climatic variability may not lower the probability of a frost event occurring during these critical formative times (Marino et al., 2011; Fu et al., 2014; Bansal et al., 2015). These are often known as “false springs”, where early warmth is followed by a return to colder temperatures. Such an event occurred in many Northern Hemisphere areas in 2017, for example,

resulting in hundreds of millions of dollars of losses to valuable woody crops such as vineyards (France, UK), blueberries and peach trees (US south-east), and hazelnut groves (Italy). Losses to forest trees have not yet been documented, but such events are similarly likely to affect areas with high proportion of young regeneration (e.g. planted areas). Paradoxically, such risks may disproportionately affect northern tree populations. While these populations may be better adapted to fall frost by setting bud earlier, they have a tendency to flush early and begin cambial division due to lower warmth accumulation requirements (Howe et al., 2003; Azad, 2012; Perrin et al., 2017). Variations in phenology may also lead to legacy effects, affecting subsequent senescence and flushing dates (Fu et al., 2014). Such risks must also be considered as warm-adapted populations are being considered for planting in more northern areas under planting designs aiming to improve drought tolerance.

2.5 Interacting Stressors: Fire, Pests and Pathogens

Changing Fire Regimes

Many aspects of the biosphere are intertwined with climate. Two main concerns under climate change are genetic maladaptation to new climates, as already discussed, and altered forest disturbance patterns (Price et al., 2013). For example, the frequency, intensity, duration and timing of forest fires may change (Dale et al., 2001). Although not necessarily linked to dead standing timber from the recent mountain beetle epidemic (Hart et al., 2015; Meigs et al., 2015), conditions are generally becoming more conducive to fire: As the fire season lengthens, forest fires are predicted to become more frequent and burn more area across much of the Canadian boreal (Wotton & Flannigan, 1993; Flannigan et al., 2005; De Groot et al., 2013; Abatzoglou & Williams, 2016; Harvey, 2016).

Lodgepole Pine Forest Pests and Pathogens

Climate also affects the lifecycles of forest pests as they are dependent on temperature (Carroll et al., 2003) and are sensitive to climate effects (Volney & Fleming, 2000). For example, colder temperatures historically limited mountain pine beetle (*Dendroctonus ponderosae*) along the -40°C isotherm (Safranyik, 1978). Older lodgepole pine are the preferred host species to mountain pine beetle (Williamson et al., 2009), with faster growing populations being potentially more vulnerable to attack (Yanchuk et al., 2008; de la Mata et al., 2017). Warmer temperatures, especially warmer winters (Williamson et al., 2009), are therefore facilitating range expansion of mountain pine beetle into new areas of the widespread species distribution of lodgepole pine (Carroll et al., 2003). Further, warmer conditions may enable a doubling of annual breeding cycles (more generations per year), further facilitating the pace of disturbance (Mitton & Ferrenberg, 2012), with potential to expand their range across the boreal (Safranyik et al., 2010).

Depending on precipitation patterns, forest pathogens may also become more problematic under warmer conditions. For example, occurrences of needle blight often affecting lodgepole pine (*Dothistroma* spp) are linked to climate (Welsh et al., 2014) and could become more prevalent if precipitation and moisture do not decrease under warming (Woods et al., 2016). If precipitation does decrease under climate change, however, then the incidence of *Armillaria* spp root disease could increase in forests (Klopfenstein et al., 2009; Sturrock et al., 2011). *Armillaria* is problematic for lodgepole pine tree regeneration (Dempster, 2017). According to Dempster (2017), other causes of mortality in lodgepole pine include Western gall rust (*Endocronartium harknesii*), Warren's root collar weevil (*Hylobius warreni*), Atropellis canker (*Atropellis piniphila*), and blister rusts (*Cronartium* spp.). Therefore, lodgepole pine trees of all ages may be affected by various biotic interactions under climate change.

Cumulative Stresses

Trees experiencing stress have also been linked to increased susceptibility to biotic stressors: There may be increasing synergistic impacts among disturbance and maladaptation due to cumulative stresses on the tree (Aubin et al., 2016). Cumulative stresses from drought and insects can make trees more susceptible to subsequent pest invasions (Hogg et al., 2002). Low precipitation can lower tree vigour, reducing the production of defensive resins against mountain pine beetle (Safranyik et al., 2010). As described above, moisture stress can also lead to higher initial susceptibility to *Armillaria* root disease (Dempster, 2017), which may limit lodgepole pine regeneration. Furthermore, *Armillaria* is a primary pathogen that weakens the tree; it then becomes more vulnerable to secondary agents (Sturrock et al., 2011). Disturbance regimes can also interact with extreme climatic events, impacting forests; post-fire regeneration may be limited under the increasingly dry conditions predicted in the future (Hogg & Wein, 2005). Although uncertainties exist due to possible interactions, multiple changes to disturbance patterns are expected by the end of this century (Price et al., 2013). Such non-climatic factors may limit range expansion under climate change, as found for soils and seed predation by Brown (2014).

2.6 Human-Assisted Adaptation of Forests to Future Climates

Human Intervention: Matching Populations to Future Climates

To overcome challenges of forest maladaptation to future climates, assisted migration plans have been proposed (Peters & Darling, 1985; St Clair & Howe, 2007; Aitken et al., 2008; Pedlar et al., 2012). These involve the intentional translocation of individuals northward or higher in elevation to re-align them with their optimal climates (Pedlar et al., 2012); mixing a

proportion of southern seed sources into local populations (St Clair & Howe, 2007). This involves using genecology studies to “mimic” natural migration patterns to realign tree populations with projected future climates (St Clair & Howe, 2007). In addition to maintaining forest productivity, it also has implications for the ability of a forest stand to regenerate. For example, a transfer of lodgepole pine seed sources from lower-elevation sites upslope has been recommended to improve seed recruitment under warmer conditions (Conlisk et al., 2017).

There is growing interest on the topic of such human intervention reforestation strategies, as highlighted by intensifying scholarly debate and an increasing number of definitions (Hewitt et al., 2011; Hällfors et al., 2014). For example, assisted migration is an umbrella term that can encompass assisted colonization (Hoegh-Guldberg et al., 2008; Gray et al., 2011), assisted range expansion (Aitken & Whitlock, 2013), or assisted gene flow (Aitken & Whitlock, 2013). Assisted colonization involves long-distance transfers of species of conservation concern and is conducted as a “species rescue” strategy (Gray et al., 2011; Pedlar et al., 2012). These are associated with more risks (e.g. of species invasion or unintended consequences), which are often discussed in conservation-oriented literature (Mueller & Hellmann, 2008; Ricciardi & Simberloff, 2009; Vitt et al., 2009, 2010). Assisted range expansion proposes moving populations from the leading edge of a species range in accordance with climate projections, helping to establish new leading edges more rapidly (Ste-Marie et al., 2011; Pedlar et al., 2012; Aitken & Whitlock, 2013). Finally, assisted gene flow, also referred to as assisted population migration, involves translocations within current species ranges (Ste-Marie et al., 2011; Pedlar et al., 2012; Aitken & Whitlock, 2013; Williams & Dumroese, 2013). Assisted gene flow would be the most commonly-used approach for most commercial tree species and would help to maintain ecosystem services (Pedlar et al., 2012). In the context of post-harvest reforestation, for example,

planting could be conducted with pre-adapted populations procured from more southern or lower-elevation sites. It is considered less risky because planting is done with the same species, with modification of allele frequencies for highly polygenic traits (MacLachlan et al., 2017).

One intention associated with assisted gene flow is to promote genetic diversity, thereby increasing the ability of the system to adapt to uncertain futures (Ukrainetz et al., 2011). Many of the elements involved in assisted gene flow already form part of modern forest management, for example, tree breeding, seed procurement, and planting of improved seed (Pedlar et al., 2012). It is also more economically feasible because the necessary infrastructure is in place for regular management; only minor modifications to the system would need to be made (Pedlar et al., 2012).

Risks, Objections and Social Acceptability of Assisted Gene Flow in a Forestry Context

As forests begin to incur damage that is attributed to climate change, assisted gene flow is being discussed more frequently as a possible solution. Some argue that the risk of reduced forest health and growth warrant change in reforestation policies; others argue that the risks of inaction outweigh those of action.

Risks of implementing assisted gene flow include inadvertently introducing maladaptation through improper alignment of populations and their climate optima. By missing the mark, the concern is that cold damage could be incurred. For example, Benito-Garzón *et al.* (2013) cite widespread cold-induced damage and mortality in pine plantations covering vast areas of south-western France. Provenances were transferred from the Iberian Peninsula due to their superior performance but succumbed to a severe frost event in 1985. Some authors suggest a mismatch of photoperiod could possibly affect phenology (Way & Montgomery, 2015),

although temperature is considered to be more important to budbreak than photoperiod (Laube et al., 2014; Zohner et al., 2016). Further, short latitudinal or elevation transfers will not substantially affect photoperiodic signals (Aitken & Bemmels, 2016). Concerns have also been raised regarding overly-simplistic models of complex ecosystems. For example, in the eastern United States, spruce has been predicted to shift its range higher in elevation, but the range is actually moving downslope (Foster & D’Amato, 2015). Such discrepancy can result from species distribution models being trained using the realized niche, not the fundamental niche: Areas where the spruce had occurred were high in elevation, but this distribution reflected the fact that high-quality timber in lower elevations had been long-ago been removed for human settlement. The current distribution can thus be more heavily influenced by non-climatic factors, limiting the value of bioclimatic envelope modeling in such cases.

The risks of inaction, however, include possible decreased productivity, decreased timber supply, and increased susceptibility to pests and pathogens. Implicit is the anthropogenic-oriented value that we should aim to maintain the ecosystems in a way that allows us to meet our needs without compromising the ability of future generations to meet their needs. Some authors indicate urgency, since adaptation lags are already occurring, as evidenced by increased growth when tree populations are moved northward (Gray et al., 2011; Schreiber et al., 2013a).

While earlier studies recommended “wholesale redistribution of genotypes on the landscape” (Rehfeldt et al., 1999) over hundreds of kilometers (e.g., St Clair & Howe, 2007; Gray & Hamann, 2013), most current literature suggest more cautious approaches. For example, shorter transfer distances are recommended (Aitken & Bemmels, 2016). It is also recommended to incorporate only a percentage of pre-adapted seed sources, known as composite provenancing (e.g., Ukrainetz et al., 2011; Prober et al., 2015; Aitken & Bemmels, 2016). It has also been

suggested that planting a composite selection of seed sources would have a relatively minor effect on the landscape in western North America, where annual planting represents a relatively minor contribution. Assisted gene flow can also only be implemented for few species with large, diverse ranges, and may be further limited by local conditions such as soils.

In addition to the science, personal values determine social acceptability to human intervention in forest ecosystems under climate change. Some argue that it is best to let nature reconfigure, without human interference. This would allow and accept changing ecosystem dynamics, shifting species ranges and possible no-analog species compositions (Williams & Jackson, 2007). This emphasizes the dynamic nature of ecosystems as well as climates and ecosystems with no current equivalent (Williams & Jackson, 2007). The sense is that historic natural selection should be respected and that natural selection will continue to produce a forest that “should be”, irrespective of human needs and wants (Hajjar et al., 2014). For this reason, survey results have shown that social acceptance of assisted migration is not always positive (Hajjar *et al.*, 2014): While public and policy leaders were generally accepting of some human intervention in reforestation, the level of acceptance for using non-local seed sources was only moderate despite complex evaluation schemes that incorporated potential outcomes (Hajjar et al., 2014). There was a sense that forests could be made more adaptable and resilient through diversification using local seed sources only (Hajjar et al., 2014).

To make accurate predictions is therefore important both from a scientific and from a social acceptability perspective. To improve accuracy, broad generalizations should be avoided. This argues for more detailed studies of responses of tree species and tree populations to climate warming and extreme events. This is the approach used herein, with a focus on population responses used to weigh possible risks and benefits of reforestation options. The focus is on

establishing the need for assisted migration (or not) and delineating limits to acceptable seed transfer to prevent inadvertent maladaptation.

2.7 Provenance Trials for Studying Climate Adaptation

Provenance trials are reciprocal transplant experiments that involve collecting seed sources (provenances) from across a species range and planting them at field test sites (common gardens). Since conditions at any given test site are relatively uniform, measurements of quantitative phenotypic traits (e.g. height, growth, branchiness, etc.) from common gardens can be used to infer the genotype. Since provenances are tested across a wide range of conditions, such experiments facilitate the study of genotype by environment interactions as well.

Response functions use planting sites to gauge how tree population traits change over a broad range of environments (Fig. 2.5). Plotting the responses of different genotypes across multiple planting sites enables the determination of genotype by environment interactions: Not all seed sources will maintain their performance across all environmental conditions or over time.

Changes in the relative ranking of phenotypes can occur across space and time, known as a rank change. Different seed sources

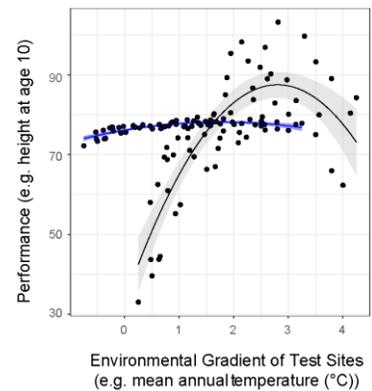


Fig. 2.5. Illustration of a response function, where performance of different seed sources is tested on several planting sites representing many conditions.

have different climatic optima (Kremer et al., 2012); phenotypic expression of different genotypes vary across a range of conditions. Transfer functions compare seed source performance at any given planting site given certain transfer distances (Fig. 2.6). These can be used to develop critical seed transfer distances, which ensure growth of at least 90% compared to local seed sources (O'Neill et al., 2017).

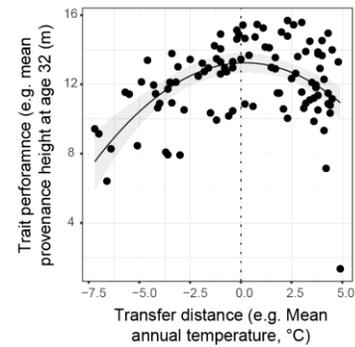


Fig. 2.6. Illustration of a transfer function, showing trait performance relative to transfer distances tested in a provenance trial.

Historically, provenance trials were designed for forest genetic purposes, e.g., to compare seed sources for timber quality and growth rates (Matyas, 1994). Due to their experimental design, it is possible to establish the optimal climate space as well as the limits of productivity for tree populations within the larger species range. More recently, they are being used to study real-world responses to climate warming since trees are grown in real field conditions. Response functions can be used to make predictions under climate change (O'Neill et al., 2008). Analyzing the responses of trees under different geographic seed transfer distances also represents climate transfer distances (Matyas, 1994; Schmidting, 1994): Transferring seeds from northern environments to warmer, southern planting sites can simulate a warmer climate that could be similar to projected warming. At the same time, transferring southern seeds to northern planting sites represents an assisted gene flow scenario and can be used to assess the limits to acceptable seed transfer. Another advantage afforded by the randomized block design used in many provenance trials is replication and statistical removal of nuisance effects due to site blocks.

Anderegg (2015) indicates that genetic provenance trials have limited utility in determining the response of natural ecosystems to changing climates due to bias oriented toward economically valuable (i.e., timber) species. However, their value as timber species is a by-

product of their ecological predominance and importance in natural ecosystems. The boreal, for example, includes only a limited number of tree species, but these are widespread across continents. Provenance trials can therefore provide information and predictive capacity under changing climates for the most important foundation species globally.

2.8 Tree Rings as Paleoarchives

Trees act as reliable bio-physio-chemical recorders: Annual growth rings in the tree xylem reflect the conditions under which they were formed. Tree rings can thus be used to infer how trees responded physiologically to varying environmental conditions (Fritts, 1976; Speer, 2010). This is useful in assessing the adaptation of tree populations to climate change. For these purposes, tree-ring series must be exactly dated. Cross-dating involves overlapping the pattern of wide and narrow tree rings from various samples of timber to develop a bridge into the past, allowing exact calendar dates to be assigned (Fritts, 1976; Speer, 2010). In this way, tree rings are absolutely dated archives of past response of trees to growth.

Tree ring science began with observations by Leonardo DaVinci that tree rings had an annual character and were linked to environmental causes (Speer, 2010). More important tree-ring observations were recorded in the mid-1700s, including reports of a frost-damaged ring from 1709, that has since become an important “marker ring” in Europe (Speer, 2010). Modern dendrochronology and recognition of the power of cross-dating began in the early 1900’s, starting with an astronomer, Andrew Ellicott Douglass (Fritts, 1976; Speer, 2010). Douglass became interested in the link between the solar sunspot cycle and its influence on terrestrial climate, suspecting tree growth could act as a climate proxy (Fritts, 1976; Speer, 2010). Edward Schulman, first a student of Douglass and later a colleague, advanced the fields of

dendrohydrology and dendroclimatology, using dendrochronology for climatic reconstructions (Fritts, 1976; Speer, 2010). Since these early times, researchers have realized the potential for acquiring different signals encoded in tree rings that can act as various proxies. On moisture-limited sites, for example, tree-ring widths often reflect precipitation, while tree ring patterns on temperature-limited sites often reflect warmth. Stable isotope ratios, functional wood anatomy data and wood density signatures from tree rings all serve as important proxies for different dendroclimatological or dendroecological studies, discussed next.

2.9 Wood Tissue Structure and Adaptive Functions

Wood Tissues and Function

Wood tissue supports several functions: structural support, water transport and carbohydrate storage. Xylem is the woody structure comprising most of the tree stem and conducts water from roots to the crown, while phloem occurs on the periphery of the stem and is responsible for transporting water and carbohydrates from the crown to the stem and roots. Both xylem and phloem are formed by a contiguous layer of meristemic tissue under the bark around the tree stem, known as the cambium. This is a specialized tissue producing new cells through division; it divides into xylem or phloem cells. This research focuses on xylem cell structure to infer function and tree water-use strategies. Coniferous xylem has two main cell types: parenchyma and tracheids.

Tracheids form most (~90 - 93%) of coniferous wood (Tyree & Zimmermann, 1983; Hacke et al., 2015) and are studied here (Fig 2.7). They are relatively thin but long cells with tapered ends; they die after formation. Tracheids have a dual role in conifers: Their lignified cell walls provide structural support while the empty cell centers (lumen) act like water pipelines. Water can flow between tracheids because they are connected to each other through small, valved openings in the double cell wall known as bordered pits (Tyree & Zimmermann, 1983; Hacke et al., 2015).

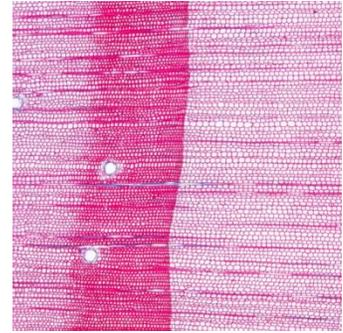


Fig. 2.7. A micro section of a tree ring boundary with latewood on the left and earlywood on the right. Such cross-sections of tracheids enable measurements of lumen diameters and cell wall thickness. Section and micrograph (20×) by David Montwé.

Bordered pits are structures that have porous edges allowing water flow between cells (margo), as well as a central, impermeable piece (torus) that closes under embolism. The thickness of the cell wall and the diameter of the lumen vary by species, genotype and between rings of the same tree (related to distance from pith or apex). They also change within the same tree ring, where tracheids formed early in the growing season (known as earlywood) have relatively larger lumen and more narrow cell walls than tracheids formed later in the growing season (known as latewood). These parameters are important for drought and water use.

Drought Tolerance through the Lens of Functional Wood Anatomy

Water flows from the roots to the leaves along what is called the Soil-Plant-Atmospheric-Continuum thanks to cohesion-driven water transport (Tyree & Zimmermann, 1983). Because leaves of plants are transpiring water during photosynthesis, this creates negative pressure (tension). Due to hydrogen bonding among water molecules, tension causes water to be pulled from the xylem and roots along a pressure gradient to the source of the lowest pressure, the leaves. The drier the atmosphere, the more water is lost from the leaves, building stronger

tension. A chain of water can tolerate tension due to the super-saturated nature of xylem cells, adhesion to the plant cell wall and the aforementioned hydrogen bonding.

Tracheids that are wider have larger lumen allowing more water flow leading to higher hydraulic efficiency, the flow rate for which can be calculated from lumen diameter using the Hagen-Poiseuille equation (Tyree & Zimmermann, 1983; Sperry et al., 2006). Hydraulic efficiency is important as it allows increased carbon assimilation through higher photosynthetic rates, helping to improve growth (Pittermann et al., 2005; Pratt et al., 2008). At the same time, however, wider tracheids are more susceptible under drier conditions: When the tension becomes too great, the water column can be broken, creating an air embolism in the cell. Such cavitation leads to loss of conductivity of the xylem. This is problematic for the tree as it reduces the transport of water to the above tissues, e.g. to the photosynthesizing crown. Such interruptions to the transpiration stream reduce leaf pressure (loss in turgor pressure), which can lead to canopy desiccation (wilting). Xylem conductivity loss can be tolerated by the tree to a point known as P_{crit} , or the critical canopy xylem pressure (Sperry et al., 2017). Beyond P_{crit} , it will not recover, leading to permanent wilting and “hydraulic failure” (Sperry et al., 2017). (Note that, in nature, trees may not necessarily succumb to hydraulic failure but instead become more susceptible to pests and pathogens, which may become the eventual agent of mortality.) In contrast, narrower xylem conduits are more resistant to cavitation (Sperry et al., 2006), but sacrifice growth through lower hydraulic efficiency.

In addition to modifications made to the size of the tracheid lumen diameter, structural support of the xylem can confer drought tolerance: Structural support protects tracheids against implosion due to increasingly negative water pressures under drier conditions (Sperry et al., 2006). Lignin surrounding cellulose polymers provides the required support (Sperry et al., 2006),

although, generally, it can be quantified as the thickness of the tracheid cell wall. Another important parameter is the thickness-to-span ratio (tb_h^2): the ratio of the thickness of the double-cell wall to lumen diameter (Hacke & Sperry, 2001). The ability to increase wall thickness is limited, and instead, the ratio is altered through lumen diameter (Pittermann et al., 2006; Sperry et al., 2006). Thicker cell walls and a higher thickness-to-span ratio increase cavitation resistance under increasingly negative water tensions during drought stress. Due to these cell wall reinforcements, for example, latewood cells are more cavitation resistant than earlywood cells. The ability of trees to acclimate to changing conditions by modifying these structures during the formation of new xylem influences their tolerance to drought as well. For example, Norway spruce has shown a high capability of responding to drought events by modifying their internal structure (Montwé et al., 2014).

Due to the selective pressure involved in reducing the problem of cavitation (Hacke et al., 2015), conifers have also evolved bordered pits (Pittermann et al., 2005). These pits allow water to flow under normal conditions. If a cell is embolized, however, the pit torus is pushed against one side – closing the pipeline between the neighboring cells. This prevents the air from seeding into other cells, which could lead to run-away cavitation. Since bordered pits cause flow resistivity (Hacke et al., 2015), tracheids with larger lumen facilitate higher hydraulic conductance through proportionally larger pits as well. At the same time, however, larger bordered pits are more prone to air seeding that can lead to embolism (Sperry et al., 2006). Smaller cells are more resistant to cavitation in part due to their proportionally smaller pits (Hacke & Sperry, 2001), but reduce water flowing to the canopy and inhibit growth.

Due to these structural adaptations, there is a well-documented safety versus efficiency trade-off (Hacke & Sperry, 2001; Pittermann et al., 2006; Gleason et al., 2016). Hydraulic

efficiency promotes growth, but incurs more risk; reduced growth is the cost of hydraulic safety. Small changes in lumen diameter strongly affect conductivity (Sperry et al., 2006). Such morphology is optimized according to local conditions and is therefore likely to be under selective (evolutionary) control; the resulting structural adaptation is tested here by studying tracheid lumen diameter and wall thickness. Given the importance of xylem traits to the water transportation system, they are of adaptive importance to tree drought tolerance, and therefore, forest performance and mortality under drought. The diversity of wood anatomy is driven in part by trade-offs in function (Sperry et al., 2006); Chapter 6 discusses intra-specific adaptations.

Tracheid Structure and Cold Tolerance

Wood anatomical features have also been linked to cold tolerance. As with drought, there is a trade-off in the size of tracheids and cold tolerance (Pittermann & Sperry, 2003; Schreiber et al., 2013b). Tracheids that have thicker cell walls and smaller centers (lumen) are less vulnerable to cold damage and freeze-thaw induced embolisms that can threaten the hydraulic pipeline in the same way as severe drought. Wider conduit diameters increase the vulnerability to freezing-induced embolism (Pittermann & Sperry, 2003; Sperry et al., 2006; Schreiber et al., 2013b). Xylem bubbles expand when the water thaws after being frozen because transpiring leaves cause tension. Trees growing in colder climates adapt by growing stem conduits with narrower lumen – but this also reduces growth. Finally, frost imprints in tree rings indicate reactions to particularly severe cold temperatures. While these tend to occur more frequently in small seedlings (Kidd et al., 2014), they can nevertheless be used as a dendrochronological tool, for example in the reconstruction of colder years due to volcanic eruptions (LaMarche & Hirschboeck, 1984). Combined with provenance trials, they can be used to indicate intra-specific genetic differences in cold tolerance, with implications for assisted migration.

Normally, samples are derived from a stem with a known height above the ground. To obtain longer chronologies, dendrochronological studies often aim to collect samples close to the ground. Many forestry-oriented studies aim to collect samples at 1.3m (diameter at breast height; DBH), since many allometric relationships for trees are based on measurements from DBH. For example, tree-ring widths at DBH can be used to calculate basal area increment (BAI), a measure of annual woody growth. BAI has the added benefit of creating a two-dimensional measure useful for tree ring studies (Biondi & Qeadan, 2008; Battipaglia et al., 2015).

A confounding variable affecting such samples used for functional wood anatomical analyses, however, relates to the fact that tracheid size varies by annual rings from the pith. In any given year, the new xylem conduit size is partially determined by the distance to the tree apex in that year (Mencuccini et al., 2007; Carrer et al., 2015). This relates to the tension and flow resistivity: larger xylem conduits are required at the base of the tree to support water flow along the pressure gradient. Therefore, there is a gradient of lumen diameter and, correspondingly, wood density, from the tree circumference to the pith (Fig. 2.8). For this reason, it may therefore be prudent to calculate the distance to the apex for each tree ring year and statistically remove its effects through inclusion as a random effect in a linear mixed effects model.

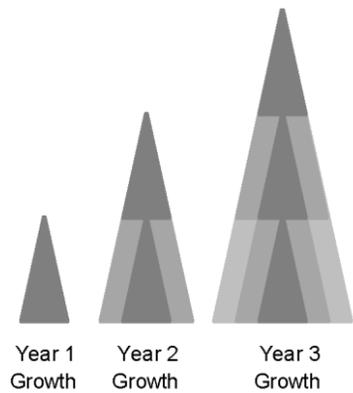


Fig. 2.8. A cartoon of tree growth showing annual variations in tracheid size represented by shades of grey (relatively smaller tracheids are dark; larger tracheids are in light). Tracheids formed in new growth each year vary in size partially due to distance to the apex, creating a gradient along a cross-section to the pith.

2.10 Stable Isotopes in Tree Rings

The Route to Carbohydrate: Stomata and the C3 Carbon Assimilation Pathway

Carbon dioxide enters leaves or needles through stomata. Stomata are pores that control gas exchange from the leaf interior and the atmosphere. The number, distribution, size, and shape vary by species. Stomatal aperture is the width of the pore opening, which most strongly affects water transpiration, known as stomatal conductance (the inverse of which is stomatal resistance). Low humidity, wind, and decreasing water potential affect stomata opening and closing, or stomatal response. If xylem conduits act as a hydraulic pipeline, the stomata act like the tap that helps control the pipeline, regulating the trade-off between carbon gain and water loss. Stomata open and close based on the behaviour of two guard cells at the opening. The behaviour of stomatal conductance optimizes the relationship between carbon uptake and water loss (Sperry et al., 2017).

There are three carbon assimilation pathways that have been evolved by plants: the C3 carbon assimilation pathway involves the Calvin-Benson cycle, the C4 pathway uses the Hatch-Slack pathway, and CAM (Crassulacean Acid Metabolism) plants alternate between both cycles. The C3 pathway is most commonly used by plants: C3 plants form the majority of plants and encompass almost all trees (Pearcy & Troughton, 1975; Leuenberger *et al.*, 1998; Still *et al.*, 2003; Sage, 2014; Sage & Sultmanis, 2016). Therefore, briefly: The Calvin-Benson cycle involves carbon dioxide being fixed directly by the enzyme Ribulose-1,5-bisphosphate-carboxylase/oxygenase (RuBisCo).

Chemical Components of Wood

Photosynthate from the crown is developed into different chemical components forming wood. These include cellulose, hemicellulose, lignin, and extractives. Cellulose is a polysaccharide (complex sugar) that exists as long chains (polymers) formed from the monosaccharide (simple sugar) glucose molecule. The chains are connected through hydrogen-bonding, which contributes further strength. Similar to cellulose, hemicelluloses also provide structural support to wood cells. However, these polysaccharides exist as short polymers made of monomers of several types of sugars other than glucose. It forms shorter, branched chains. Holocellulose is the term used to describe the total combined polysaccharide content of cellulose and hemicellulose in wood (i.e., excluding lignin or extractives).

Lignin is also a complex polymer important in cell walls of wood. It is derived from simple sugars; the exact chemical composition varies by species. This molecule forms cross-links to itself as well as other polysaccharides in the cell wall, providing additional strength. It is analogous to concrete in rebar-enforced building constructions. Lignin is created as the last step of xylogenesis prior to programmed cell death (Rossi et al., 2008). Extractives are small molecules deposited in the heartwood of a tree (the older, central part of the stem that is non-conducting). They generally occur in small amounts, but are easily extracted (hence the nomenclature) with ethanol, acetone and water.

Stable Carbon Isotopes in Tree Rings: A Stomatal Closure Signal

An isotope of an element has a different number of neutrons relative to protons: Carbon-13 has 7 neutrons and 6 protons and occurs more rarely than carbon-12; oxygen-18 is a heavier isotope of oxygen-16. These ratios are discussed using δ notation, which represents deviations from known reference materials according to: $R(\delta) = (R_{\text{sample}}/R_{\text{reference}} - 1)$, where R is the ratio

of the heavier to lighter isotope. Reference material for carbon is Vienna PeeDee Belemnite. The reference material for oxygen is Vienna Standard Mean Ocean Water. The ratios are determined through isotope ratio mass spectrometry (IRMS): After combustion (carbon) or pyrolysis (oxygen) of the samples, the matter is ionized and accelerated through a magnetic field that separates the ions based on mass and charge, which in turn facilitates the measurement of isotope ratios through the resulting spread of mass in the collection bins.

The minor differences at the atomic level develop biological significance because plants favour the lighter isotope during photosynthesis. The carbon dioxide (CO₂) molecules with the heavier isotope are physically discriminated against due to their slower diffusion rate into stomata. In C₃ plants, the heavier isotope is chemically discriminated against at the site of carboxylation by the enzyme RuBisCo, known as carboxylation resistance (Park & Epstein, 1960). The discrimination effects have been empirically shown to be -4.4‰ and -27‰ due to diffusive carboxylation resistance, respectively (Farquhar et al., 1982, 1989; Francey & Farquhar, 1982). These processes of discrimination causing altered the isotopic composition in plant tissues from natural compositions are known as fractionation (Leuenberger et al., 1998). The sugars (sucrose) resulting from photosynthesis reflect such fractionation processes, and the isotopic ratios in the final plant tissues are preserved to varying degrees (Cernusak et al., 2009). Analyzing the stable isotopic signatures in tree rings therefore enables the study of tree response to past conditions. Such fractionation processes also explain why C₃ plants have a mean $\delta^{13}\text{C}$ of -25‰, which is lower than the -8‰ $\delta^{13}\text{C}$ value in the atmosphere (Cerling et al., 1997).

Under moisture-limiting conditions, however, reduced stomatal aperture increases diffusive resistance and lowers internal leaf carbon dioxide. The ability of RuBisCo in C₃ plants to discriminate against the disfavored carbon-13 isotope is lowered; RuBisCo fixes more carbon-

13 than it would under more optimal growing conditions. The increase in carbon-13 fixation increases the ratio of carbon-13 to carbon-12 in the resulting photosynthate (the $\delta^{13}\text{C}$ values become less negative). Therefore, higher $\delta^{13}\text{C}$ values in plant tissue indicate drier conditions, and these can be retrospectively determined through tree rings. The analysis of isotope signatures in wood is therefore based on well-established, leaf-level fractionation processes for carbon (Farquhar et al., 1982; Francey & Farquhar, 1982). It is thus being increasingly used to evaluate eco-physiological responses of trees to changing environments (e.g., Saurer et al., 1997; McCarroll & Loader, 2004; Barnard et al., 2012; Hartl-Meier et al., 2014).

Water Use Efficiency: A Fertilization Effect under Higher Atmospheric Carbon?

Based on empirically-derived models, carbon isotopes can be used to estimate the ratio of carbon assimilation (A) to stomatal conductance (g_s) (Farquhar et al., 1982, 1989; Francey & Farquhar, 1982). This is a measure of carbon gain to water loss, also known as intrinsic Water Use Efficiency (WUE). Depending on definition, WUE can be used to describe processes in hours to entire seasons. Analysing the entire tree ring can provide the response averaged over the full growing season and facilitates inter-annual comparisons. Stable carbon isotope ratios from plant tissues ($\delta^{13}\text{C}_{plant}$) can then be used to solve for intercellular leaf carbon concentration (c_i), knowing: the fractionation due to kinetic diffusion at the stomata is -4.4‰ (a); the carboxylation fractionation is -27‰ (b); the atmospheric carbon concentration (c_a); and the carbon isotope ratios of the atmosphere ($\delta^{13}\text{C}_{air}$). After calculating c_i , WUE can be estimated according to empirically-derived formulas (Farquhar *et al.*, 1982, 1989; Francey & Farquhar, 1982; McCarroll & Loader, 2004; Seibt *et al.*, 2008; Tognetti *et al.*, 2014):

$$c_i = C_a [(\delta^{13}\text{C}_{plant} - \delta^{13}\text{C}_{air} + a)/(b-a)]$$

$$\text{WUE} = A/g_s = c_a [1 - (c_i/c_a)](0.625)$$

Most water transpired by plants is not used in photosynthesis; 97% of water taken up from the soil by C3 plants is considered as water loss (Raven & Edwards, 2001). Meanwhile, water loss is generally higher than carbon gain. An example cited by Leuenberger *et al.* (1998) shows that, at 20°C and 50% relative humidity, the water-vapour gradient from leaf interior to the atmosphere is 20 times stronger than the carbon dioxide gradient from the air to the chloroplast: therefore, water loss is more rapid than the uptake of carbon dioxide. Carbon dioxide must also enter the chloroplasts, so the route is longer, especially as carbon dioxide moves slowly in solution. Water molecules are also smaller than carbon dioxide molecules, leading to rates of diffusion 1.6 times higher than carbon dioxide (Leuenberger *et al.*, 1998).

In atmospheres containing higher concentrations of carbon dioxide, however, WUE is hypothesized to increase: The proportion of carbon dioxide entering the stomata and being assimilated (A) would increase relative to the water loss (g_s). Put another way, stomata can reduce stomatal aperture (g_s) and maintain the same level of carbon gain (A). The “profit maximization” theory indicates that plants instantaneously optimize their stomatal aperture for efficiency between water loss and carbon dioxide (Sperry *et al.*, 2017); an atmosphere containing more carbon dioxide would benefit C3 plants (those suffering from photorespiration) and increase biomass production. For every one ppmv (parts per million – volume) increase in atmospheric carbon dioxide (c_a), Frank *et al.* (2015) estimate an increase in leaf internal carbon concentrations (c_i) of approximately 0.76 ppmv, accounting for realized increased water use efficiencies in the 20th century.

Any positive feedback in terms of growth from fossil fuel burning due to carbon dioxide fertilization is still uncertain, however. The benefits from the increasing fertilization effect are limited under rising atmospheric carbon, while other factors affecting plant growth may

decrease. For example, photosynthetic rates may decline past optimal temperature thresholds, thereby leading to reduced carbon gain and productivity. Photosynthesis and respiration respond to temperature differently (e.g., Turnbull et al., 2001). For example, a very rough generalization provided by Leuenberger et al. (1998) indicates that photosynthesis stops at 0 °C, has optimal rates at around 20-35 °C, declines after 40 °C and stops around 50 °C. Meanwhile, plant respiration rates are low, but begin to increase after about 20 °C and continue increasing. Therefore, a heat limit is reached where the two overlap: approximately 45 °C (Leuenberger et al., 1998). Water stress combined with higher respiration may thus reduce possible benefits of increased atmospheric carbon, which seems to have been confirmed for the last decade (Zhao & Running, 2010). Thus, even if water use efficiency increases, there may be no corresponding increase in growth – this is often debated in the literature (Korner, 2005; Girardin et al., 2011; Brienen et al., 2012, 2017; Babst et al., 2014; Lévesque et al., 2014; van der Sleen et al., 2014), especially since the magnitude and direction can change with different future scenarios for carbon and climate (Gea-Izquierdo et al., 2017).

Although it is uncertain whether or not atmospheric carbon fertilization will increase growth, it raises an important aspect of the problem relying on WUE derived from stable carbon isotopes alone: one cannot be certain regarding the relative rate of change between carbon gain (A) and stomatal conductance (g_s). In addition to stomatal conductance, a change in the photosynthetic rate may also alter this proportion, for example, or the two could act in tandem. This underscores the importance of analyzing stable oxygen isotope ratios to provide a more complete understanding of past physiological responses.

Stable Oxygen Isotopes in Tree Rings: A Proxy for Transpiration

Unlike carbon, whose only assimilation pathway is through carbon dioxide from the atmosphere, oxygen in the tree is primarily derived from water (H_2O). The water molecules can occur in different forms based on the isotopes of the atoms forming the molecule: water formed with oxygen-16 (H_2^{16}O) is more common compared to water made with oxygen-18 (H_2^{18}O) given natural abundances. Roots do not cause fractionation in H_2^{18}O versus the more typical H_2^{16}O : the water taken up by trees reflects soil water. Differences in stable oxygen isotope ratios relative to a standard ($\delta^{18}\text{O}$) within tree rings can also be accounted for through enrichment during transpiration (Dongmann et al., 1974): Since oxygen-18 is heavier, it does not diffuse from the stomata as readily as oxygen-16. The $\delta^{18}\text{O}$ values are therefore positive, reflecting enrichment, with higher $\delta^{18}\text{O}$ ratios indicating higher transpiration.

Transpiration, and the corresponding oxygen isotope ratio of water in the leaf, is highly dependent on relative humidity of the atmosphere, or its inverse, vapour pressure deficit. Given the involvement of leaf-level H_2O in photosynthesis, the leaf-level isotope ratios are reflected in the plant carbohydrates produced. Tree rings provide a useful tool to retrospectively study these responses. Because oxygen isotope ratios react to stomatal aperture (g_s) independent to changes in photosynthesis (A), a dual-isotope approach therefore allow more precise inferences regarding stomatal responses (Saurer et al., 1997; Scheidegger et al., 2000; Barbour, 2007; Barnard et al., 2012). It is nevertheless important to recognize possible caveats: post-assimilation changes to the isotope ratio can occur on the pathway from the leaf to the site of wood formation, and higher transpiration can reduce the effects of leaf H_2^{18}O enrichment due to flushing with xylem water, known as the Peclét effect (Barbour et al., 2004; Farquhar et al., 2007; Offermann et al., 2011; Gessler et al., 2013, 2014; Treydte et al., 2014). Interested readers are referred to a wealth of

literature on this topic (e.g., Saurer *et al.*, 1997, 2008; Scheidegger *et al.*, 2000; Barbour *et al.*, 2000, 2001, 2004, 2016; Roden *et al.*, 2005; Barbour, 2007; Voltas *et al.*, 2008; Battipaglia *et al.*, 2008; Barnard, 2009; Offermann *et al.*, 2011; Tene *et al.*, 2011; An *et al.*, 2012; Holzkämper *et al.*, 2012; Roden & Siegwolf, 2012; Sohn *et al.*, 2012, 2014; Barnard *et al.*, 2012; Gessler *et al.*, 2013, 2014; Hartl-Meier *et al.*, 2014; Haupt *et al.*, 2014; Barbour & Song, 2014; Tognetti *et al.*, 2014; Treydte *et al.*, 2014; Loucos *et al.*, 2015; Cernusak *et al.*, 2016; Lavergne *et al.*, 2017).

2.11 Combining Growth, Functional Wood Anatomy and Dual-Isotopes in a Provenance Trial Setting

In addition to providing an experimental design that simulates climate change and assisted gene flow scenarios, provenance trials facilitate relative comparisons where tree-ring analyses may otherwise be limited. For example, the analysis of stable oxygen isotope ratios in tree rings may also not necessarily reflect leaf-level processes, but soil water as well. The hydrology of planting sites also varies in relation to precipitation and surface evaporation. This can in turn affect the oxygen isotope ratio in water taken up by the tree, which may be used during the formation of wood compounds. This makes stable oxygen isotopes in tree rings useful for a diversity of dendrochronological reconstructions (e.g. Treydte *et al.*, 2006, 2007, 2014). However, it can therefore also cause limitations for ecophysiological interpretations.

Under the experimental design afforded by provenance trials, however, this is less of a concern. All genotypes are grown together in common gardens where environmental conditions are relatively homogenous. Replication through blocks reduces the influence of heterogeneity further and facilitates statistical accounting for any site differences in hydrological patterns. Similarly, any change in isotopic signatures due to demographics is also accounted for with this

experimental design. For example, Brienen et al. (2012, 2017) found biases in dendrochronological conclusions due to demographics. Under typical forest conditions, trees grow through shade stress into drought stress as they reach the canopy, which could influence the stable isotope signatures (Brienen, 2016). Xylem anatomy can also be influenced by shade stress, for example, by reducing lumen diameter (Schoonmaker et al., 2010). This is accounted for by using the provenance trial setting, where trees were grown in a common age structure, so relative comparisons among populations are possible. This is also accounted for using the randomized block design here.

2.12 Physiological Behaviors

When combining the understanding of such leaf-level processes with functional wood anatomy analyses, we can infer a tree's general behaviour related to water use. Further combining the annual resolution afforded by tree rings with genetic provenance trials allows us to assess how different populations have responded to drought in the past. This indicates if their physiological responses will remain effective under climate change.

Physiological Risks and Trade-offs: Isohydry and Anisohydry

Water regulation via stomatal control entails a trade-off between carbon assimilation and water loss. Under dry conditions, the plant will close stomata to prevent extreme negative stem water potential that could risk long-term damage to the plant's hydraulic system through embolism (McDowell et al., 2008). If dry conditions continue, however, the plant risks carbon starvation because a tree's metabolism continues to use carbon (McDowell et al., 2008). This behavior is known as isohydry: the ability to maintain stable mid-day water potentials (Klein, 2014). This type of response involves maximizing growth under optimal conditions, but is

susceptible to prolonged dryness. On the opposite end of a physiological continuum is anisohydry, where plants allow a greater fluctuation of stem water potentials (Klein, 2014). This behavior involves leaving stomata open for longer to allow continued carbon gain, but this increases risks of hydraulic failure (McDowell et al., 2008). Often, this approach is combined with narrower, sturdier stem conduits to withstand negative water potential. Such physiological adaptations can therefore have large impacts on productivity and survival. Further, these behaviors are also not acting in isolation: the trees rarely die of hydraulic failure, but first succumb to biotic stressors.

Differences in these two types of physiological behaviors can be illustrated in a co-occurring Utah juniper (*Juniperus osteosperma*) and Piñon pine (*Pinus edulis*) from the Piñon-juniper forest ecosystems in the south-western United States (McDowell et al., 2008; Hacke et al., 2015). Juniper occurs on lower, drier sites. Pine occurs more frequently in higher elevation sites that receive more precipitation. Juniper trees have narrower conduits, and are considered more drought tolerant. These trees sacrifice some growth due to a lower ability to conduct water to the photosynthesizing crown. It tends to be a more risk-averse strategy. In contrast, the pines maximize growth due to high sap flows when moisture is available, but close their stomata under drought. This avoids too much negative pressure because their xylem is more susceptible to cavitation. These cavitation-avoidance behaviors can partially explain the ranges of these species: pine occurs on higher elevation sites that are less moisture limited than the valleys where Juniper occurs (Hacke et al., 2015).

Many studies compare isotope signatures as well as the spectrum of isohydry and anisohydry among species (McDowell et al., 2008; Hartl-Meier et al., 2014; Klein, 2014; Altieri et al., 2015; Anderegg, 2015), but within-species comparisons are rare (Anderegg, 2015). Intra-

specific differences in the physiological behaviors can be elucidated by combining stable isotope and functional wood anatomy from tree samples taken from genetic provenance trials: If planting sites experience naturally occurring extreme events such as drought, the differences in responses (adaptation or maladaptation) can further be determined.

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3 Growth and Drought Tolerance of Lodgepole Pine

3.1 Publication

Montwé D, Isaac-Renton M, Hamann A, Spiecker H (2016) Drought tolerance and growth in populations of a wide-ranging tree species indicate climate change risks for the boreal north. *Global Change Biology*, **22**(2):806-815.

3.2 Abstract

Choosing drought-tolerant planting stock in reforestation programs may help adapt forests to climate change. To inform such reforestation strategies, we test lodgepole pine (*Pinus contorta* Doug. ex Loud. var *latifolia* Englm.) population response to drought, and infer potential benefits of a northward transfer of seeds from drier, southern environments. The objective is addressed by combining dendroecological growth analysis with long-term genetic field trials. Over 500 trees originating from 23 populations across western North America were destructively sampled in three experimental sites in southern British Columbia, representing a climate warming scenario. Growth after 32 years from provenances transferred southward or northward over long distances was significantly lower than growth of local populations. All populations were affected by a severe natural drought event in 2002. The provenances from the most southern locations showed the highest drought tolerance but low productivity. Local provenances were productive and drought tolerant. Provenances from the boreal north showed lower productivity and less drought tolerance on southern test sites than all other sources, implying that maladaptation to drought may prevent boreal populations from taking full advantage of more favorable growing conditions under projected climate change.

3.3 Introduction

Since the glaciers of the last ice age retreated in western North America, tree species have slowly re-colonized from glacial refugia, and have become adapted to local environments over generations (Aitken et al., 2008). Such adaptations include drought tolerance mechanisms, which are important in areas like the interior plateaus of western North America, where climates are characterized by dry and hot summers. Tolerances of tree populations in these areas can potentially be exceeded as climates continue to warm (St Clair & Howe, 2007; Allen et al., 2010; Alberto et al., 2013). Maladaptation may be an issue not just for the populations at the southern and low elevation range limit, but may be observed throughout the distribution of wide-ranging species, where sub-populations are normally adapted to local climate environments (Aitken & Whitlock, 2013). Already, reduced tree productivity and survival is considered to be a major threat in the northern hemisphere due to drought (Allen et al., 2010; Peng et al., 2011; Anderegg et al., 2013). To mitigate such negative impacts, management strategies to increase the adaptive capacity of forests are urgently needed.

Lodgepole pine (*Pinus contorta* Dougl. ex. Loud. var *latifolia* Englm.) is one of the most widespread tree species in western North America. It is an ecologically and economically important tree, and is of particular concern for climate change adaptation. Lodgepole pine can grow in environments where mean annual temperatures are as low as -5 °C and as high as 12 °C, covering over 4000 km of latitude in western North America (Little, 1971): sub-populations occurring within this range are adapted to very different climate environments (Rehfeldt et al., 1999). Under projected climate change, populations that cannot migrate to matching climatic conditions within close distance, e.g. up in elevation, may become increasingly maladapted to new climate environments (Aitken & Whitlock, 2013). The general increase in temperatures is

expected to be associated with an increase in severe drought events across the interior plateaus of western North America. For example, what used to be a once in a 100-year drought event during the climate normal period from 1961 to 1990 might be expected to occur once every 5 years by the 2080's (Christidis et al., 2014). Reforestation with more drought tolerant planting stock may therefore be a prudent climate change adaptation strategy for this region.

Choosing appropriate planting material for reforestation is a tool that forest managers can use to adapt forests to changing conditions (St Clair & Howe, 2007; O'Neill et al., 2008a). Normally, reforestation programs geographically constrain their seed movement under the paradigm that local provenances are optimally adapted (Illingworth, 1978). However, moving planting stock northward or higher in elevation to re-align the population with the climate environments that they are adapted to is now being considered by some managers (O'Neill et al., 2008b). This concept is known as assisted migration, and has been suggested as a means to conserve species with declining populations or to maintain forest health and productivity. One major concern over assisted migration, however, relates to long transfer distances. If populations are moved to habitats far outside of their historical range, they may react in unpredictable or invasive ways, or they may introduce new pests and pathogens to an area (Marris, 2009; Ricciardi & Simberloff, 2009). In forestry, where populations are primarily moved within their existing range or somewhat beyond the leading edge of the distribution, assisted migration prescriptions, also referred to as assisted gene flow in this context (Aitken & Whitlock, 2013), may pose fewer risks. Northward transfers may nevertheless result in planting stock being more susceptible to cold injury (e.g., Benito-Garzón et al., 2013).

The most suitable provenances for reforestation efforts are often determined through provenance trials that have been established for the most important forest tree species.

Provenance trials involve testing multiple populations from across a species range across the planting environments of interest, similar to common gardens in agriculture (Morgenstern, 1996). Through observation of the phenotype in a constant environment, these experiments can be used for assessing genetic differences and the interaction between the genotype and the environment. Beyond the initial intention, these trials have also emerged as opportune climate change laboratories (Matyas, 1994; Schmidting, 1994; Carter, 1996; Leites et al., 2012): testing a northern seed in a warmer climate in the south can indicate how northern forests will react under projected climate change. This now offers the opportunity to re-evaluate historic geographic-based seed transfer limits and select the best provenances for a warmer, more drought-prone climate.

The IUFRO-Illingworth lodgepole pine provenance trial (Illingworth, 1978) that we analyze in this study is arguably the largest provenance trial series in existence, and it has been previously used to infer potential climate change impacts. Rehfeldt *et al.* (1999) were the first to assess the optimal climatic niche space of provenances and potential climate change impacts. Subsequently, complex universal response functions were used to assess growth response (Wang et al., 2006a; O'Neill et al., 2008a), and tree-ring analysis on selected provenances to extend these response functions has also been carried out (McLane et al., 2011a, 2011b). These studies indicated that climate change will generally increase growth of lodgepole pine, especially in the northern regions of its distribution. Since then, the mountain pine beetle epidemic that has decimated millions of hectares of lodgepole pine forests in western North America has also destroyed many sites of this provenance trial, affording the opportunity to destructively sample trees from these experiments for whole-tree analysis of inter-annual variation in growth.

Here, we use height, diameter and basal area increments in over 500 trees originating from 23 populations across western North America that were destructively sampled on three experimental sites in southern British Columbia, selected for representing the warmest and driest environments in the IUFRO trial series that comprises 60 test sites. We focus on a seven-year period that includes an extended drought in 2002. The specific objectives were to: 1) assess genotype by environment interactions by evaluating climate-growth relationships for each population; 2) quantify drought resistance, resilience and recovery of different populations using height and basal area growth to evaluate potential trade-offs among different populations; and 3) suggest reforestation strategies by considering performance and trade-offs in all populations.

3.4 Materials and Methods

Experimental Design

When the Illingworth lodgepole pine provenance trial was developed over 40 years ago, it was designed for determining the best planting stock from a productivity perspective, so not all of the original 153 provenances were tested at all 60 sites (Illingworth, 1978). We therefore selected 23 provenances that were widely planted, representing four climatic regions of western North America: the United States (US), British Columbia's Southern Interior (SI), British Columbia's Central Interior (CI), and the Northern region of British Columbia and Yukon (N). Five or six provenances were chosen to be climatically representative of these four regions (Fig. 1, Tabs. 1 & 2). To evaluate drought response under climate change, we focussed on the three planting sites in the southern interior region of British Columbia with the warmest and driest climate conditions (Fig. 1).

Field and Laboratory Measurements

Each of the three test sites is represented by two blocks, where provenances are planted in 9-tree plots arranged in a randomized complete block design. The trees were planted in 2.5×2.5 m spacing. To minimize the effects of varying competition on growth, we tried to select trees that, by the time of death, had still been surrounded by neighboring trees. At each block, four trees were cut and measured per provenance for a total of 513 trees (a few blocks did not contain a sufficient number of dead trees for sampling). Because most trees were approximately 32-35 before being killed by the mountain pine beetle, they were roughly mid-rotation age. After a tree was felled and de-limbed, we used an Eslon tape to measure tree length. Stem disks were collected at 0.3 and 1.3 m above ground and at each tree's half-height. Since the exact year of tree death at each site was not known, a few living trees from each site were cut and stem disks taken for cross-dating purposes.

All of the approximately 1400 stem disks were first sanded with a large belt-sander (LZG-S 10/7/14, Langzauner GmbH, Austria), then scanned (ScanMaker 9800XLplus, Microtek, USA). The scanned images were then imported for analysis in WinDendro (Regent Instruments Inc, 2012). Tree-ring width, earlywood width and latewood width were measured for four radii per disk. Automatic measurements generated by the software were inspected and manually corrected as necessary. Cross-dating was also conducted in WinDendro using a reference period based primarily on living trees to date each tree and align the tree-ring responses.

Calculation of Drought Indices

This study focuses on the years 1999-2005, which covers an extended drought period that occurred in 2002. Tree-ring widths were transformed into basal area increments using the *bai.out*-function of the *dplr*-package (Bunn, 2008) in the R programming environment (R Core

Team, 2013). This transformation removes the dimension related trend in tree-ring width series. The three years prior to the year 2002 are used as pre-drought normal basal area growth conditions, while a three year post-drought period allows the evaluation of population recovery and resilience (Lloret et al., 2011). Specifically, drought resistance is the ability of a tree to resist growth loss due to drought, and is calculated by dividing each tree's basal area increment in 2002 by its average growth during the pre-drought period. Drought recovery represents the ability of a tree to recover after drought, and is estimated by dividing each tree's average basal area increment during the post-drought period by its growth in the drought year. Drought resilience is the ability to achieve pre-drought performance, calculated by division of post-drought growth by the pre-drought performance. Similarly, relative resilience accounts for the damage that the tree incurred during drought, and is calculated by subtracting resistance from resilience (Lloret et al., 2011).

Statistical Analyses

Since our field work was conducted in 2013 and 2014, but most trees had died between 2006 and 2008, some trees had begun to rot at the lower part of the trunk and sometimes taking a stem disk at 1.3 m height was not possible. Occasionally, the top annual height increments were missing because the brittle, dry top was broken while felling the tree in the dense stand. If mortality was low in a particular provenance, it was also not always possible to sample four trees in a block. Due to these missing values and a consequently slightly unbalanced design, we calculated best linear unbiased estimates (BLUEs) of height and basal area increments of regions and provenances within regions. When estimating BLUEs for regions, block and provenance effects were defined as random. When estimating provenance means, block was random. The mixed model was implemented with the *asreml* package (Butler et al., 2009) for the R

programming environment (R Core Team, 2013). For the analysis and illustration of the region and provenance main-effects, blocks within sites were considered as replicates. Subsequent post-hoc tests were carried out using the `ghlt`-function from the `multicomp` package (Hothorn et al., 2008), applying Bonferonni's adjustments to control experiment-wide α -levels.

To infer adaptation with respect to source climate of provenances, we ran regression analyses of height and drought indices as a function of relevant climate variables of the provenances' source locations. Source and planting site climate variables were derived using version 4.62 of the `ClimateWNA` software package (Wang et al., 2006b; Hamann et al., 2013), which interpolates climate based on the parameter-elevation regressions of independent slopes model (Daly et al., 2008). We used the 1961-1990 period as a baseline climate-normal which we assume to represent each population's adapted climate. This period precedes a significant anthropogenic warming signal and is also preferred as reference climate because of an abundance of weather station data for this period. Tested climate variables include: mean annual temperature ($^{\circ}\text{C}$), mean summer temperature from June to August ($^{\circ}\text{C}$), mean coldest month temperature ($^{\circ}\text{C}$), minimum winter temperature ($^{\circ}\text{C}$), minimum spring temperature ($^{\circ}\text{C}$); summer heat-moisture-index (SHM); mean annual precipitation (mm); and mean growing season precipitation from May to September (mm).

3.5 Results

Source Climate and Growth of Populations

Populations varied significantly in height and diameter at 1.3 m after 32 years until dying by mountain pine beetle between 2006 and 2008 (Tab. 3). The northern population and US population had significantly lower height and smaller diameters than the central interior and

southern interior populations. In contrast, the mean total heights of the southern and central interior populations were not significantly different from each other. Furthermore, the central population transferred to a warmer climate at the southern sites slightly underperformed the local source, although not significantly (Tab. 3).

The strongest predictors of population growth were temperature variables, especially variables indicating cold (Fig. 3.2). Relationships were generally parabolic in shape as expected from niche theory, and the top three source climate predictor variables were mean annual temperature ($R^2=0.64$, $p<0.001$), mean coldest month temperature ($R^2=0.49$, $p=0.001$), and minimum winter temperature ($R^2=0.41$, $p=0.004$). The populations from the cold and warm extremes of the range (northern and US populations, respectively) represent the tails of the parabola, where poor growth is associated with high and low temperatures of population origin. The best lodgepole pine performance in southern British Columbia appears to be associated with mean annual temperatures of approximately 2 to 3 °C. Provenances from the central and southern interior regions represent these climates well, as they occur at the top of these parabola-shaped relationships. A significant linear relationship was also found between height and a logarithmically-transformed source summer heat-moisture index ($R^2=0.38$, $p=0.008$) and mean summer precipitation ($R^2=0.47$, $p=0.002$).

Population Differences in Drought Tolerance

The drought event of 2002 was initiated by low precipitation during June, July and August and led to a drop in basal area increments in all populations (Fig. 3.3). We found significant differences in drought response between populations for all four drought tolerance indices (Tab. 4). Differences in drought resistance, recovery and relative resilience can be mainly attributed to the US and N populations. The US provenances unexpectedly showed a greater

decrease in growth than the other provenances, but showed a better rate of recovery and the ability to achieve a higher level of pre-drought basal area growth (Tab. 4). The opposite trend is seen in the northern population, which exhibits higher drought resistance than the US population, but showed the slowest recovery, resilience and relative resilience of all four populations (Tab. 4). The central and southern interior populations were not significantly different from each other, and generally appeared comparatively drought tolerant: they showed higher drought resistance, medium recovery, higher resilience, and medium resilience relative to their resistance (Tab. 4).

Drought Tolerance and Source Climates

The relationship between source climate of populations and their drought tolerance was found to be linear (Fig. 3.4). Populations originating in regions with warmer winters have higher relative drought resilience. Mean annual temperature had an R^2 of 0.64 ($p=0.007$), but provenance mean coldest month temperature showed the highest R^2 of 0.69 ($p=0.001$). The minimum temperature in winter and minimum temperature in spring demonstrated similar predictive power ($R^2=0.67$, $p=0.002$). Relative drought resilience showed a significant relationship with the average source summer heat-moisture index when log transformed. The provenances from drier climates, indicated by a higher index value, showed higher relative resilience ($R^2=0.42$, $p=0.008$). The average source mean summer precipitation showed a weak, negative linear relationship when log transformed, indicating that provenance's relative resilience decreased for populations originating from wetter climates ($R^2=0.29$, $p=0.008$). Since the points are colored by region, it is also possible to see how these trends hold true at broader regional scales (Fig. 3.4).

Relationship between Growth and Drought Tolerance

Productivity as indicated by height by the end of 2006 shows an unexpected parabolic relationship with relative drought resilience (Fig. 3.5). The provenances from the US region had low productivity, but higher drought tolerance, indicating an expected trade-off between tolerance to drought at the cost of growth. The northern provenances show no evidence of a trade-off with both low growth and low drought tolerance. Both the central and southern interior populations again show high similarity in their response, both with a surprising ability to withstand long-term consequences of drought while being comparatively productive.

3.6 Discussion

Source Temperature Predicts Productivity

In the southern part of British Columbia's interior, water limitation is a defining factor for plant growth, especially in the desert-like valleys (Nelson et al., 2011). As latitudes increase in the interior, climates become colder and less dry. The lack of significant differences in height and drought tolerances between the southern and central interior populations was therefore unexpected, and indicates adaptation to temperature rather than water availability. The central and southern interior populations occur at the top of the parabolas in Fig. 3.2, which suggests that these provenances show the optimal growth and drought tolerance in southern British Columbia planting environments. This response could relate to the climatic transfer distance, since the central and southern populations both did not show large climatic differences to the planting sites. Although the central interior population showed slightly lower height growth (Tab. 3), it did not show smaller basal area increments (Fig. 3.3).

Anomalously dry periods have occurred several times over the life-time of the provenance trial (Fig. 3.3a), but they have not always been associated with a corresponding decrease in basal area growth (Fig. 3.3b). Most of these dry periods did not occur during the critical part of the year when growth was underway: summer droughts can have greater impact than dry periods that happen outside of this time. Given the high predictive power of cold-driven climate variables, some visible drops can likely be attributed to cold conditions, or a combination of cold and dry conditions. The drought in 2002, however, was a summer drought and led to a clear drop in basal area growth across all populations (Fig. 3.3b).

Trade-offs Indicate Differing Adaptive Strategies

Drought resilience is arguably the most important drought response index because the failure of a tree to regain its previous potential after an extreme event often precedes mortality (Scheffer et al., 2001; Allen et al., 2010). On the other hand, low resilience might point to an effective adaptive strategy that shuts down growth under unfavorable conditions, but prevents structural damage that might compromise long-term productivity. If such a mechanism existed, it should lead to better performance under future droughts, which could not be assessed in this study due to mortality. Relative resilience is also important; because it incorporates the damage incurred during drought. Negative values of relative resilience would indicate a decreasing growth trend after drought, which is often preceding mortality (Lloret et al., 2011). The ability of how fast a tree can recover may become more important with drought events expected to become increasingly frequent under projected climate change (Christidis et al., 2014). If a tree is slow to recover, it would also be indicative of a problem with the reduced growth often preceding mortality.

To more effectively interpret the drought response results, it is important to consider productivity before choosing seed sources for reforestation. Our results indicate that a typical trade-off only applies to the most southern (US) lodgepole pine population. The results in Fig. 3.5 suggest comparatively good drought resilience at the expense of height growth. This type of strategy could involve investments into a more safety-oriented stem hydraulic system (Hacke & Sperry, 2001; Hacke et al., 2001), with increased tracheid wall thickness, reduced cell lumen size and more resistant pit membranes in order to reduce risk of cavitation that can permanently disrupt the water supply to the crown (Tyree & Zimmermann, 2002). The response of the US population in the post drought period appears plastic, with comparatively fast recovery and high relative resilience. A high degree of stomatal control appears to be a likely alternative mechanism, while a combination of both strategies is also possible (Hartmann, 2011).

Contrary to expectations of substantial trade-offs between hydraulic safety and productivity, the central and southern interior populations show an unexpected ability to tolerate drought and to maintain comparatively good long-term growth (Fig. 3.5). Guy & Holowachuk (2001) concluded that the most productive and water-use efficient genotypes come from more moderate climates, which appears to also be the case in this study. This strategy might, however, nevertheless involve a trade-off that has not yet been revealed for lack of a truly exceptional drought during the study period. Maladaptation could become more significant if drought events increase in frequency and severity under expected climate change (Bréda et al., 2006).

Boreal Provenances Appear At-Risk

As productivity and drought tolerance were negatively related in the US population, we had expected a similar growth versus drought trade-off in the northern population. Recent research has indicated a link between cold-adaptation and drought-adaptation in seedlings

(Bansal et al., 2015a, 2015b) and mature Douglas-fir trees (Montwé et al., 2015). Cold-adapted trees also invest into thicker cell walls and narrower lumen sizes that are more resistant to freeze-thaw-induced embolisms (Schreiber et al., 2013a, 2013b). Instead, the most northern population showed no evidence of such benefits in this study, as had both poor drought resilience and low height and diameter growth.

An important inference of this study is therefore that boreal populations may not be able to take advantage of more favorable growing conditions under projected climate change. Although previous research has suggested that northern populations may benefit disproportionately from climate warming (Wang et al., 2006a, 2010; O'Neill et al., 2008a), the lack of drought tolerance shown in this study appears to put these populations at high risk.

Implications for Assisted Migration

Because provenances from the central interior, southern interior and the United States regions appear generally drought tolerant and productive, our results suggest that they may be used for reforestation under a warming climate. However, the result that height growth can be predicted by temperature hints at the need for cautious interpretation. Warmer source location's winter and spring temperatures predict increased height growth (Fig. 3.2). In addition, Fig. 3.3 also shows several drops in basal area growth that were not associated with early summer drought, for example, in 1991. This could be the effect of a frost event that damaged the cambial tissue, or a freeze-thaw event that causes cellular embolisms (Mayr et al., 2006). A similar event seems to have occurred in 1993. The US population had been a top performer until this event, but a rank change occurred in 1993, and the US population never recovered such competitive growth levels. This is also suggesting that results from young provenance trials should be assessed carefully. The population-specific response to frost appears to be an important follow-

up study and we propose using micro sections of tree-rings to search for poorly lignified or damaged cells due to frost damage (Piermattei et al., 2015). Tree growth can also be influenced by differences in photo-period (Wheeler, 1979). A shorter photo-period could potentially negatively influence the success of seed transfers and should be tested if transfers across latitudinal distances are envisaged.

In British Columbia's southern interior, the paradigm that local is best appears to remain valid, at least temporarily. Southern provenances planted in southern British Columbia represent good drought tolerance and productivity, and can be recommended for continued planting. The US provenances were incorporated into our sampling design to test the viability of an assisted migration prescription, should the southern interior region be shown to need it. Although it appears that it might not be currently necessary, our sample material missed recent dry summers. With increasing frequency of dry growing seasons, forest managers may wish to consider using a small percentage of more drought resilient planting stock (Prober et al., 2015). Such a drought-averse strategy reduces the risk of mortality or forest health issues, but represents a compromise with productivity.

3.7 References

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3.8 Figures and Tables

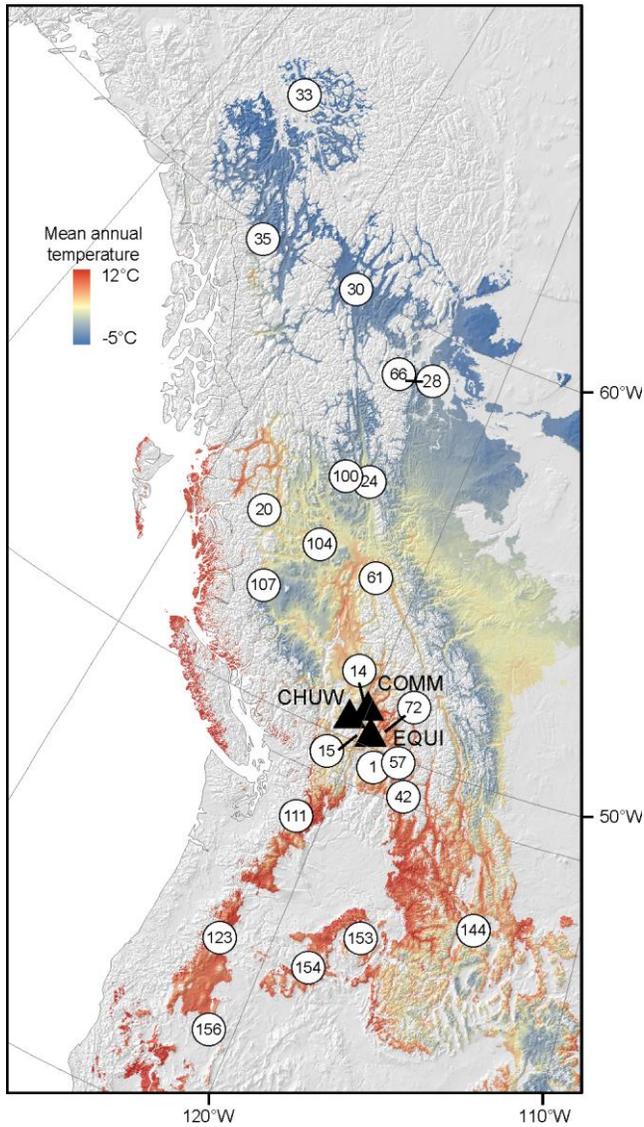


Fig. 3.1. Map of western North America showing the lodgepole pine (*Pinus contorta* Dougl. ex. Loud. Englm.) species distribution, colored by mean annual temperature. The planting sites are represented by black triangles. Each circle represents a provenance, numbered according to Illingworth (1978).

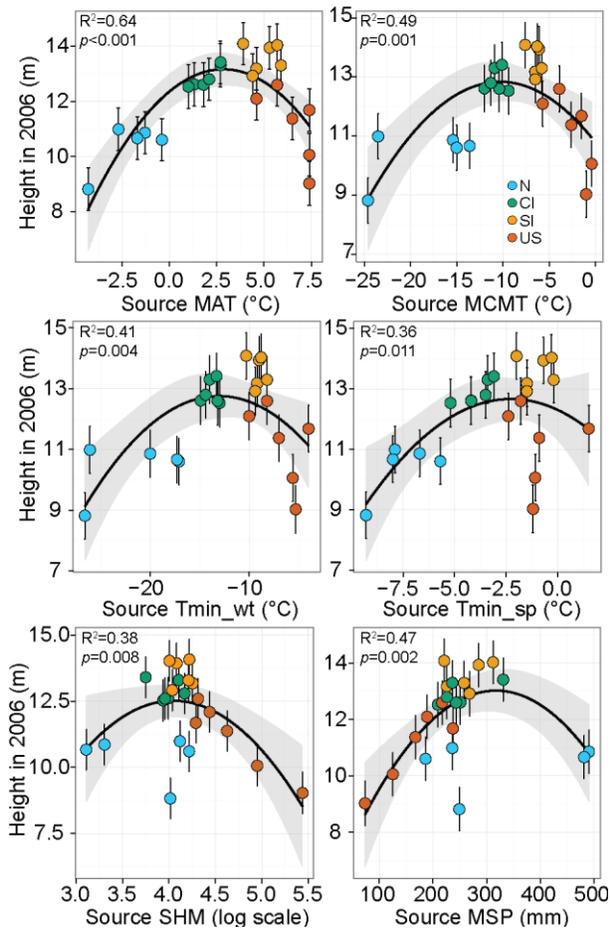


Fig. 3.2. Height of lodgepole pine provenances at three test sites in southern British Columbia and the relationship to their climate of origin. Each data point represents a provenance replicated on 6 blocks, and it is colored by origin (north = blue, central interior = green, southern interior = orange, and United States = red). Climate variables here represent the mean annual temperature (°C); Mean coldest month temperature (°C); coldest temperature in winter (°C); coldest temperature in spring (°C), summer-heat-moisture index, and mean summer precipitation (mm). Error bars represent the standard error of the mean, while gray ribbons outlines the 95% confidence intervals.

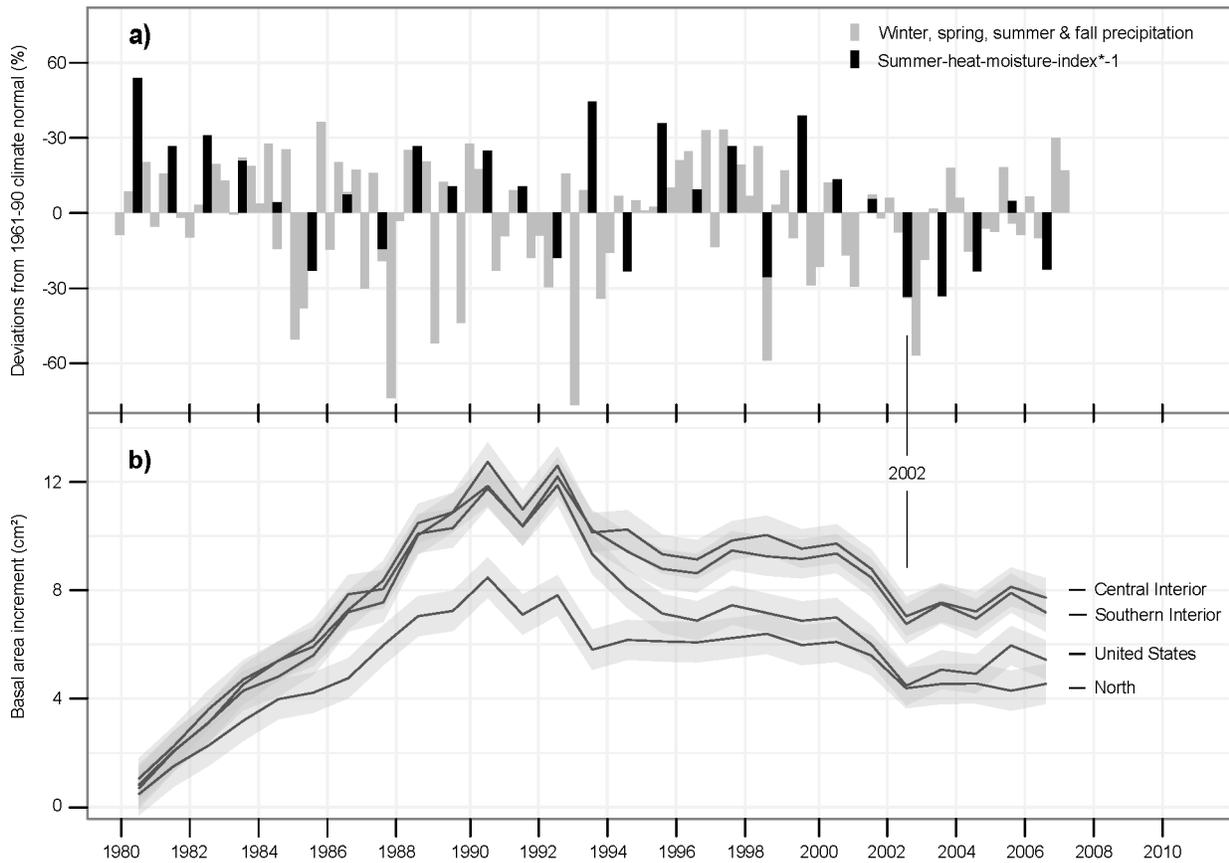


Fig. 3.3. Climate anomalies and basal area increments. Panel a) shows anomalies in moisture conditions over the study period. Grey bars represent deviations in winter, spring, summer and fall precipitation (mm) and black bars overlying summer precipitation represent deviations in the summer-heat-moisture index as negative index values, both relative to their respective mean values during the 1961-1990 baseline period. Panel b) shows basal area increments, or the area represented by the new ring's formation, in cm² for the four regions over the lifetime of the provenance trial.

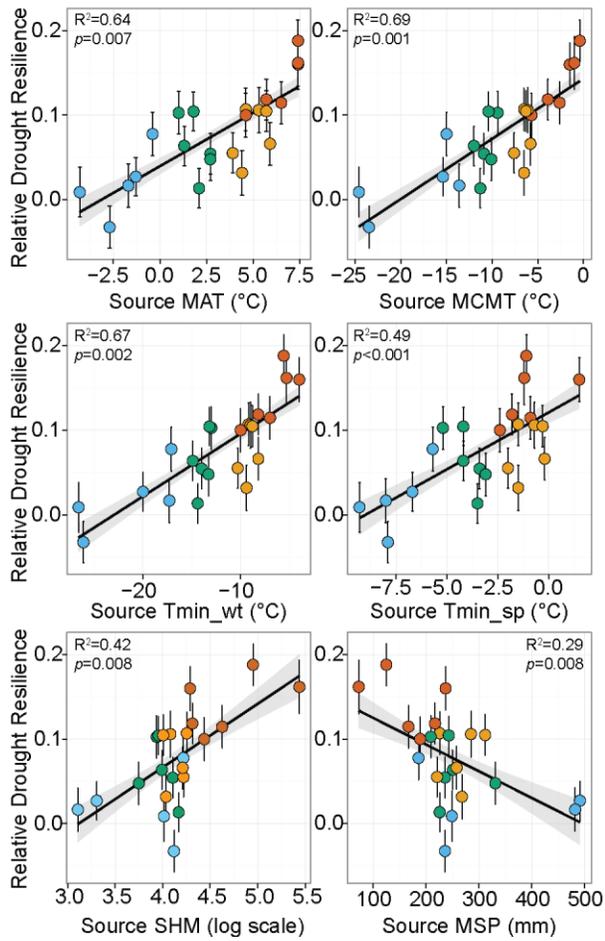


Fig. 3.4. Relative drought resilience of four lodgepole pine populations compared to source climate (1961-1990 climate normal). Each data point represents a provenance, and it is colored by population (north = blue, central interior = green, southern interior = orange, and United States = red). Climate variables here represent the mean annual temperature (°C); mean coldest month temperature (°C); coldest temperature in winter (°C); coldest temperature in spring (°C), summer heat-moisture index (SHM), and mean summer precipitation (MSP, mm). Error bars represent the standard error of the mean, while gray ribbons outlines the 95% confidence intervals.

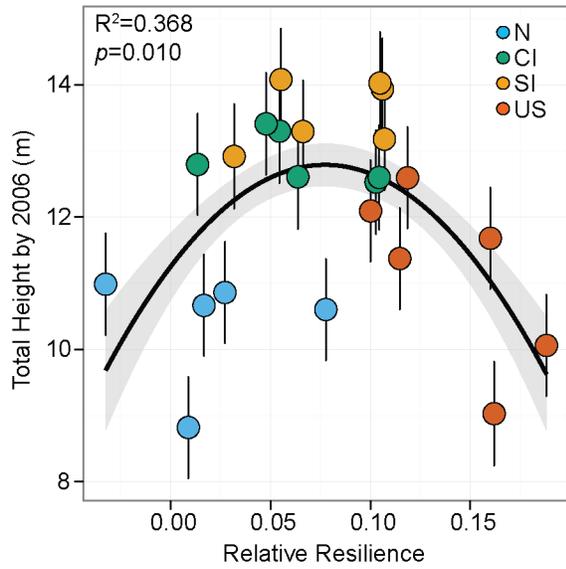


Fig. 3.5. The trade-off relationship height and relative resilience to drought in lodgepole pine. Productivity is measured by height in meters by the end of the study period (2006), and drought tolerance is represented by relative drought resilience, or the ability to recover to previous growth levels relative to the damage incurred during drought. Each data point represents a provenance, and it is colored by population (north = blue, central interior = green, southern interior = orange, and United States = red).

Tab. 3.1. Average source climate variables from the 1961-1990 normal period are shown for provenances. *Region* refers to the assigned population based on geographical origin (N = north, CI = central interior, SI = southern interior, US = United States). *Prov* refers to the provenance corresponding to the Illingworth provenance trial's numbering system (Illingworth 1978). Latitude (*Lat.*) and longitude (*Long.*) is given in decimal degrees; and elevation is given in meters above sea level (*Elev.*). Climate variables include: mean annual temperature in °C (*MAT*); mean warmest month temperature in °C (*MWMT*); mean coldest month temperature in °C (*MCMT*); temperature difference is a measure of continentality and is given in °C (*TD*); mean annual precipitation is given in mm (*MAP*); mean summer precipitation is given in mm (*MSP*); annual heat:moisture index (*AHM*); summer heat:moisture index (*SHM*); Reference Evaporation (*Eref*); and climate moisture deficit is given in mm (*CMD*). Climate data derived from ClimateWNA v 4.62 (Wang et al. 2006b).

Region	Prov.	Lat.	Long.	Elev.	MAT	MWMT	MCMT	TD	MAP	MSP	AHM	SHM	Eref	CMD
N	33	63.30	-136.47	876	-4.3	13.8	-24.6	38.4	423	249	14	55	369	131
N	30	59.98	-128.55	640	-2.7	14.5	-23.5	38.0	449	236	16	62	442	197
N	35	59.80	-133.78	789	-0.4	12.6	-15.0	27.6	361	186	27	68	410	203
N	28	58.67	-124.17	762	-1.3	13.4	-15.4	28.8	689	491	13	27	446	7
N	66	58.65	-124.77	1173	-1.7	10.8	-13.6	24.4	675	482	12	22	336	0
CI	24	55.95	-123.80	686	2.1	14.6	-11.3	25.9	469	226	26	65	504	246
CI	100	55.80	-124.82	762	1.3	13.6	-12.0	25.6	522	251	22	54	470	181
CI	20	54.13	-127.23	937	1.8	12.7	-10.4	23.1	551	243	21	52	484	208
CI	104	54.02	-124.53	732	2.7	14.3	-10.9	25.2	506	236	25	61	545	267
CI	61	53.87	-121.80	838	2.7	14.1	-10.1	24.2	750	331	17	43	530	155
CI	107	52.50	-125.80	1311	1.0	10.7	-9.4	20.1	822	209	13	51	486	211
SI	14	50.97	-120.33	1059	3.9	15.0	-7.6	22.6	466	221	30	68	580	304
SI	72	50.70	-119.18	777	5.7	17.1	-6.3	23.4	735	312	21	55	681	256
SI	15	50.05	-119.65	1067	4.4	15.2	-6.5	21.7	665	268	22	57	588	247
SI	57	49.90	-118.20	579	5.9	17.4	-5.8	23.2	659	258	24	67	687	329
SI	1	49.58	-119.02	1006	4.6	15.9	-6.5	22.4	547	226	27	70	609	319
SI	42	49.18	-117.58	998	5.3	16.9	-6.1	23.0	858	285	18	59	672	265
US	111	47.78	-120.93	762	7.4	17.3	-1.5	18.8	1535	237	11	73	663	261
US	144	46.67	-113.67	1524	4.6	16.0	-5.7	21.7	408	189	36	85	670	426
US	153	45.63	-117.27	1311	5.7	16.2	-3.9	20.1	510	217	31	75	795	439
US	154	44.53	-118.57	1494	6.5	17.1	-2.6	19.7	676	167	24	102	801	491
US	123	44.38	-121.67	1006	7.4	16.8	-1.0	17.8	427	73	41	230	909	692
US	156	42.30	-120.78	1615	7.4	17.6	-0.4	18.0	583	125	30	141	881	560

Tab. 3.2. Planting site climate variables from the 1961-1990 normal period are shown for all field tests. *Site code* refers to the abbreviated site name from the Illingworth lodgepole pine provenance trial (Illingworth 1978). Latitude (*Lat.*) and longitude (*Long.*) is given in decimal degrees ; and elevation is given in meters above sea level (*Elev.*). Climate variables include: Mean annual temperature in °C (*MAT*); mean warmest month temperature in °C (*MWMT*); mean coldest month temperature in °C (*MCMT*); temperature difference is a measure of continentality and is given in °C (*TD*); mean annual precipitation is given in mm (*MAP*); mean summer precipitation is given in mm (*MSP*); annual heat:moisture index (*AHM*); summer heat:moisture index (*SHM*); Reference Evaporation (*Eref*); and climate moisture deficit is given in mm (*CMD*). Climate data derived from ClimateWNA v 4.62 (Wang et al. 2006b).

Site code	Lat.	Long.	Elev.	MAT	MWMT	MCMT	TD	MAP	MSP	AHM	SHM	Eref	CMD
CHUW	50.58	-120.62	1430	3.1	14.0	-7.1	21.1	482	241	27.2	58.1	527	232
COMM	50.92	-120.07	1370	3.1	13.9	-7.5	21.4	522	256	25.1	54.3	532	217
EQUI	50.37	-119.6	1370	3.3	14.2	-7.3	21.5	686	301	19.4	47.2	557	188

Tab. 3.3. Height and diameter at 1.3 m of four lodgepole pine populations after 32 years of growth. Standard error of the mean is provided in parentheses. Different letters behind the values indicate significant differences between groups ($\alpha=0.05$).

Variable	North	Central interior	Southern interior	United States
Height (m)	10.4 a (0.76)	12.9 b (0.75)	13.6 b (0.75)	11.2 a (0.75)
Diameter (cm)	13.2 a (0.75)	16.5 bc (0.73)	16.4 bc (0.73)	15.1 bc (0.73)

Tab. 3.4. Drought indicator values (larger values are better) for resistance, recovery, resilience, and relative resilience for four regions ordered by latitude. Standard error of the mean is provided in parentheses. Different letters behind the values indicate significant differences between provenances ($\alpha=0.05$).

Drought index	North	Central Interior	Southern Interior	United States
Resistance	0.73 a (0.019)	0.74 a (0.018)	0.75 a (0.019)	0.64 b (0.019)
Recovery	1.03 a (0.021)	1.09 ab (0.019)	1.11 b (0.019)	1.23 c (0.020)
Resilience	0.75 b (0.019)	0.80 a (0.018)	0.83 a (0.018)	0.78 ab (0.019)
Relative Resilience	0.02 b (0.012)	0.06 a (0.010)	0.08 a (0.011)	0.14 c (0.011)

4 Reducing Contamination Risk in Wood Samples for Isotope Analysis

4.1 Publication

Isaac-Renton M, Treydte K, Schneider L (2016) Contamination risk of stable isotope samples during milling. *Rapid Communications in Mass Spectrometry*, **30**(13):1513-1522.

4.2 Abstract

Isotope analysis of wood is an important tool in dendrochronology and ecophysiology. Prior to mass spectrometry analysis, wood must be homogenized, and a convenient method involves a ball mill capable of milling samples directly in sample-tubes. However, sample-tube plastic can contaminate wood during milling, which could lead to biological misinterpretations. We tested possible contamination of whole wood and cellulose samples during ball-mill homogenization for carbon and oxygen isotope measurements. We use a multi-factorial design with two/three steel milling balls, two sample amounts (10 mg, 40 mg), and two milling times (5 min, 10 min). We further analyzed abrasion by milling empty tubes, and measured the isotope ratios of pure contaminants. A strong risk exists for carbon isotope bias through plastic contamination: polypropylene deviated from the control by -6.77‰. Small fibers from PTFE filter bags used during cellulose extraction also present a risk as this plastic deviated by -5.02‰. Low sample amounts (10 mg) showed highest contamination due to increased abrasion during milling (-1.34‰), which is further concentrated by cellulose extraction (-3.38‰). Oxygen isotope measurements were unaffected. A ball mill can be used to homogenize samples within their test tubes prior to oxygen isotope analysis, but not prior to carbon or radiocarbon isotope analysis. There is still a need for a fast, simple and contamination-free procedure.

4.3 Introduction

Stable carbon and oxygen isotope signatures ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values) of tree-rings are parameters useful in dendroclimatology, dendroecology, ecophysiology and biology since they are datable to specific calendar years and are sensitive to environmental variation. They are increasingly being used as proxies for past climate changes; to understand tree response to drought and thinning; to explain genetic differentiation in water use efficiency; and in interpreting forest response under changing atmospheric conditions and climates (Guy & Holowachuk, 2001; McCarroll & Loader, 2004; Treydte et al., 2009; Saurer et al., 2014; Sohn et al., 2012; Saurer et al., 2012; Jansen et al., 2013; Lévesque et al., 2013; Rinne et al., 2013; Hartl-Meier et al., 2014; Kress et al., 2014; Frank et al., 2015; Giuggiola et al., 2016). These important analyses are based on the fact that environmental conditions influence stomatal control and photosynthetic activity, which in turn affects isotope ratios in synthesized macromolecules later archived in the tree-rings. Such relationships are built on well-established understanding of carbon isotope fractionation processes (Farquhar et al., 1982, 1989; Francey & Farquhar, 1982) and a more recent understanding of oxygen isotope fractionation processes in plants (Saurer et al., 1997; Scheidegger et al., 2000; Barbour, 2007; Offermann et al., 2011; Gessler et al., 2013, 2014; Treydte et al., 2014).

All methods of processing wood samples for isotope analysis require homogenization prior to mass spectrometry analysis (Borella et al., 1998; Laumer et al., 2009). There is no ideal homogenization method, however. For example, ultra-centrifugal mills are commonly used for tree-ring stable isotope research (Treydte et al., 2009; Hartl-Meier et al., 2014; Tognetti et al., 2014), but samples must be laboriously cut into fine pieces with a scalpel before milling to avoid burning and unintentional fractionation. Material collection and cleaning of the mill are both

cumbersome and time consuming processes, and may result in high loss of sample material (Laumer et al., 2009). Ultra-sonic homogenization is an alternative process, but only works for small cellulose samples (<10 mg) (Laumer et al., 2009; Hangartner et al., 2012). This procedure involves placing the extracted cellulose into a sample tube with deionized water, and homogenizing it with ultra-sonic waves (Laumer et al., 2009). This alternative method requires freeze drying, however, and is therefore not faster. Other methods exist but are usually limited, for example: grinding with wig-L-bug mill; small drill bits; or require very specialized and often expensive equipment that are only appropriate for some applications (e.g. UV-laser ablation); cryo-mill; and cellulose cross-sections made from a microtome (Helle & Schleser, 2004; Schulze et al., 2004; Kagawa et al., 2006a, 2006b, 2015; Roden & Ehleringer, 2007; Fichtler et al., 2010; Sohn et al., 2014, 2012, Schollaen et al., 2014, 2015).

Due to these limitations, a ball mill fitted with a sample-tube holder is one of the most convenient methods of homogenization for isotope analysis. A ball mill is faster than using an ultra-centrifugal mill because larger wood pieces can be milled, and hence, less time is required to cut wood into small pieces with a scalpel. Since the wood can remain in the original sample tube, there is no loss of material and time can be saved: steel milling balls are simply added to each tube, and multiple samples can be placed in a tube holder and milled simultaneously. The resulting homogenized material is then ready for cellulose extraction or ready for mass spectrometry of whole wood (resins and extractives may have to be removed first (McCarroll & Loader, 2004; Gaudinski et al., 2005; Boettger et al., 2007; Roden & Ehleringer, 2007)). After a recent study found no evidence of isotope fractionation due to heating from friction of wood samples during ball milling, the ball mill was explicitly recommended for tree-ring isotope research (Riechelmann et al., 2014).

Such a ball mill configuration became a matter of concern, however, after unpublished isotope data showed atypical patterns: Radiocarbon measurements of the same samples indicated the presence of “dead carbon”. Isotope contamination might stem from sample tube abrasion during milling, which could taint the sample with plastic. Since most standard sample tubes are made of polypropylene (PP) plastic derived from fossil fuels, they therefore have specific isotope signatures. Fossil fuels represent the remains of prehistoric plants with low $\delta^{13}\text{C}$ values, although the exact $\delta^{13}\text{C}$ signatures of fossil fuel types can vary (Suess, 1955; Zondervan & Meijer, 1996; Dean et al., 2014). If the inside of a plastic sample tube is ground during ball milling and this does indeed cause plastic contamination, the isotope signature could be altered. This could lead to biological or climatological misinterpretations. A thorough literature review produced no information on isotope signatures of polypropylene sample tubes.

Here, we evaluate the suitability of a common ball mill design for continued use as a fast and contamination-free method of homogenizing wood for isotope measurements. First, we assess the potential for plastic contamination to alter isotope values by measuring the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values of polypropylene-based sample tubes. Second, we observe possible sample tube abrasion under different milling configurations. Third, we quantify any loss of polypropylene plastic during cellulose extraction, and test the remaining isotope contamination on both cellulose and whole wood. Finally, we assess homogenization alternatives.

4.4 Materials and Methods

Tree-Ring Selection and Separation

For this study, we chose a large stem disk (~80 cm in diameter) of a Norway spruce (*Picea abies* L.H. Karst.) that was free of any damage and had a large ring (~6 mm). Taking

samples from the same ring was important because a constant isotope signature was required to ensure that any variation in isotope values would be based on procedure alone. Because the stem disk was so large and the ring was so wide, enough material for all treatments could be taken from within ~4 cm, thereby reducing the potential of isotope value variation along the same ring (Leavitt, 2010). The stem disk was sawed into a section about 1 cm thick for easier cutting, and the surface was cleaned with a scalpel. The wide ring was separated and finely cut into small pieces using a scalpel under a stereomicroscope (Leica Wild M3B, Wetzlar, Germany).

Experimental Design

To determine the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values of the pure contaminants, we analyzed eight samples of polypropylene plastic cut from sample tubes: four underwent the full laboratory procedure for cellulose extraction and four were weighed directly into tin capsules for mass spectrometry. Each sample was from a new sample tube, but all sample tubes were from the same batch of standard 2.0 mL laboratory polypropylene sample tubes (Rotilabo-safety reaction tubes, Item No.: NA16.1, Carl Roth GmbH, Karlsruhe, Germany). As part of a thorough study, we additionally evaluated possible contamination through fibers of the PTFE filter bags often used for cellulose extraction: we prepared samples of polytetrafluoroethylene plastic (PTFE) by cutting pieces from a F57 filter bag (Ankom Technology, Macedon, USA). While different extraction methods can be used (Gaudinski et al., 2005; Boettger et al., 2007), a common technique involves placing the samples inside such filter bags made of PTFE, then exposing the samples to NaOH and NaClO₂ to remove extractives and lignin (Gaudinski et al., 2005). However, PTFE fibers can occasionally become embedded in the sample and may go unnoticed before mass spectrometry. Similar to polypropylene, this could cause isotope contamination because PTFE is also made of fossil fuels. The chemical structures of polypropylene $[(\text{C}_3\text{H}_6)_n]$

and PTFE [(C₂F₄)_n] contain no oxygen atoms, so only $\delta^{13}\text{C}$ values would be expected to be affected by plastic contamination. We nevertheless measured $\delta^{18}\text{O}$ values of a small subset to verify that no unexpected source of fractionation existed.

To test polypropylene contamination of wood due to ball milling, we used a multi-factorial design involving two milling times (5 min and 10 min), two different numbers of balls (2 or 3 balls), two sample amounts (10 mg and 40 mg) and two measurement types (whole wood and cellulose). These categories were chosen because they represent a range of common applications of the ball mill for homogenizing wood for stable isotope analysis. Each category contains four samples (Tables 1 and 2). Samples were carefully weighed to a precision of 0.01 mg using an analytical balance (Model XS105, Mettler Toledo, Greifensee, Switzerland) before being placed into 2.0 mL polypropylene sample tubes. We initially compared the sample tubes by Carl Roth with Eppendorf sample tubes, both of which are made of polypropylene plastic, but a visual assessment found no apparent difference. We therefore continued with the Carl Roth sample tubes that are standard in our laboratory.

The ball mill used was the “Mixer Mill” by Retsch (MM 200, Retsch GmbH, Hann, Germany). To test the base level of abrasion of each treatment on the sample tube itself, we ran empty sample tubes under two time treatments (5 min and 10 min) with differing numbers of milling balls (2 or 3 balls). The abrasion from these sample tubes was detected visually and a binary label of “opaque” (abrasion present) or “clear” (abrasion absent) was assigned (Fig. 1). For the control, four 40 mg samples were milled with the ultra-centrifugal mill (ZM 2000, Retsch GmbH, Germany) with a 0.25 mm mesh size to ensure homogeneity (Borella et al., 1998; McCarroll & Loader, 2004; Laumer et al., 2009). This mill is a suitable control because it is all-metal and therefore free from any potential plastic contamination. All samples were then

separated into cellulose and whole wood samples, and samples for both $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ measurements were packed for mass spectrometry.

We also conducted a follow-up experiment to test if the addition of liquid to the sample tube prior to ball-milling could reduce sample tube abrasion without inhibiting sample homogenization. We determined the suitability of distilled water and 96 % ethanol. We initially observed that sample tubes milled with 1 mL of liquid remained “optically clear”, but subsequent runs produced very poor homogenization results. Continued re-milling was not considered because it could increase abrasion and plastic contamination. Follow-up mass spectrometry on a subset of treatments with highest contamination potential indicated that some samples were significantly different from the control (data not shown). We therefore do not pursue this option further and cannot recommend its use without further investigation.

Cellulose Extraction

Half of the samples were whole wood (Table 2), which were packed directly into tin capsules after milling. The other half underwent the cellulose-extraction procedure after milling. The latter samples were packed into PTFE filter bags (F57, Ankom Technology, USA) and cellulose was extracted by applying a modified Jayme-Wise Holocellulose Isolation Method (Gaudinski et al., 2005; Boettger et al., 2007). For a 10 mg sample, this typically involves a wash with a 5 % NaOH solution two times for 2 hrs at 60 °C followed by a wash with 7 % NaClO₂ for 30 hrs at 60 °C. We assumed the reaction to be a linear process, and initially scaled the chemical exposure time according to sample weight. We found, however, that the percent of the sample that was extracted varied substantially after completing an initial round of cellulose extraction. This likely arose from a high variability in particle size after milling. In a normal study design, a

researcher would likely mill such a heterogeneous sample again to ensure particle uniformity and equal cellulose extraction rates (Borella et al., 1998). Since we tested the effect of milling time, we chose not to re-mill any such samples. To allow us to nevertheless compare the cellulose from such heterogeneously milled samples, we attempted to ensure a consistent rate of extraction by processing the samples until at least only 45 % of the original sample mass remained. As a further precaution, the final extraction rate was accounted for in the model (see below).

Measurements of Isotope Ratios

For $\delta^{13}\text{C}$ measurements, samples were combusted to CO_2 at 1080 °C using a EURO EA Elemental Analyzer (EuroVector, Milan, Italy). For $\delta^{18}\text{O}$ measurements, samples were pyrolyzed to CO at 1400 °C using a high-temperature oxygen analyzer (HEKAtech, Wegberg, Germany). Stable isotope ratios were determined by isotope ratio mass spectrometry (Delta V Advantage Mass Spectrometer, Thermo Scientific, Bremen, Germany). All isotope measurements were conducted at the WSL Central Laboratory (Birmensdorf, Switzerland) at a precision of ± 0.02 ‰ for carbon and ± 0.3 ‰ for oxygen. $\delta^{13}\text{C}$ values were referenced to Vienna Pee Dee Belemnite (VPDB) and $\delta^{18}\text{O}$ values were referenced to Vienna Standard Mean Ocean Water (VSMOW) according to the following formula: $R = (R_{\text{sample}} / R_{\text{standard}} - 1)$, where R is the ratio of the heavy to light isotope (Farquhar et al., 1982; McCarroll & Loader, 2004).

Statistical Analysis

All analyses were conducted in the R statistical programming environment (R Core Team). To test differences in abrasion among the empty sample tubes after milling, we used binary logistic regression. Statistical analysis of the multi-factorial design relies on a simple linear model ANOVA implemented with the *lm* function in the base package in R. This analysis

was first completed to compare different amounts of losses among treatments after cellulose extraction, using percent remaining as a response variable after logit transformation (Warton & Hui, 2011) using the *logit* function in the *car* package. We then analyzed cellulose and whole wood separately to account for slightly different predictor variables. Both of these models use $\delta^{13}\text{C}$ values as the response variable, and predictor variables include amount (10 mg or 40 mg), the number of steel milling balls (2 or 3), and the milling time (5 min or 10 min). The percent of the sample's mass after cellulose extraction was included in the cellulose model as a covariate. In the whole wood model, the percent of carbon was included as a covariate. A similar model was run on a smaller subset with $\delta^{18}\text{O}$ values as the response variable. To calculate the proportional contribution of the three main treatments (amount, number of balls and milling time) to the total explained variance, we estimated the partial eta-squared (η_p^2) using the *etaSquared* function from the *lsr* package on the ANOVA outputs. Post-hoc tests were completed with the *TukeyHSD* function, which uses Tukey's Honest Significant Difference to account for experiment-wide alpha inflation.

4.5 Results

Sample Tube Abrasion and Isotope Signatures of Potential Contaminants

The link to polypropylene as a contaminant is through sample tube abrasion, and the results of the abrasion test can be seen in Figure 1. This figure can be used to evaluate the level of abrasion present: the appearance of empty sample tubes after milling with different numbers of balls (2 or 3 balls) for different durations (5 min and 10 min) are assigned a binary label of abrasion. Those assigned a label of "clear" indicate little to no abrasion, while those assigned a label of "opaque" suggest high abrasion (Fig. 1). The visual results together with the results of

binary logistic regression show that there are significant differences among groups ($p=0.008$). The sample tubes from the 3 ball - 10 min category showed the most abrasion, while the sample tubes from the 2 ball - 5 min category showed the least.

Effect of Cellulose Extraction on the Amount of Material

Cellulose extraction did not reduce the amount of polypropylene plastic in samples (Fig. 2, Tab. S1 in Supplementary Information). The yield after cellulose extraction of the wood samples depended on the type of mill and the amount of material that was milled (Fig. 2, Tab. S1 in Supplementary Information). The control samples, which were milled with the all-metal, ultra-centrifugal mill, showed a high remaining amount with low variability between samples. Similarly, those samples that were milled in the ball mill with more material (40 mg) had more material remaining and less variability in its amount after cellulose extraction. The samples ball-milled with less material (10 mg) showed a significantly higher loss of material after cellulose extraction, combined with higher variability. The samples that were ball-milled for 10 minutes showed the least amount remaining after cellulose extraction. In fact, the only three categories that were significantly different in their amount from the control were 10 mg - 2 ball - 10 min ($p<0.001$), 10 mg - 3 ball - 10 min ($p<0.001$) and 10 mg - 3 ball - 5 min ($p=0.029$).

Carbon and Oxygen Isotope Signatures in Contaminants, Milled Wood and Cellulose

We found no oxygen atoms in either polypropylene or PTFE (Tab. 1). However, both plastics contain carbon atoms, and our analyses showed distinct $\delta^{13}\text{C}$ signatures (Tab. 2). For polypropylene, the mean $\delta^{13}\text{C}$ value was -30.6‰ . For PTFE, the mean $\delta^{13}\text{C}$ value was -28.9‰ . These values are -6.8‰ and -5.0‰ lower than the mean $\delta^{13}\text{C}$ value of our wood control sample (-23.8‰), respectively.

The $\delta^{13}\text{C}$ values were similar among the control samples and the large (40 mg) samples milled with the ball mill (Fig. 3a and 3b, Tab. 2). Post-hoc tests further confirmed no statistical difference between the 40 mg samples and the control for both cellulose and whole wood. In comparison, the small samples (10 mg) milled with the ball mill showed generally more deviation from the control and more variability of their $\delta^{13}\text{C}$ values (Fig. 3a and 3b, Tab. 2). Both cellulose and whole wood samples milled for longer (10 min) and with less material (10 mg) showed significantly lower $\delta^{13}\text{C}$ values compared to the control samples (Fig. 3a and 3b, Tab. 2). The number of balls, time and material all significantly contributed to the model ($p < 0.001$). In the cellulose model, the yield (% remaining) also contributed significantly to the model ($p < 0.001$). Using $\delta^{13}\text{C}$ values as the response variable in both cellulose and whole wood models, the effect size for the sample amount (10 mg or 40 mg) was found to be of higher relevance ($\eta_p^2 = 0.505$ in both) than the effect size for the number of steel milling balls (2 or 3 balls), which was weaker in both cellulose and whole wood models ($\eta_p^2 = 0.204$ and 0.263 , respectively). The effect of milling time (5 min or 10 min) was also weaker in both cellulose and whole wood models ($\eta_p^2 = 0.341$ and $\eta_p^2 = 0.252$, respectively).

A comparison of the $\delta^{13}\text{C}$ values of whole wood and cellulose indicated a relatively consistent offset (Fig. 3c): $\delta^{13}\text{C}$ values in cellulose were less negative compared to $\delta^{13}\text{C}$ values in whole wood. This offset was most prominent and consistent in samples that were milled with more material (40 mg), where they deviated on average by -1.053‰ (Table 2). These values fall within the range of previously published work on wood-cellulose comparisons of conifers (Gori et al., 2013; Weigt et al., 2015). Interestingly, the offset became reduced in the samples milled with less material (10 mg) and even reversed in the treatment with the highest abrasion: the 10 mg - 3 ball - 10 min category showed cellulose being even more depleted $\delta^{13}\text{C}$ values than

whole wood (Fig. 3c). In the 10 mg samples, the mean offset was only -0.190‰ because individual offsets were not consistent in direction (Table 2).

4.6 Discussion

Sample Tube Abrasion and Heterogeneity of Particle Size in Milled Wood

Our milling test with empty sample tubes showed that abrasion can occur. We also demonstrated that the intensity of abrasion increases when using 3 instead of 2 balls and when extending the milling time from 5 to 10 minutes. This is the first evidence that there is indeed a risk for contamination of wood samples with plastic during the ball mill procedure.

During cellulose extraction, samples of similar mass unexpectedly showed different amounts of loss. This serendipitously provided a means to estimate variations in yield created under our milling configurations. Here, we confirm the importance of particle size on the yield and rate of cellulose extraction (Borella et al., 1998). The reduction in yield can result from both higher exposure (smaller particles have higher surface area) and direct loss through the PTFE filter bag used for cellulose extraction (porosity of 25 μm). The control samples milled with the ultra-centrifugal mill showed the highest remaining amount and least variability. This suggests that the ultra-centrifugal mill produces particles that are relatively large compared to the particle sizes produced with the ball mill, but uniform.

Contamination Concentration due to Cellulose Extraction

Since the yield of plastic after cellulose extraction remained the same, plastic is not lost during extraction, in contrast to the non-cellulose components of wood which are lost. The concern, therefore, is that cellulose extraction can exacerbate the effects of plastic contamination

by increasing the plastic-to-sample ratio. Since both plastics had more negative $\delta^{13}\text{C}$ values than the cellulose from the control samples used in this study, we would therefore expect that any increase in the plastic-to-sample ratio would result in more negative $\delta^{13}\text{C}$ values than usual. This effect was not seen in the large (40 mg) samples because the well-known offset in $\delta^{13}\text{C}$ values of cellulose and whole wood (Borella et al., 1998) remained consistent among treatments. However, the plastic-contamination effect was visible in the samples milled with less material (10 mg). These samples showed a decreased whole-wood to cellulose offset, and even a reversal of the offset occurred in the category with the highest abrasion (10 mg - 3 balls - 10 min). This trend change provides evidence that smaller samples do incur plastic contamination and that the plastic-to-sample ratio increases after cellulose extraction.

Recommendations and Possible Alternatives

A ball mill equipped with holders to mill wood samples in polypropylene sample tubes may be suitable for $\delta^{18}\text{O}$ analysis: no oxygen atoms are present in plastic, and hence, we found no evidence that $\delta^{18}\text{O}$ values would be affected by plastic contamination. We also did not detect any evidence of fractionation from heating, which is in line with a previous experiment (Riechelmann et al., 2014). Due to the importance of particle size on yield after cellulose extraction, however, milling larger amounts of sample may be preferred.

Using a ball mill is not advisable for milling wood prior to $\delta^{13}\text{C}$ radiocarbon measurement. Changes of isotope values due to plastic contamination were most concerning for smaller amounts (10 mg). An increase in the plastic-to-sample ratio during cellulose extraction further contributes to erroneously low $\delta^{13}\text{C}$ values in these smaller samples. We cannot recommend the ball mill for milling larger sample amounts, either. Our results showed that $\delta^{13}\text{C}$

values in larger sample amounts (40 mg) in both whole wood and cellulose did not deviate significantly from the control, but there is potential for contamination if the samples are re-milled since the abrasion in sample-tubes increases with milling time. In a follow-up test, milling very large sample amounts (40 - 90 mg) for 10 minutes within their test tubes was often ineffective, and re-milling for another 10 minutes did not improve the homogenization. However, with each successive milling treatment, the probability of sample-tube abrasion and isotope contamination increases, even if the risk had been initially low. We found similarly poor homogenization when milling samples with liquid. Accordingly, we cannot recommend milling with a ball mill, in direct disagreement with recommendations made by a previous study (Riechelmann et al., 2014).

Several modifications to the current ball-mill configuration can reduce or eliminate the potential for plastic contamination. For example, the wood samples could be transferred into inert milling containers made of materials such as ceramic, metal, or possibly the more abrasion-resistant PTFE sample tubes. Using these types of containers is, however, also a slow procedure since only two inert containers can be run simultaneously, as compared to milling up to 16 sample tubes at once. Time-savings are also lost because the sample must be transferred in and out of the container, and the milling containers must be carefully cleaned between samples. Nevertheless, the ball mill fitted with inert containers remains one of the most simple and least problematic (albeit slow) options for homogenizing wood prior to isotope measurements (Tab. 5).

The all-metal ultra-centrifugal mill remains a good option for homogenization, but is time-consuming, and often much of the sample is lost (Tab. 5). Sample loss is primarily due to a loose fit of the mechanical mill pieces and the fact that it was designed for much larger sample

volumes. We therefore tested a smaller version of the milling pieces specially designed by the manufacturer for smaller samples, described as a “small-sample-converter-kit”. However, this small-sample-converter-kit did not solve the problem of sample loss because it also had loose-fitting parts. Such loss of sample is especially problematic when there is little sample material to begin with (<10 mg) and when cellulose extraction is done afterward, because a further 60 % loss of mass can occur.

If a researcher chooses to work with cellulose, they would be advised to use the ultra-sonic homogenization method (Laumer et al., 2009) (Tab. 5). This method is, however, also limited in that it can only homogenize cellulose samples less than 10 mg. These cellulose samples must be soaked in 1 mL of deionized water for this process to work, and care must be taken to minimize and standardize the treatment length. Over-heating can otherwise occur, potentially increasing the exchange of oxygen atoms between the sample and water (Schollaen et al., 2015). Ultra-sonic homogenization of small samples of whole wood is not an option: We found no change after applying a full-power, two-minute ultra-sonic treatment on two water-logged whole wood samples. Much more energy is apparently needed to break the lignin molecules than the cellulose chains.

In conclusion, there is a clear need for simple, fast, effective and contamination-free methodologies to homogenize wood prior to isotope analysis. This would further facilitate tree-ring isotope research aimed at answering important biological, ecophysiological, and dendrochronological questions.

4.7 References

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4.8 Figures and Tables

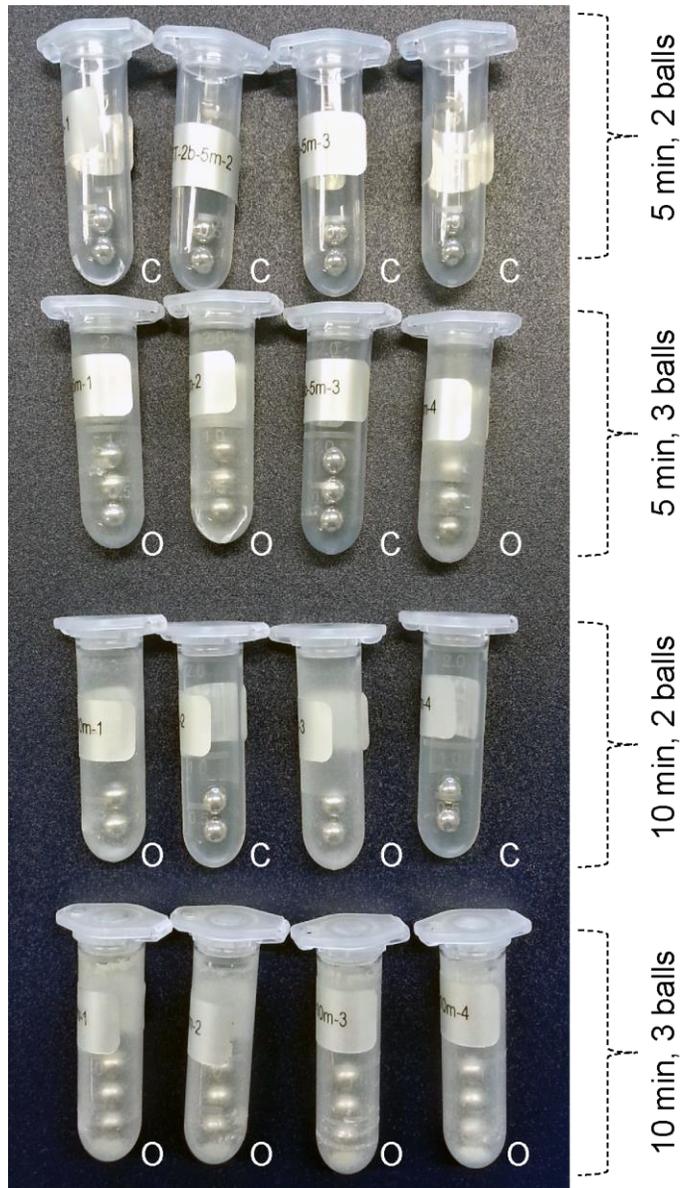


Fig. 4.1. The results of the plastic abrasion test by milling empty sample tubes by time and number of balls added. A white letter to the bottom-right of each tube shows the assigned level of abrasion, a binary response variable. Abrasion is clearly visible in test-tubes that appear opaque, denoted by “O”. Tubes that are clear indicate no abrasion, labelled with a “C”. The sample tubes that were milled for 10 min using 3 balls showed the highest abrasion because they were all opaque. In contrast, the tubes milled for 5 min with only 2 balls were all clear, showing the least abrasion.

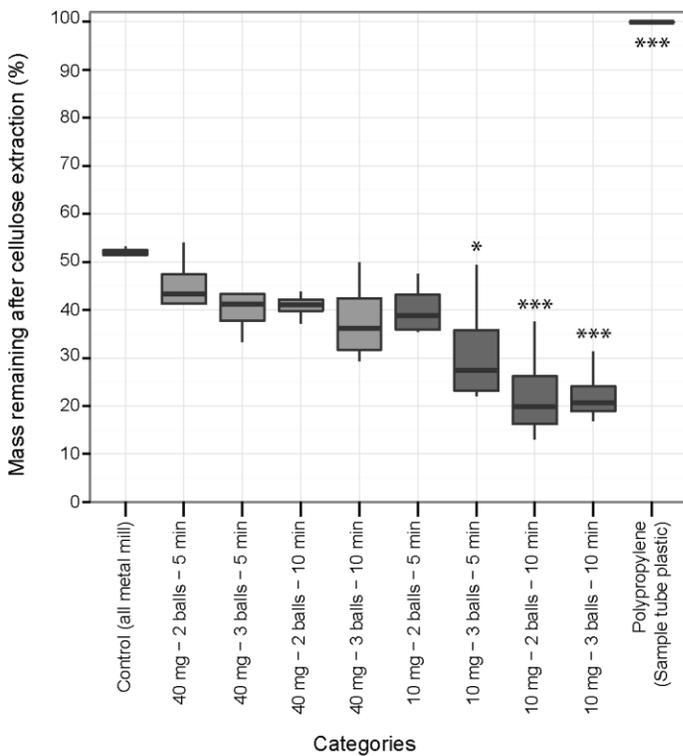


Fig. 4.2. Boxplots of the percent of sample mass remaining after the first round of cellulose extraction (they were later extracted until <45% of the sample mass remained). The inter-quartile range of 40 mg samples are shown in light grey while the inter-quartile range of 10 mg samples are shown in dark grey. Each category represents four samples. Stars represent categories that are significantly different from the control (*** = $p < 0.001$, * = $p < 0.05$).

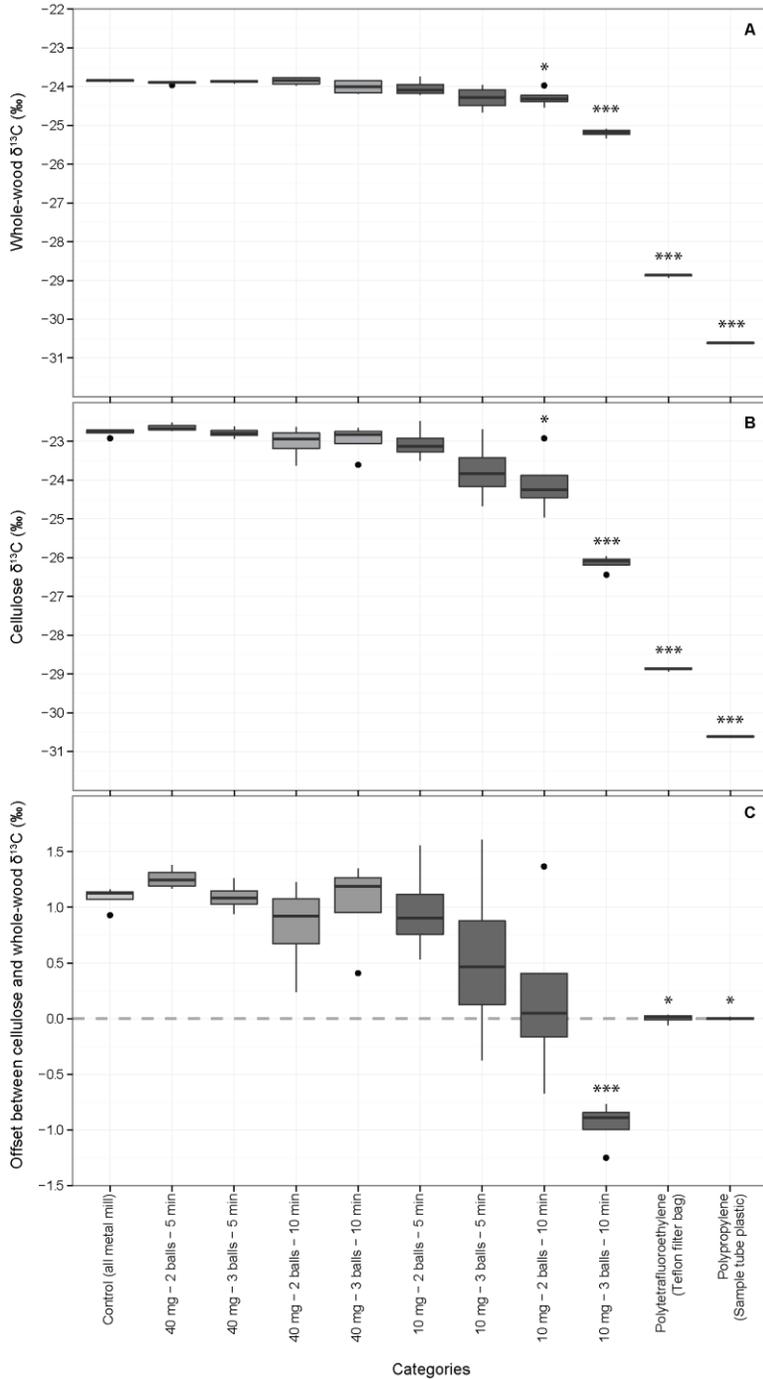


Fig. 4.3. Boxplots of the distribution of $\delta^{13}\text{C}$ values of treatments, represented as ‰ deviations from VPDB. Panel A displays the $\delta^{13}\text{C}$ values from whole wood samples in each treatment category. Panel B shows the $\delta^{13}\text{C}$ values from cellulose samples in each treatment category. Panel C shows the $\delta^{13}\text{C}$ value offset between whole wood and cellulose in each treatment category. The inter-quartile range of the control samples, which were milled with an all-metal mill, is displayed in light grey. The inter-quartile range of samples milled with 40 mg are shown in darker grey. The inter-quartile range of 10 mg samples are shown in the darkest shade of grey. Each category represents four samples. Stars represent categories that are significantly different from the control (*** = $p < 0.001$, * = $p < 0.05$).

Tab. 4.1. Mean $\delta^{18}\text{O}$ values and standard error for polytetrafluoroethylene plastic (PTFE), polypropylene plastic, and wood that underwent different ball milling treatments. The ball mill is denoted by BM, the ultra-centrifugal mill is represented by CM. Sample weights (*Weight*) are in milligrams (mg), and the milling time (*Time*) is in minutes. The control is marked with a star, and the offsets to this control are provided (*Mean $\delta^{18}\text{O}$ Offset*). Standard error is denoted by *SE*, while the number of samples is denoted by *N*. The difference of each group to its respective control can be evaluated with the p-value adjusted with a Bonferroni correction to compensate for experiment-wise alpha inflation (*adj. p-value*).

Material	Mill	Weight (mg)	No. Balls	Time (min)	Mean $\delta^{18}\text{O}$ (‰)	Mean $\delta^{18}\text{O}$ Offset (‰)	SE	N	adj. p-value
Polypropylene	—	—	—	—	No value	—	—	4	
Polytetrafluoroethylene	—	—	—	—	No value	—	—	4	
Cellulose*	CM	40	—	—	29.17	—	0.19	4	—
Cellulose	BM	10	2	5	29.02	-0.16	0.17	4	0.865
Cellulose	BM	40	2	5	29.08	-0.09	0.27	4	0.951

Tab. 4.2. Mean $\delta^{13}\text{C}$ values and standard error for polytetrafluoroethylene plastic (PTFE), polypropylene plastic, and wood that underwent different ball milling treatments. The ball mill is denoted by BM, the ultra-centrifugal mill is represented by CM. Sample weights (*Weight*) are in milligrams (mg), and the milling time (*Time*) is in minutes. The control is marked with a star, and the offsets to this control are provided (*Mean $\delta^{13}\text{C}$ Offset*). Standard error is denoted by *SE*, while the number of samples is denoted by *N*. The difference of each group to its respective control can be evaluated with the p-value adjusted with a Bonferroni correction to compensate for experiment-wise alpha inflation (*adj. p-value*).

Material	Mill	Weight (mg)	No. Balls	Time (min)	Mean $\delta^{13}\text{C}$ (‰)	Mean $\delta^{13}\text{C}$ Offset (‰)	SE	N	adj. p-value
Polypropylene	—	—	—	—	-30.61	-6.77	0.01	4	—
Polytetrafluoroethylene	—	—	—	—	-28.87	-5.02	0.02	4	—
Whole wood*	CM	40	—	—	-23.85	—	0.01	4	—
Whole wood	BM	10	2	10	-24.29	-0.44	0.12	4	0.024
Whole wood	BM	10	2	5	-24.03	-0.18	0.10	4	0.828
Whole wood	BM	10	3	10	-25.19	-1.34	0.05	4	<0.001
Whole wood	BM	10	3	5	-24.30	-0.45	0.16	4	0.020
Whole wood	BM	40	2	10	-23.86	-0.01	0.05	4	>0.999
Whole wood	BM	40	2	5	-23.90	-0.05	0.02	4	>0.999
Whole wood	BM	40	3	10	-24.01	-0.16	0.09	4	0.914
Whole wood	BM	40	3	5	-23.87	-0.02	0.02	4	>0.999
Cellulose*	CM	40	—	—	-22.77	—	0.05	4	—
Cellulose	BM	10	2	10	-24.09	-1.33	0.43	4	0.013
Cellulose	BM	10	2	5	-23.06	-0.30	0.21	4	0.992
Cellulose	BM	10	3	10	-26.14	-3.38	0.10	4	<0.001
Cellulose	BM	10	3	5	-23.76	-0.99	0.41	4	0.122
Cellulose	BM	40	2	10	-23.03	-0.27	0.21	4	0.996
Cellulose	BM	40	2	5	-22.64	-0.13	0.05	4	>0.999
Cellulose	BM	40	3	10	-22.98	-0.21	0.21	4	>0.999
Cellulose	BM	40	3	5	-22.79	-0.01	0.07	4	>0.999

Tab. 4.3. Analysis of variance for the cellulose model. Reported values include the sum of squares (*SS*), the mean square (*MS*), degrees of freedom (*df*), the calculated test statistic (*F-value*) and the p-value (*p-value*). Residuals refer to variation that is not explained by the model.

Source of Variation	SS	MS	df	F-value	<i>p</i> -value
Mill	2.23	2.24	1	27.03	<0.001
Balls	4.00	4.00	1	48.26	<0.001
Time	8.01	8.01	1	96.71	<0.001
Amount	15.81	15.81	1	190.84	<0.001
Percent Remaining	5.07	5.07	1	61.24	<0.001
Interaction (Balls:Time:Amount)	8.29	2.07	4	25.01	<0.001
Residuals	2.15	0.08	26		

Tab. 4.4. Analysis of variance for the whole wood model. The fixed effects are shown, and the amount of carbon present in the sample was specified as a random effect. Reported values include the sum of squares (*SS*), the mean square (*MS*), degrees of freedom (*df*), the calculated test statistic (*F-value*) and the p-value (*p-value*). Residuals refer to variation that is not explained by the model.

Source of Variation	SS	MS	df	F-value	p-value
Mill	0.38	0.39	1	14.00	<0.001
Balls	0.82	0.83	1	29.86	<0.001
Time	0.77	0.78	1	28.03	<0.001
Amount	2.35	2.35	1	85.16	<0.001
Carbon Amount	0.06	0.06	1	2.15	0.155
Interaction (Balls:Time:Amount)	1.53	0.38	4	13.80	<0.001
Residuals	0.72	0.028	26		

Tab. 4.5. Options for homogenizing wood and cellulose prior to stable- and radio-carbon isotope analyses. As seen in the *Notes* column, there is a trade-off between the speed of the procedure and the potential for plastic contamination.

Homogenization Options	Sample Category				Notes
	Cellulose		Whole-Wood		
	10 mg	40 mg	10 mg	40 mg	
Ball mill, samples milled in plastic containers	—	✓	✓	✓	<ul style="list-style-type: none"> All check-marks are grey because there is some risk of contamination involved. Using a ball mill with water or ethanol can help reduce polypropylene contamination. It can still not be recommended, however, because samples milled in liquid were not milled properly, requiring re-milling, thereby increasing the risk of sample-tube abrasion and plastic contamination.
Ball mill, samples milled in inert containers	✓	✓	✓	✓	<ul style="list-style-type: none"> All check-marks are black, because there is no risk of plastic contamination while using ceramic or metal containers. This is a time-consuming option since samples must be moved from sample-tubes into the inert containers (usually max. of 2/run), the powder collected and returned into the sample tubes, and the containers must be cleaned. Unlike the ultra-centrifugal mill, wood does not need to be as finely cut prior to milling.
Ultra-sonic Homogenizer	✓	—	—	—	<ul style="list-style-type: none"> Ultra-sonic easier than microtome for small (<10 mg) cellulose samples. Ultra-sonic homogenizer did not work on water-logged whole wood samples.
Ultra-centrifugal Mill	—	✓	✓	✓	<ul style="list-style-type: none"> This is a time consuming option because samples are removed from sample-tubes and dropped into the rotating milling pieces and forced through a fine mesh. Although the milling is almost instantaneous, collecting the powder across the mill surfaces and returning it to sample tubes is slow. Cleaning all surfaces between samples takes approx. 7-10 minutes. The check-mark is also grey for the ultra-centrifugal mill for the small samples because much sample is lost.
Microtome	✓	—	✓	—	<ul style="list-style-type: none"> Microtoming is also slow and problematic for small amounts, which is why the check-mark is grey for 10 mg samples.

4.9 Supplementary Information

Tab. 4.S1. Cellulose extraction data: The weight packed into the filter bag (*Weight In*) deviates from the original sample weight (*Weight*) because some sample is lost during milling. If the sample weight after the initial cellulose extraction (*Initial Weight In*) represented more than 45 % of the original weight (*% Initially Remaining*), then cellulose extraction was continued until all samples were <45 % remaining (*Weight Out Final*; *% Final Remaining*). *Initial Extraction* and *Final Extraction* represent the extraction time.

Sample ID	Mill	Weight	Balls	Time	Replicate	Weight In (mg)	Initial Weight Out (mg)	Weight Out Final (mg)	% Initially Remaining	% Final Remaining	Initial Extraction (hrs)	Final Extraction (hrs)
CM-40 mg-1	CM	40 mg	NA	NA	Rep1	16.21	8.46	7.58	52.2	46.8	30	50
CM-40 mg-2	CM	40 mg	NA	NA	Rep2	18.01	9.27	8.07	51.5	44.8	30	50
CM-40 mg-3	CM	40 mg	NA	NA	Rep3	22.31	11.46	10.68	51.4	47.9	30	50
CM-40 mg-4	CM	40 mg	NA	NA	Rep4	20.01	10.64	9.69	53.2	48.4	30	50
BM-10 mg-2b-5m-1	BM	10 mg	2b	5m	Rep1	6.52	2.72	2.72	41.7	41.7	15	15
BM-10 mg-2b-5m-2	BM	10 mg	2b	5m	Rep2	5.53	1.99	1.99	36.0	36.0	15	15
BM-10 mg-2b-5m-3	BM	10 mg	2b	5m	Rep3	6.95	3.3	2.97	47.5	42.7	15	20
BM-10 mg-2b-5m-4	BM	10 mg	2b	5m	Rep4	6.64	2.35	2.35	35.4	35.4	15	15
BM-10 mg-3b-5m-1	BM	10 mg	3b	5m	Rep1	5.99	1.32	1.32	22.0	22.0	15	15
BM-10 mg-3b-5m-2	BM	10 mg	3b	5m	Rep2	6.14	1.92	1.92	31.3	31.3	15	15
BM-10 mg-3b-5m-3	BM	10 mg	3b	5m	Rep3	5.1	1.2	1.2	23.5	23.5	15	15
BM-10 mg-3b-5m-4	BM	10 mg	3b	5m	Rep4	7.12	3.51	2.84	49.3	39.9	15	25
BM-10 mg-2b-10m-1	BM	10 mg	2b	10m	Rep1	6.16	1.38	1.38	22.4	22.4	15	15
BM-10 mg-2b-10m-2	BM	10 mg	2b	10m	Rep2	5.32	0.92	0.92	17.3	17.3	15	15
BM-10 mg-2b-10m-3	BM	10 mg	2b	10m	Rep3	6.2	2.33	2.33	37.6	37.6	15	15
BM-10 mg-2b-10m-4	BM	10 mg	2b	10m	Rep4	5.99	0.78	0.78	13.0	13.0	15	15
BM-10 mg-3b-10m-1	BM	10 mg	3b	10m	Rep1	6.19	1.34	1.34	21.6	21.6	15	15
BM-10 mg-3b-10m-2	BM	10 mg	3b	10m	Rep2	5.69	0.96	0.96	16.9	16.9	15	15
BM-10 mg-3b-10m-3	BM	10 mg	3b	10m	Rep3	6.32	1.98	1.98	31.3	31.3	15	15
BM-10 mg-3b-10m-4	BM	10 mg	3b	10m	Rep4	6.24	1.22	1.22	19.6	19.6	15	15
BM-40 mg-2b-5m-1	BM	40 mg	2b	5m	Rep1	19.37	7.97	7.97	41.1	41.1	30	30
BM-40 mg-2b-5m-2	BM	40 mg	2b	5m	Rep2	19.92	8.24	8.24	41.4	41.4	30	30
BM-40 mg-2b-5m-3	BM	40 mg	2b	5m	Rep3	19.82	8.96	8.01	45.2	40.4	30	40
BM-40 mg-2b-5m-4	BM	40 mg	2b	5m	Rep4	19.41	10.49	8.97	54.0	46.2	30	50
BM-40 mg-3b-5m-1	BM	40 mg	3b	5m	Rep1	20.78	8.99	8.99	43.3	43.3	30	30
BM-40 mg-3b-5m-2	BM	40 mg	3b	5m	Rep2	20.48	8.89	8.89	43.4	43.4	30	30
BM-40 mg-3b-5m-3	BM	40 mg	3b	5m	Rep3	20.52	8.04	8.04	39.2	39.2	30	30
BM-40 mg-3b-5m-4	BM	40 mg	3b	5m	Rep4	19.8	6.6	6.6	33.3	33.3	30	30
BM-40 mg-2b-10m-1	BM	40 mg	2b	10m	Rep1	20.54	8.35	8.35	40.7	40.7	30	30
BM-40 mg-2b-10m-2	BM	40 mg	2b	10m	Rep2	20.82	8.66	8.66	41.6	41.6	30	30
BM-40 mg-2b-10m-3	BM	40 mg	2b	10m	Rep3	20.64	7.68	7.68	37.2	37.2	30	30
BM-40 mg-2b-10m-4	BM	40 mg	2b	10m	Rep4	22.01	9.64	9.64	43.8	43.8	30	30
BM-40 mg-3b-10m-1	BM	40 mg	3b	10m	Rep1	20.19	6.56	6.56	32.5	32.5	30	30
BM-40 mg-3b-10m-2	BM	40 mg	3b	10m	Rep2	18.38	7.34	7.34	39.9	39.9	30	30
BM-40 mg-3b-10m-3	BM	40 mg	3b	10m	Rep3	18.26	5.36	5.36	29.4	29.4	30	30
BM-40 mg-3b-10m-4	BM	40 mg	3b	10m	Rep4	18.9	9.42	8.17	49.8	43.2	30	50
TT-1	NA	NA	NA	NA	Rep1	14.71	14.72	14.72	100.1	100.1	30	30
TT-2	NA	NA	NA	NA	Rep2	13.57	13.54	13.54	99.8	99.8	30	30
TT-3	NA	NA	NA	NA	Rep3	14.51	14.5	14.5	99.9	99.9	30	30
TT-4	NA	NA	NA	NA	Rep4	14.04	14.02	14.02	99.9	99.9	30	30

5 Reducing Image Artifacts for Faster Analysis of Wood Micro Sections

5.1 Publication

Montwé D, Isaac-Renton M, Hamann A, Spiecker H (2015) Using steam to reduce artifacts in micro sections prepared with corn starch. *Dendrochronologia*, **35**:87-90.

5.2 Abstract

Preparation of micro sections to measure cell wall thickness and lumen diameter is a widely used method in the fields of dendroecology, dendroclimatology and tree physiology. Efficient sample preparation and image analysis is critical for studies with long time series and large sample sizes. Recently, there have been substantial improvements in micro section preparation techniques, including a corn starch-based non-Newtonian fluid treatment. This method reduces cell wall damage during cutting with a microtome, which in turn decreases artifacts during image analysis. Although this procedure does in fact improve sample quality, we found starch grains sometimes to be difficult to remove and to cause artifacts during image analysis. This technical note outlines a simple, fast and effective steam treatment that causes starch gelatinization and a reduction in the number of starch grain artifacts.

5.3 Introduction

Measuring cell wall thickness and lumen diameter in xylem cells requires the preparation of micro sections, typically 10-20 μm thick. Measurements of these microscopic structures are important in dendroecology, dendroclimatology, and tree physiology research (Fonti et al., 2010). In tree physiology, cell wall thickness and cell lumen diameter are closely correlated with cavitation resistance (Hacke et al., 2001), and can therefore be used to infer drought resistance of tree species or populations. In dendroecology, this information enables the assessment of tree species' abilities to acclimate to changing environmental conditions and their overall suitability to increased drought severity under climate change (Bryukhanova & Fonti, 2013). In dendroclimatology, time series of cell parameters can serve as proxies for climate reconstructions (Wimmer, 2002). The proportion of cell walls to cell lumen is closely related to wood density (Wassenberg et al., 2014), which is an important proxy for summer temperatures (Briffa et al., 2004).

Preparing micro sections from large stem diameters, representing long dendrochronological time series, is a challenging and labor-intensive task, however. These obstacles have led to the development of alternative preparation techniques, including the use of high-precision diamond-fly-cutters for reflected light microscopy (Spiecker et al., 2000), but also to advances in microtome techniques. Sliding microtomes using common cutter blades have emerged as being capable of producing high-quality micro sections, while also being much easier to maintain than earlier alternatives (Gärtner et al., 2014, 2015a). These instruments are intended to develop long anatomical time series, with a single micro section covering multiple tree-rings (Gärtner et al., 2015a, 2015b).

One remaining issue, however, is that secondary cell walls of tracheids are sensitive to pressure induced by the cutting blade, often causing them to be pushed into the cell lumen (Schneider & Gärtner, 2013). Such damage to the cell structure can create problems for automatic image analysis, which are time-consuming to manually remove. Until recently, this problem was addressed by embedding the sample in paraffin wax, for example (Feder & O'Brien, 1968). However, this procedure is time-consuming and the wax must be removed before staining (Schneider & Gärtner, 2013).

As an alternative method to reduce cutting artifacts in the development of long time series of anatomical properties in tree-rings, Schneider and Gärtner (2013) proposed using a non-Newtonian fluid to stabilize the cell structure. A fluid mixture of corn starch and water is applied to the surface of the sample prior to the microtome cut. The effectiveness comes from the fluid's non-Newtonian properties. While being applied with a brush, the mixture is liquid, and flows into the empty cell lumen. Under pressure, the starch grains form a solid structure, stabilizing the cell walls. As a result, artifacts due to deformed cell walls can be mitigated. We applied this method to lodgepole pine (*Pinus contorta* Douglas ex Loudon) samples. The corn-starch mixture worked as expected and did indeed minimize the aforementioned artifacts. We found the corn starch solution efficient, easy to use, and to improve accuracy of the image analysis.

However, during our sample preparation, we sometimes found it difficult to completely wash the corn starch grains out of the cells. We found that the starch grains were more easily removed from the larger earlywood cells, leading to good contrast between cell wall and cell lumen, but the removal could be more problematic in the narrower latewood cells. Here, the grains were held in place by friction and adhesion, and even long and intensive rinsing could not always overcome these forces. Examples of remaining starch grains are shown in Fig. 5.1 and

become especially visible under higher magnification. The dark, crystalline centers of the grains are especially visible. Sometimes, it appears that the grains also take up Safranin stain, which could lead to poorer contrast in the image analysis stage. We found these grains to interfere with the automatic detection of WinCELL Version 2013a (Regent Instruments Inc, 2013), despite trying to eliminate the starch by setting appropriate filters. Often, the grains had to be removed by manual image manipulation. Although more sophisticated tools for image manipulation may better filter the remaining starch grains, we found their complete removal, without altering other structures in the image, to be laborious.

Starch gelatinization by inducing heat is a common procedure in the food industry (Bauer & Knorr, 2005). By adding water and applying heat, the intermolecular bonds of the starch are broken and more water molecules can link to hydrogen bonding sites. Therefore, the starch grains change their crystalline structures to a gelatinous, viscous solution (Zobel, 1984). This reaction may be useful when starch grains need to be completely removed from the micro sections. In this technical note, we describe how a steaming procedure can be used to dissolve and wash out the starch before staining. This presents an additional step to the methodology proposed by Schneider and Gärtner (2013). This procedure could be useful, where starch grains remain abundant after rinsing and interfere with image analysis, and where image processing to remove starch grains may cause other inaccuracies.

5.4 Materials and Methods

Stem disks of lodgepole pine were collected in a 40-year old provenance trial located in the vicinity of Kamloops, British Columbia, Canada, at an elevation of approximately 1,400 m a.s.l. (Illingworth, 1978). The air-dry disks were already sanded to allow for measuring of tree-

ring width and latewood proportions. In preparation of the wood anatomical measurements, 1 cm wide, diagonal cross-sections were marked on the surface of the disks. Subsequently, the cross-sections were sawed from the disks using a standard circular saw. The resulting samples were then trimmed to a thickness of 1.5 cm with a small circular saw. The saw cuts were also made perpendicular to the fibers, to ensure a vertical orientation of the tracheids (Gärtner & Schweingruber, 2014). These samples were split at the pith, yielding two pieces per disk, between 4 cm and 7 cm long.

Prior to cutting micro sections with the microtome, the corn starch solution was prepared as a mixture of corn starch, water and glycerol in the ratio of 10g:8ml:7g (Schneider & Gärtner, 2013). Following this step, the sample was placed in the object holder of a GSL1-microtome (Gärtner et al., 2014), was softened by applying water with a brush, and then transverse micro sections were cut. The first few cuts were needed to remove the top layer of fibers damaged by the saw. We then changed the blade (A170, NT-Cutter Japan) and applied the corn starch solution with a brush to the surface of the sample (Schneider & Gärtner, 2013; Gärtner et al., 2015a). We aimed to produce sections of a thickness of approximately 10-20 μm , which were subsequently placed on a glass slide. To wash out the starch grains, the section was rinsed for four minutes by pumping water through the sample with a pipette (Gärtner & Schweingruber, 2014) and subsequently stained as described below.

Our simple laboratory set-up for steaming the micro sections is illustrated in Fig. 5.2. A lab grade, 2.5 l stainless steel container is placed on a hot plate filled with about 500 ml of distilled water. A metal rack is placed in the bottom of the bowl so that the top sits just above water level. It is important that the glass slide remains well above water to prevent the sample from being washed off the slide and damaged by the boiling water. A lid is used to close the

container and the water is heated to boiling. Once boiling, the glass slide supporting the micro section can be placed on the rack with laboratory tweezers or forceps. The sample remains in the container for 1 min before being removed. The hot steam acts upon the sample by causing the starch grains to lose their crystalline structures and to form a liquid solution. To save time, multiple samples can be steamed together. When fresh micro sections cannot be steamed immediately, they can be temporarily embedded in glycerol to prevent drying out as suggested by Gärtner and Schweingruber (2014). Before steaming, the glycerol should be washed out, because it increases the gelatinization onset temperature (Tan et al., 2004).

After steaming, the samples were rinsed with water to remove the gelatinized starch liquid. Subsequently, the samples were stained and dehydrated according to standard procedure (Gärtner & Schweingruber, 2014). A few drops of a 1:1 mixture of Safranin and Astra Blue were applied with a pipette. After five minutes, the staining solution was washed away with water, and the micro sections were dehydrated in an ascending series of ethanol 70%, 96% and 99% to remove the rest of the surplus stain (Gärtner & Schweingruber, 2014). Excess ethanol was removed with a paper towel, and micro sections were then embedded in glycerol and water.

Digital photos were obtained using a Nikon Eclipse Ni-E upright motorized microscope with an automatic scanning-table and a color camera with a resolution of 5 megapixels and 12-Bit color depth (Nikon DS-Fi2). Micrographs were taken with 200x magnification and automatically merged to form a large image with the stitching function in the NIS-Elements software, Version 4.20.01 (Nikon Corporation, 2014). For illustrating the problem and the effectiveness of the proposed treatment, we also show how the samples appear in the widely used software WinCELL.

5.5 Results and Discussion

Two micro sections from the same lodgepole pine wood sample are contrasted in Fig. 5.3. Both were treated with corn starch during cutting with the microtome, but only one had an additional steaming treatment before both were stained. As seen in the two left panels in Fig 5.3 (A & C), several starch grains remained present after the rinsing, staining and dehydration stages. The smaller cells in the latewood, or cells cut toward their tapered end, seemed to be primarily affected. These cells were measured incorrectly in WinCELL, meaning the complete analysis of a year's growth would not be possible without manual correction. Although WinCELL allows the image contrast to be increased and filters to be applied, we found these functions to be insufficient in removing the image artifacts and to consistently help in correctly identifying cell lumen and cell wall thickness. We found that steaming of samples was less time-consuming than manual removal in image analysis software. Efforts to use image manipulation to select and remove artifacts with various settings often discarded either too little or too much from the cell wall, which biased cell wall measurements. Since every micro section and resulting image might have slightly different properties, the parameters may also need to be adjusted from slide to slide, making it difficult to standardize. In contrast, the images on the right side of Fig. 5.3 (B & D) correspond to the steam treatment and contain fewer obvious artifacts. Under very high magnification, some gelatinized starch was visible, but caused no artifacts during image analysis.

The first step to reducing the presence of starch grains is rinsing the sample thoroughly with water after cutting with the microtome (Tardif & Conciatori, 2015). We tested several rinsing methods, including a spray from a typical laboratory squirt bottle, ordinary tap-rinsing, or rinsing and/or soaking in a Petri-dish. The most effective method for our samples, however,

involves placing a pipette on top of the section and pumping water through the sample, as recommended by Gärtner and Schweingruber (2014). Although starch grains can be reduced to a low number after several minutes of rinsing, we found that more than 4 minutes did not further reduce the abundance of grains. This may vary for other species or samples with a different proportion of latewood. We should also note that in the case of the steaming treatment, it is important to again rinse the sample with water. This reduces the presence of gelatin which could reduce the quality of the micro section.

Although steaming is reported to increase the crystallinity of cellulose (Ito et al., 1998), and heat can plasticize lignin and hemicelluloses (Inoue et al., 1993), we found no indication that the short steam treatment damaged the cellular structure. Prolonged heat treatment at high temperatures can distort wood, as shown in an 8-hr heat treatment at 200 °C by Priadi and Hiziroglu (2013). Steam treatments are, however, also used to soften wood specimens prior to cutting with a microtome (Tardif & Conciatori, 2015). It appears unlikely that brief steam treatments under normal atmospheric pressures will compromise the cell structure of an unrestrained micro section. That said, it may still be generally advisable to treat all samples consistently and to thoroughly document the sample preparation to avoid potential bias in subsequent measurements.

We conclude that the additional steam treatment for xylem micro section preparation only requires a minor investment in time and equipment. We consider cellular damage by the presented steaming method to be of low concern. Instead, steaming leads to improved section quality and more accurate image analysis without the need for image manipulation that may lead to other artifacts. This gelatinization procedure may therefore be a useful, additional step to the method introduced by Schneider and Gärtner (2013).

5.6 References

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5.7 Figures and Tables

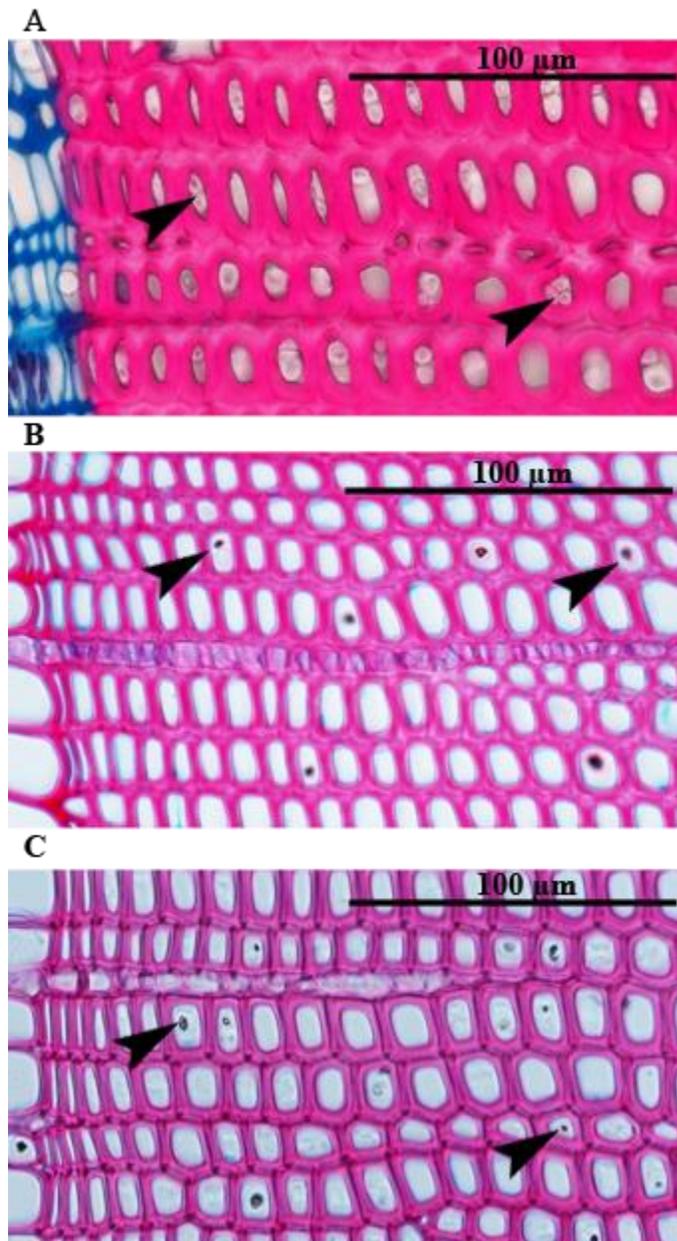


Fig. 5.1: Three examples of micro sections of stained xylem cells made from lodgepole pine samples (*Pinus contorta* Douglas ex Loudon). Two black arrows per panel highlight examples of starch grains that remain after rinsing, staining and dehydration. The center of the grains appears darker, especially in panels B and C. The starch grains sometimes absorbed Safranin during the staining stage, as seen in Panel B.

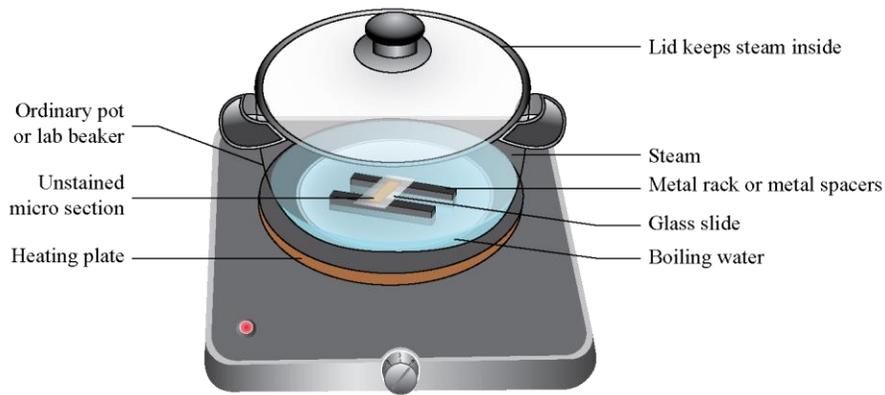


Fig. 5.2: Schematic view of steaming a micro section. A pot or beaker is placed on a heating plate, a metal rack or a substitute placed inside. The water level in the container should not exceed the height of the rack. After the water is boiling, wet micro sections on glass slides can be placed on top of the rack. The lid helps to keep the steam inside.

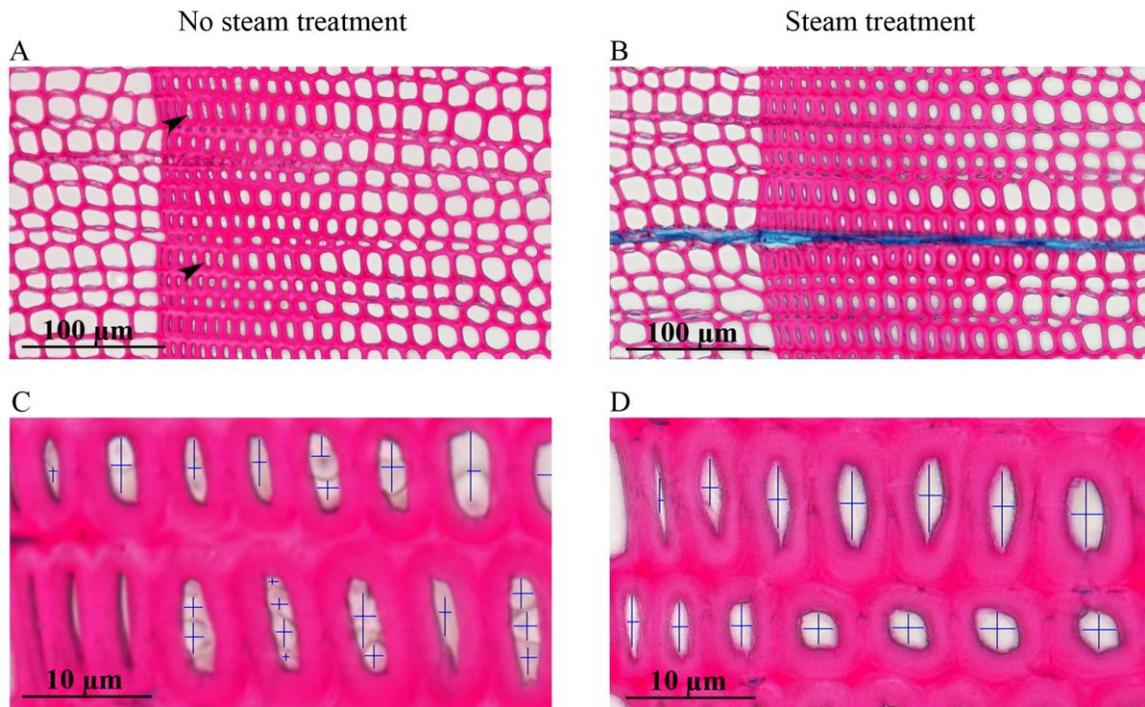


Fig. 5.3: Panels A and C show transverse sections of tree-rings from the same wood sample close to the border between latewood and earlywood photographed at 200x magnification. Images on the left (A & C) correspond to samples that were rinsed, stained and dehydrated and did not undergo a steam treatment. The arrows in panel A highlight two examples of remaining starch grains. The images on the right (B & D) display samples that underwent an additional 1-minute steam treatment. The lower panels are higher magnification micrographs of the top panels and show the cell lumen detection in WinCELL (Version 2013a).

6 Physiology of Lodgepole Pine Drought Tolerance

6.1 Abstract

Forests in leading edge areas of species distributions may not show expected increased primary productivity under climate warming, instead being limited by climatic extremes such as drought (Barber et al., 2000; Montwé et al., 2016). Looking beyond tree growth to underlying physiological mechanisms is fundamental for accurate predictions of forest responses to climate warming and drought stress (Anderegg et al., 2016). Here, we show that drought tolerance of lodgepole pine (*Pinus contorta* Doug. Ex. Loud) is linked to properties of the xylem anatomy and stomatal regulation. These physiological mechanisms were inferred by combining dual-isotope analysis, functional wood anatomy, and growth from genetic transplant experiments over multiple decades. Populations from central parts of species distribution were most productive and exhibited an isohydric behavior to cope with drought stress: large xylem conduits facilitate photosynthesis when water is available, while moderate drought tolerance is achieved through stomatal responsiveness. Cavitation-resistant xylem in seed sources from the distribution's trailing edge confer drought tolerance. When exposed to warmer climates, northern seed sources showed lower drought tolerance and growth due to a suite of physiological maladaptations. Due to drought, therefore, northern areas may not profit from strong warming and could instead benefit from cautious implementation of assisted migration. Since risks of seed transfer vary across species distributions, however, a cautious and population-oriented approach to climate-based seed transfer is required to address climate change impacts on wide-ranging tree species.

6.2 Introduction

Covering about a third of the Earth's land surface, forests support communities, play a key role in carbon and water cycles, and influence climate forcings and feedbacks (Bonan, 2008; Kurz et al., 2008). Predicting the capacity of trees to cope with warming and drought is therefore an urgent and important challenge under climate change. Due to selective pressures, wide-ranging tree species often represent several sub-specific populations that have developed unique multi-trait polygenic adaptations to local climates (Alberto et al., 2013; Aitken & Bemmels, 2016). As a consequence, climate change is causing a mismatch of locally adapted populations to new environmental conditions (Davis & Shaw, 2001; Valladares et al., 2014). Although trees have generally high plasticity (Alberto et al., 2013), drought additionally causes substantial forest productivity losses and mortality (Ciais et al., 2005; Allen et al., 2010; Peng et al., 2011; Choat et al., 2012; Anderegg et al., 2016). Such droughts are anticipated to become more frequent and severe as greater climatic variability coincides with the higher evaporative demand from general warming (Ciais et al., 2005; Reyer et al., 2013; Christidis et al., 2014). The rate of decoupling between adaptations and environmental change may exceed the ability of trees to acclimate, migrate through seed dispersal or gene flow, or adapt through natural selection (Loarie et al., 2009; Talluto et al., 2017).

To reduce the negative consequences of growing forest maladaptation under climate change, a percentage of pre-adapted seed sources from warmer, drier areas within the same species distribution could be planted (Aitken & Bemmels, 2016). While this could help realign populations with their climatic optima, careful assessment of adaptation is critical to effectively evaluate potential risks. Adaptation, maladaptation and the suitability of seed transfer can be assessed using provenance trials (Alberto et al., 2013; Aitken & Bemmels, 2016). These are

reciprocal transplant experiments that test multiple seed sources in common garden settings to reveal genetic differences in performance. These experiments also act as real-world climate change laboratories: Moving seeds southward exposes trees to warmer climates that may resemble projected warming while northward seed transfers test proposed climate-based seed transfers. Such trials represent substantial investments over multiple decades and are hence usually reserved for valuable trees. While this may represent a bias toward timber trees species, it does not limit their value in predicting responses in natural ecosystems, as is sometimes argued (Anderegg, 2015): Their economic value is a by-product of their ecological value as these tree species form the foundation of widespread forest ecosystems. This is the case for lodgepole pine (*Pinus contorta* Dougl. ex Loud.), which covers an estimated 1.3 million km² area across western North America (Schroeder et al., 2010). This conifer is useful for studying the effects of climate change and assisted gene flow as its extensive distribution stretches as far north as the Yukon territory and as far south as the state of Colorado (Fig. 6.1f).

Two primary mechanisms trees use to acclimate to changing moisture conditions involve construction of new xylem cells forming the hydraulic pipeline (Fig. 6.1d) and regulation of transpiration through stomata (Fig. 6.1e). Conifers discriminate against the heavy carbon-13 isotope relative to the more abundant light carbon-12 isotope, while leaf water becomes enriched in the heavy oxygen-18 isotope because the light H₂¹⁶O molecules transpire more readily than H₂¹⁸O (Saurer et al., 1997; Barbour, 2007). These fractionation processes change under drought stress, however, when stomatal aperture is reduced to minimize water loss. With an awareness of other factors (Saurer et al., 1997; Gessler et al., 2014; Treydte et al., 2014), the response of stomata to drought can thus be cautiously inferred through the analysis of resulting photosynthate's isotopic ratios, discussed in relation to a standard (i.e., $\delta^{13}\text{C}$ or $\delta^{18}\text{O}$). The

balance between water loss and carbohydrate uptake is also partially determined through xylem structure because trees can modify conduit size and cell wall reinforcement of new xylem. Xylem adaptations can be accurately described through observations of anatomical structural changes in hydraulic diameter and wall thickness of xylem conduits. Combined, xylem morphology and stomatal responsiveness contribute to the range of physiological behaviors trees use to cope with water limitation: from isohydry to anisohydry. Isohydric plants are able to maintain stable mid-day water potentials by closing stomata under water limitation (McDowell et al., 2008). Anisohydric plants allow more variable water potentials due to changing dryness by being able to keep stomata open under drier conditions to maintain photosynthesis by having xylem properties to tolerate increasing water tension (McDowell et al., 2008).

Here, we test the ability of different tree populations to physiologically acclimate to warmer and drier conditions under climate change. We evaluate long-term growth performance and drought tolerance to a severe spring drought that occurred in western North America in 2002. We further investigate physiological mechanisms underlying response to drought, and whether the ability to acclimate to drier conditions relates to intra-species isohydric or anisohydric responses.

6.3 Methods

Experimental Design and Field Measurements

All trees used in this study were grown in a genetic field trial established in 1974 for lodgepole pine. We study seed sources (provenances) grouped into four geographic regions (populations): the boreal North representing the leading edge (LE); Central Interior (CI) and Southern Interior (SI); and the far south, representing the trailing edge (TE) (Figs. 6.1f, 6.S1).

Each of these regional populations is represented by five provenances (Figs. 6.1f, 6.S1, Tab. 6.S2), grown on three planting sites in British Columbia's southern interior (Tab. 6.S1) and replicated on two blocks per site (Fig. 6.S1) in a randomized block design (Fig. 6.S1). Growth and drought tolerances are based on 4 trees per provenance. Functional wood anatomy and stable isotope analyses were conducted on a sub-sample of 120 trees: one tree of median height per provenance \times 5 provenances per regional population \times 3 sites \times 2 blocks. See Supplementary Information for more detail.

Field and Tree-Ring Measurements

We measured height as well as annual height increments (distance between branch whorls). All tree-ring, wood anatomical and isotopic analyses presented here are based on stem disks taken from diameter at breast height (1.3 m). After sanding and scanning these disks, we measured annual radial growth on four radii per stem disk using Windendro (version 2016) to a precision of 0.01 mm, and derived basal area increment (BAI). Stem disks were then cut into two adjacent sections of \sim 1cm along a radius to the pith: one each for functional wood anatomical and stable isotope analyses. A period of 10 years from 1996 to 2005 was chosen for wood anatomical and isotopic analyses as it captured a spring drought event occurring in 2002. Drought tolerance is discussed in terms of relative resilience, or the ability to achieve pre-drought growth (BAI) after accounting for the drought's initial impact on growth (see Supplementary Information).

Functional Wood Anatomy

We cut micro sections of 10-20 μ m thickness and prepared them for image analysis. Micrographs were taken at 200 \times magnification with a Nikon Eclipse Ni-E upright microscope.

Images were cleaned as necessary using Nikon NIS-Elements (version 4.20.01) and Adobe Photoshop CS4 software. In four cell rows per tree ring, we measured cell wall thickness and cell lumen diameter (d). Lumen diameter was used to calculate the average hydraulic diameter ($\sum d^5 / \sum d^4$) for each ring. See Supplementary Information for more detail.

Stable Isotope Analyses

We first confirmed through a pilot study that resin-extracted whole-wood from an entire tree ring was most suitable for stable carbon and oxygen isotope analyses in our samples (Figs. 6.S1, 6.S2). After homogenizing individual resin-extracted tree rings (Retsch MM 2000 ultra-centrifugal mill), samples were combusted/pyrolysed prior to Isotope Ratio Mass Spectrometry. Isotope ratios are reported in δ notation, representing deviations from known reference materials. The level of precision is $\pm 0.02\text{‰}$ for carbon and $\pm 0.4\text{‰}$ for oxygen. Intrinsic Water Use Efficiency (WUE) was derived from carbon isotope ratios based on empirically-derived formulas and annual estimates for atmospheric carbon concentrations. See supplementary information for more detail.

Statistical Analyses

Linear mixed effects models were used for testing differences among the four regional populations: regional population was specified as the fixed effect while random effects included year, provenance and site. Distance from the apex was included as a random effect for models involving functional wood anatomical traits. The Benjamini & Hochberg method was used to correct the false discovery rate in multiple comparisons and climate correlation (Benjamini & Hochberg, 1995). Growth correlations to annual site climate variables (1996 - 2005) tested basal area and height increments while growth correlations to the climates of seed origin (1961-1990

long-term average) were based on total height and diameter in 2005. See Supplementary Information for more detail.

6.4 Results

Results show pronounced differences in growth and physiology among tree populations over time (Figs. 6.1a, 6.S3). Over the length of the study, seed sources from the central part of the lodgepole pine distribution demonstrate good growth and reliably higher hydraulic diameter and $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values (Fig. 6.1a). In contrast, seed sources from northern areas of the lodgepole pine range show consistently lower growth, hydraulic properties of the xylem and $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values (Fig. 6.1a). Meanwhile, seed sources from the far south had lower growth than central and southern interior populations (Fig. 6.1a). They also have moderate values in all physiological traits, except they have relatively thicker cell walls (Fig. 6.1a). These responses are linked to adaptations to source climate (Fig. 6.1b, Tab. 6.S1) as well as the conditions on the planting sites (Fig. 6.1c, Tab. 6.S2). Growth, tracheid wall thickness and $\delta^{18}\text{O}$ showed stronger latitudinal clines of adaptation linked to temperature and growing season conditions (Fig. 6.1b). In contrast, the functional physiological traits were found to be more linked to site conditions than could be explained by climate of seed origin (Fig. 6.1c, Tabs. 6.S3, 6.S4). Correlations between $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ are positive, although varying by population (Fig. 6.S4), suggesting that $\delta^{13}\text{C}$ is governed by stomatal conductance here (Saurer *et al.*, 1997; Scheidegger *et al.*, 2000; Tognetti *et al.*, 2014).

The two populations from the central part of the lodgepole pine species distribution showed the highest height and diameter growth, $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values, hydraulic diameter and tracheid wall thickness (Fig. 6.2, Tab. 6.S6). They also show moderate drought tolerance relative

to other populations. These traits were almost always significantly different from the northern population (Fig. 6.2, Tab. 6.S6). As seen in Fig 6.1a and Fig 6.3, the dual-isotope signatures of central and southern interior populations show a high degree of plasticity, or the ability to acclimate to changing climatic conditions. Under drier conditions, however, these populations do not modify their xylem structure to the same degree that the trailing edge (far south) population does (Figs. 6.1, 6.3).

The far south population shows higher drought tolerance and moderate productivity relative to other populations (Fig. 6.2, Tabs. 6.S5, 6.S6). This response is linked to a significantly lower hydraulic diameter compared to the most productive populations (Fig. 6.2, Tabs. 6.S5, 6.S6). These trees also had lower WUE, $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values relative to the central populations, although these were not significantly different (Fig. 6.2, Fig. 6.S6). While they showed low variation in stable isotope values under drought conditions (Figs. 6.1, 6.3), they had the highest ability to adjust their hydraulic diameter, lumen diameter and wall thickness (Fig. 6.S4, Tab. 6.S5).

When northern seed sources originating from the leading edge of the lodgepole pine distribution are grown on southern planting sites, simulating climate warming, they perform poorly (Figs. 6.1a, 6.2, Tab. 6.S5). Their height and diameter growth is significantly lower than all other populations (Tab. 6.S6). A suite of low values for all physiological variables (Figs. 6.1a, 6.2, Tab. 6.S5) was also significantly different from the two high-performance central populations in most cases (Tab. 6.S6). These seed sources also showed the lowest ability to modulate their response in terms of isotopic signatures or xylem parameters (Fig. 6.3).

6.5 Discussion

Although lodgepole pine is considered a drought tolerant species as a whole, there are complex variations in intra-species growth and responses to drought. Within this wide-ranging tree species distribution, the full range of isohydric to anisohydric behaviors is shown in different populations. The physiological mechanisms underlying these responses are equally diverse and can be attributed to both leaf-level and wood anatomical processes.

The responses of the central populations suggest isohydric behaviors to cope with water limitation. While growth is maximized under optimal conditions, these trees cope with water limitation with more responsive stomata, suggesting some ability to adapt to future drought. This is indicated by relatively high $\delta^{18}\text{O}$ values, which point to higher transpiration: Assuming no differences in rooting patterns, relative differences among populations' $\delta^{18}\text{O}$ signatures point to transpiration, not water source, due to the common garden approach and, hence, homogenous soil water conditions. Higher transpiration is also likely facilitated by larger tracheid lumens, which form an efficient hydraulic pathway to the photosynthesizing crown. The increased transpiration indicates that, while moisture is available, trees from central areas in the lodgepole pine distribution have a competitive advantage in photosynthesis and growth potential. At the same time, cavitation risk appears to be modulated through stomatal closure under drought, helping to explain their moderate drought tolerance. However, isohydric behaviors are often not successful under prolonged drought, as it can cause the depletion of carbon reserves, eventually leading to starvation (McDowell et al., 2008). While these central areas therefore appear not to be immediately in need of assisted gene flow, the point at which the local forests begin to decline depends on the rate and magnitude of climate change as well as interactions with other biotic and abiotic factors.

Trees from the southern, trailing-edge population showed some ability to adjust their hydraulic diameter, lumen diameter and wall thickness. Combined with their inferred stomatal response, this physiological response explains the classic growth *versus* drought tolerance trade-off exhibited by these trees. However, these provenances were grown on planting sites in the southern interior of British Columbia, not *in situ*. With these data, we can only indicate what could happen by incorporating a percentage of these seed sources in central areas of the lodgepole pine distribution. Such an assisted gene flow scenario could increase drought tolerance in central areas, but would reduce forest productivity.

The low productivity of the northern population relative to other populations may be partially explained by less transpiration, evidenced by lower values of $\delta^{18}\text{O}$ and the narrow hydraulic diameters. These trees also have thin tracheid walls, which are associated with a higher risk of cavitation to the water pipeline (Hacke et al., 2001). Cavitation could result in air embolisms in the stem, further reducing the ability of water to reach the crown. Sustained stomatal conductance under drought (as indicated by stable isotopes), combined with low cell wall reinforcement to compensate for increasing xylem tension, would likely cause the northern population to be more cavitation prone. This could further explain their inability to cope with drought and warming, and why they did not regain pre-drought growth levels. Interestingly, these data also show no evidence of an expected dual adaptation to cold and drought tolerance, which rely on similar mechanical properties (Bansal et al., 2016).

Under predicted warmer and drier climates in the boreal north, where temperature warming could exceed 5 °C by the end of the century, the low potential to respond to strong warming and drought may become a problem for vast areas of northern pine forests. These results contribute to a growing number of negative predictions for the western areas of the boreal

north (Barber et al., 2000; Girardin et al., 2016; Montwé et al., 2016) that contradict earlier research suggesting increased growth in northern latitudes (Keeling et al., 1996; Myneni et al., 1997). Our data also suggest that range erosion may not only occur in the rear edge, but could create “extinction debts” at northern tree species margins as well (Talluto *et al.*, 2017)

6.6 Conclusion

Our data suggest that increased forest health issues, productivity losses and carbon-cycle feedbacks will not be uniform across the species distribution and could occur in northern areas first in the case of lodgepole pine. While introducing pre-adapted alleles to the leading edge may help maintain forest health and productivity, assisted gene flow would cause high opportunity costs for minimal gain in central areas of the species distribution: deploying pre-adapted seed under assisted gene flow prescriptions cannot be universally applied without risks; a cautious and population-oriented approach is needed.

6.7 References

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6.8 Figures

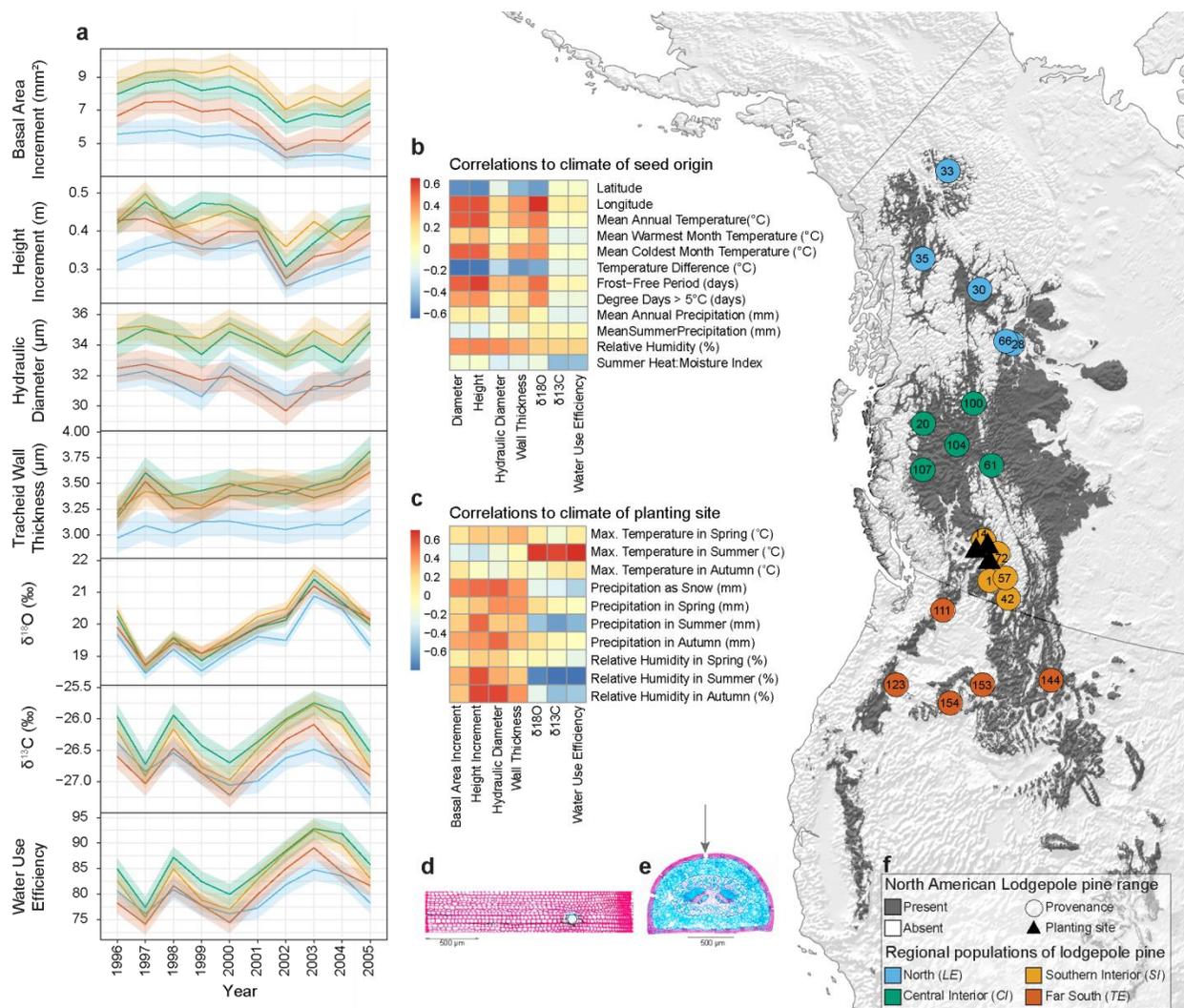


Fig. 6.1. Variations in growth and physiology of lodgepole pine (*Pinus contorta*) in relation to climate and provenance. a) Growth, functional wood anatomical traits, stable isotope values and $\delta^{13}\text{C}$ -derived intrinsic Water Use Efficiency (A/g_s). Lines and standard errors are colored according to the regional population within the species distribution: blue represents the leading edge (northern) population; green represents the central interior population; yellow represents the southern interior population; and orange represents the trailing edge (far south) population ($n = 1170$: 4 populations \times 5 provenances \times 1 tree \times 3 sites \times 2 blocks \times 10 years, minus 3 trees). b) Correlations of growth and physiological traits to climate of seed origin. These were based on total height and diameter at age 32 and were correlated to long-term climates from 1961-1990 ($n = 117$). c) Correlations of growth and physiological traits to annual climate variables at the planting sites. These were based on annual height and basal area increments related to weather on the planting sites over the 10 years ($n = 600$). d) Stained micro cross-section of a lodgepole pine showing one tree ring (pith to the left). e) Micro cross-section of a pine needle with two clearly visible stomatal openings (one highlighted by an arrow). f) Lodgepole pine distribution (dark grey) in western North America and the experimental design.

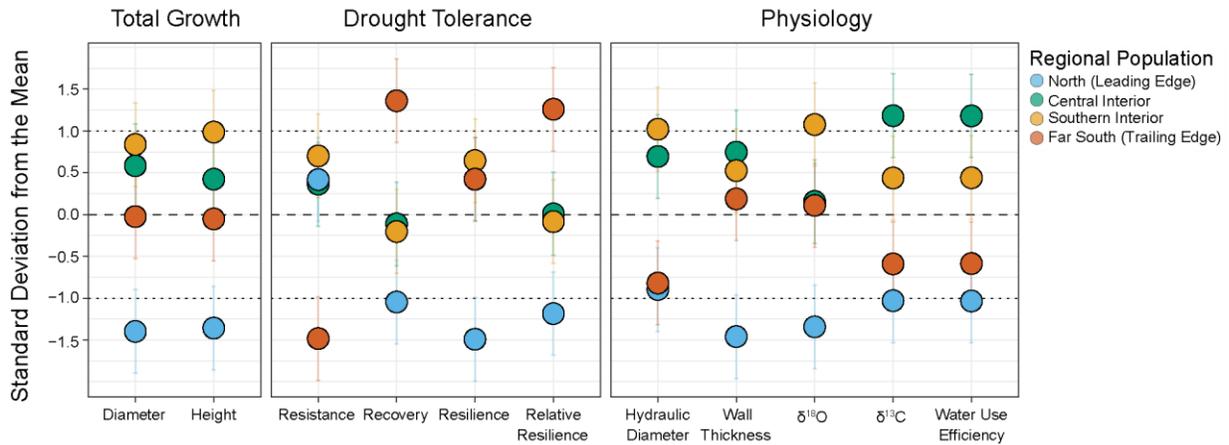


Fig. 6.2. Genetic adaptations revealed from growth, drought tolerance and physiology. Each dot represents the average response in units of standard deviation to show relative rankings among the four regional populations: blue represents the leading edge (northern) population; green represents the central interior population; yellow represents the southern interior population; and orange represents the trailing edge (far south) population. Response is tested across three planting sites in British Columbia's southern interior, so a positive climate transfer distance exists for northern populations (tests climate warming) while a negative climate transfer distance exists for southern populations (tests assisted gene flow prescriptions). Bars are standard errors of the mean.

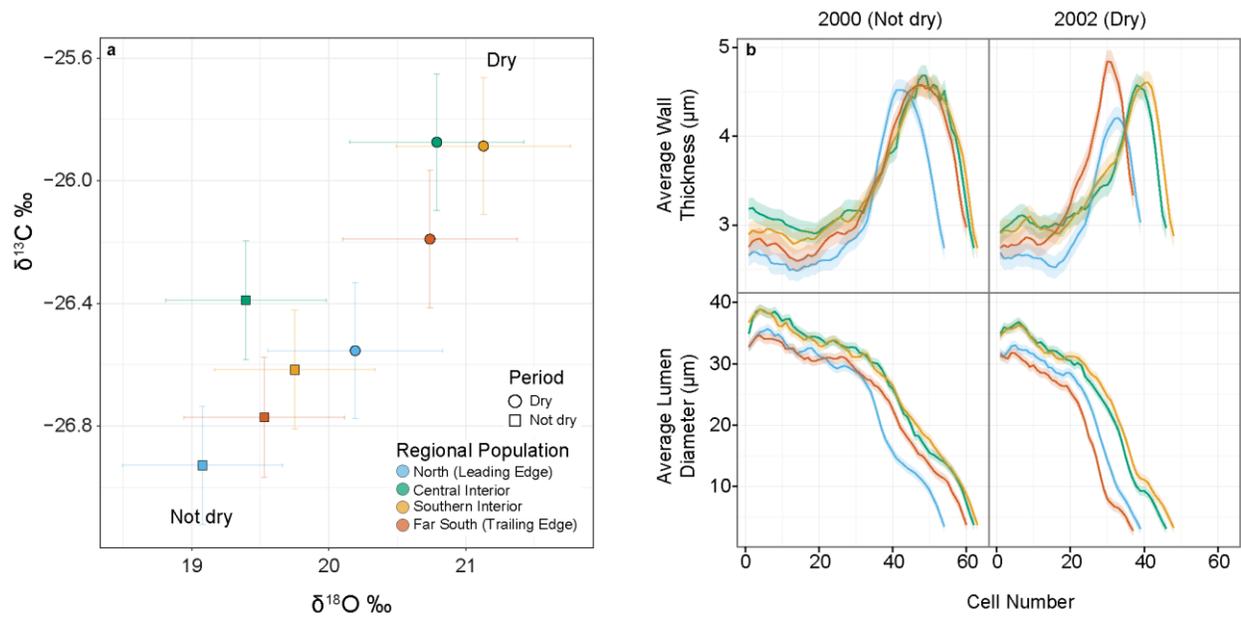


Fig. 6.3. Plasticity among populations of lodgepole pine. a) Response of stable isotopes in years of different moisture levels. b) Average tracheid wall thickness and lumen diameter as a function of cell number under different moisture conditions. Each of the regional populations are coloured according to Fig. 6.1. Error bars (a) and ribbons (b) are standard errors of the mean.

6.9 Supplementary Information: Methods

Experimental Design and Field Measurements

All trees used in this study were grown in a genetic field trial established in 1974 for lodgepole pine (*Pinus contorta* Doug. ex. Loud.) (Illingworth, 1978). Many planting sites incurred high mortality in 2006 due to the mountain pine beetle epidemic. In 2013 and 2014, we were permitted to fell dead trees within this valuable multi-decade experiment, allowing us to accurately measure growth parameters and collect stem disks. From this larger experiment, we developed a complete randomized block design representing seed sources from across the distribution, which stretches north-south over 4000 km. Seed sources were grouped into four large geographic regions (Fig. 6.S1, Tab. 6.S1): the Leading Edge (LE), which represents provenances from the boreal north; British Columbia's Central Interior (CI) and Southern Interior (SI), which are both found in the central parts of the lodgepole pine distribution; and the Trailing Edge (TE), which represents provenances from the far south of the lodgepole pine distribution. Seed sources (provenances) from these four regions are collectively referred to as populations.

Each of these regional populations is represented by five seed sources (provenances), which were tested on three planting sites in British Columbia's Southern Interior that had incurred high beetle-caused mortality (Tab. 6.S2). In turn, each site contains two blocks, which act as replications: the location of the seed source plot was randomized within the block (Fig. 6.S1c). Growth and drought tolerance data rely on 4 trees per seed source per block (Montwé et al., 2016). Due to the high financial and labour costs involved in inferring physiological traits from tree rings, we selected the tree of median height for further stable isotope and functional

wood anatomical analyses (1 tree / provenance / block). Three samples were not available, hence; most of the wood anatomical and isotopic analyses therefore rely on a sub-sample of 117 trees (1 tree/provenance × 4 regions × 3 planting sites × 6 blocks - 3 trees).

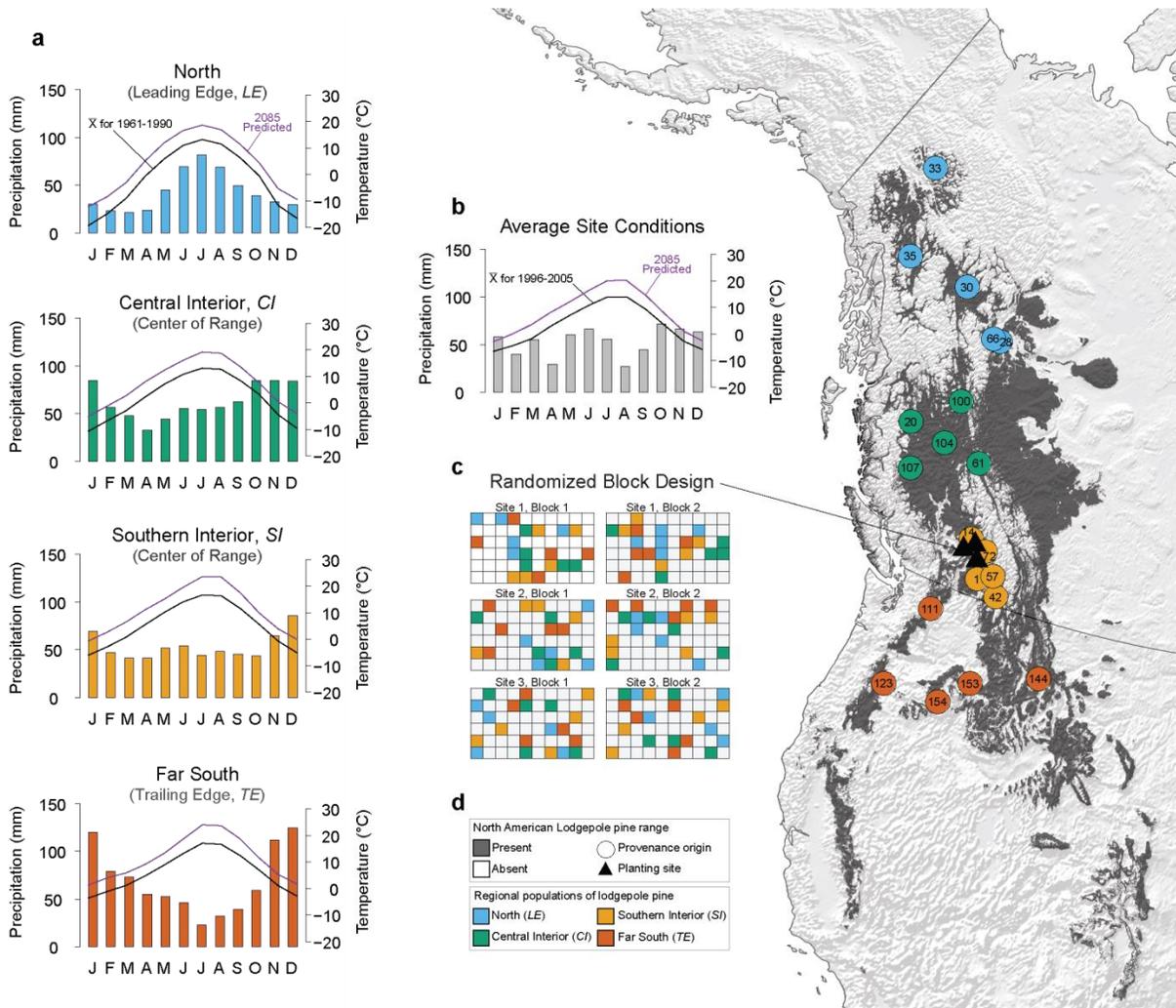


Fig. 6.S1. Study design and climates across the species distribution of lodgepole pine (*Pinus contorta*). a) Climate graphs of precipitation (bars) and temperature (lines) for the average climates of the four regional populations b) Climate graph showing average conditions across the planting sites, where bars show precipitation and lines show temperature. c) The experiment relies on a complete randomized block design, where each provenance was planted in a randomized pattern across two blocks per site. d) Map and legend, where dark grey shows the lodgepole pine range, circles represent seed sources numbered according to the original design by Illingworth, and the three planting sites are depicted as black triangles.

Field and Tree Ring Measurements

After felling and de-limbing each tree, we cut stem disks for further growth and physiological analyses. With an Esilon tape, we measured height and annual height increment, indicated by the distance between branch whorls. In the field, we confirmed the accuracy of height increment counts by comparing them to the number of tree rings from stem disks at a given height. The accuracy of height increments for the 120 trees was verified against the same tree's tree-ring widths, with some minor and consistent modifications made as necessary. The analyses of wood anatomy and isotope ratios are based on the stem disk taken from diameter at breast height (DBH; 1.3m). These disks were sanded with increasingly fine sand-paper grit and scanned at 1200 dpi (ScanMaker 9800XLplus; Microtek, Hsinchu, Taiwan). Tree-ring widths were measured on four radii per stem disk using the semi-automatic tree-ring software Windendro, version 2012. From these measurements, we calculated DBH as double the average radius length, and calculated basal area increment as the average increase in annual growth. These stem disks were then cut into two adjacent sections of ~1 cm along a radius to the pith: one was used for functional wood anatomical and the other for stable isotope analyses.

Drought Indicators and Period of Study

A study period of 10 years from 1996 to 2005 was chosen as it captured a spring and summer drought event occurring in 2002. Compared to the 1961-1990 climate normal, this drought represented a drop of a standard deviation in mean annual precipitation, while standard deviations from mean precipitation in June, July, August and September were -0.61, -0.62, -0.90 and -0.75, respectively. An illustration of climate and growth over the life of the provenance trial is provided in an earlier publication (Montwé et al., 2016). Designation of these years as pre-drought, drought and post-drought are important for the super-epoch analysis involved in

calculating the four drought indicators as coined by Lloret et al. (2011): Resistance refers to the drop in growth in the drought year relative to the three preceding years; recovery is the speed with which growth resumes; resilience is the ability to return to pre-drought growth; and relative resilience is the ability to return to pre-drought growth relative to the severity of the growth drop during the drought year. These variables were defined with regard to basal area increment to be consistent with an earlier study (Montwé et al., 2016), where 1999-2001 are considered pre-drought years, 2002 is the drought year, and 2003-2005 are the post-drought years. Although 2003 was also considered warm, it was not quite as dry in the early part of the growing season when growth is critical. Since basal area increment had already begun to recover in 2003, by definition, it was considered a post-drought year.

Sample Preparation for Wood Anatomical Analysis

Wood anatomical analyses rely on 117 trees, since 3 trees out of the design were unsuitable for micro sections. After applying a corn-starch solution to the wood sections (Schneider & Gärtner, 2013), we cut micro sections of 10-20 μm thickness using a GSL-1 microtome (Gärtner et al., 2014). The samples were washed, steamed (Montwé *et al.*, 2015a), rinsed and bleached (Gärtner & Schweingruber, 2014). The samples were then soaked in a stain made of equal portions of Safranin and Astrablue for five minutes, rinsed with distilled water, and washed with increasing concentrations of ethanol (Gärtner & Schweingruber, 2014). After applying Canada balsam, the samples were covered with a glass cover slip and placed in an oven at 60 °C for 8 hours to solidify. Micrographs were taken at 200 \times magnification at a resolution of 5 megapixels and 12-bit colour depth using a Nikon Eclipse Ni-E upright microscope and automatically stitched together with the NIS-Elements software, version 4.20.1. Images were further cleaned as necessary in Photoshop (Adobe CS4) or WinCell, version 2013. For each tree

ring, four cell rows were measured in WinCell to measure average cell lumen diameter and wall lengths. We subsequently calculated wood anatomical analyses which provide data on and insight into physiological processes. These include the hydraulic diameter ($\sum d^5 / \sum d^4$) for each ring, a measure of the xylem's potential as a water pipeline to the photosynthesizing crown. Cell lumen and cell wall thickness profiles were calculated according to procedure described by Montwé et al. (2015b). Finally, relative comparisons among populations are possible since differences in xylem anatomy due to shade stress (e.g. possible reductions in lumen diameter (Schoonmaker et al., 2010)) are accounted for by using the provenance trial setting, where trees were grown in a common age structure.

Pilot Study: Sample Preparation for Stable Isotope Analysis

We first conducted a pilot study to confirm the most suitable methodology for annually-resolved isotopic analysis on our lodgepole pine samples. It has long been recognized that wood components vary isotopically (Wilson & Grinsted, 1977; Leuenberger et al., 1998; Macfarlane et al., 1999; McCarroll & Loader, 2004; Cernusak et al., 2009; Gessler et al., 2014). Cellulose is often used for stable isotope analysis due to its stability and immobility (Macfarlane et al., 1999; Gaudinski et al., 2005; Boettger et al., 2007). It is already formed during the early phase of cell development and, hence, likely reflects more timely isotopic signatures. In contrast, lignification can be delayed by weeks or months (Wang et al., 2000; Cuny et al., 2015), so lignin molecules may represent a lagged signal (Wilson & Grinsted, 1977; Helle & Schleser, 2004). However, cellulose extraction is time-consuming, so whole-wood is preferred when there is a consistent offset. For lodgepole pine, Guy and Holowachuk (2001) previously reported a strong relationship between carbon isotope signatures in cellulose and whole sapwood. However, we needed to test the reliability of this signal over time in tree rings (the key component in our study) from trees

that had been dead-standing for 5-8 years for both oxygen as well as carbon isotope ratios. We also wanted to exclude the influence of blue-stain fungus (*Ophiostoma* spp.) affecting many of our lodgepole pine samples on the isotope ratios, as reported for other conifers (English et al., 2011). This pilot additionally compared latewood to the entire ring to test if earlywood cells were formed with stored photosynthate (Helle & Schleser, 2004), possibly leading to different climatic correlations (Kagawa et al., 2006a, 2006b; Barnard et al., 2012).

The pilot study was based on tree rings covering 7 years (1999 – 2006, inclusive), representing four trees from one provenance grown on one planting site block. Tree rings were separated with a scalpel under the stereo-microscope (Leica Wild M3B, Wetzlar, Germany). Whole-wood samples were homogenized with an ultra-centrifugal mill (ZM 2000, Retsch GmbH, Germany) and placed in Teflon sample bags. To remove possible resins and extractives (mobile and potentially causing isotope bias), the bags were washed continuously with 96%-grade ethanol for 24 hours in a Soxhlet apparatus. For cellulose samples, tree rings were placed into Teflon bags and hollocellulose was extracted following a modified Jayme-Wise method (Gaudinski et al., 2005). This procedure involved placing the samples in a bath of 5% Sodium Hydroxide (NaOH) for 2 hours at 60 °C, then thoroughly rinsing the samples, and then placing them in a bath of 7% Sodium Chlorite (NaClO₂) and glacial acetic acid for 30 hours at 60 °C (average time for samples weighing 10 mg). One far outlier was removed (entire-ring, whole-wood in 2002 for one tree for $\delta^{18}\text{O}$).

Cellulose and whole-wood year-to-year variations of our pilot samples were highly correlated ($r = 0.92$ for $\delta^{13}\text{C}$; $r = 0.85$ for $\delta^{18}\text{O}$), but, as expected, the absolute values were significantly different ($p < 0.001$ for both $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) (Fig. 6.S2). Isotope ratios for latewood and the entire ring were also highly correlated for $\delta^{13}\text{C}$ ($r = 0.88$) and significantly different ($p =$

0.009). The correlations between latewood and the entire ring were weaker for $\delta^{18}\text{O}$ ($r = 0.39$), but these groups were not significantly different from each other for $\delta^{18}\text{O}$ ($p = 0.701$). Latewood, however, shows higher variability. This can likely be attributed to inconsistent bias introduced when separating the latewood from the earlywood with a scalpel: the split was assessed visually and therefore somewhat arbitrary. Using the entire ring was therefore considered more suitable, especially as these had stronger correlations with climate. This pilot study therefore confirmed that using the entire tree-ring and extracted whole-wood were most suitable for stable isotope ratio analyses.

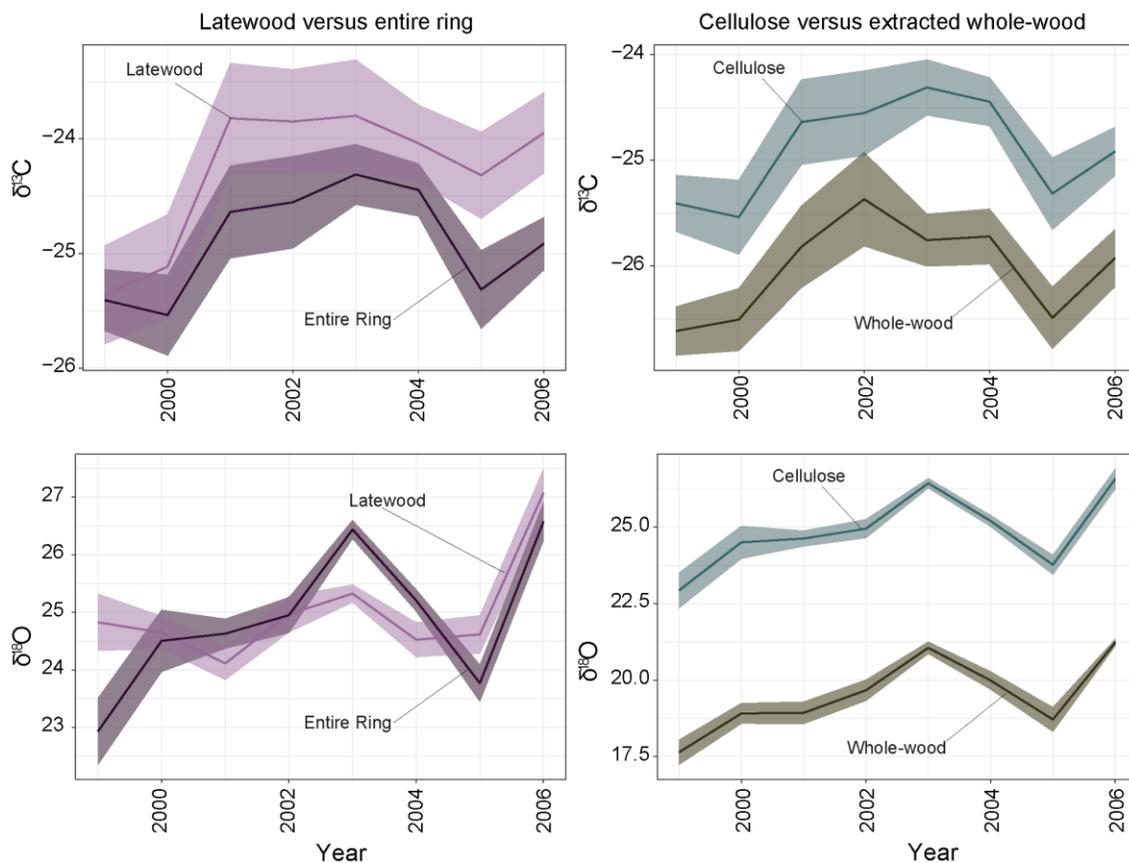


Fig. 6.S2. Pilot study results comparing materials and methodologies for stable carbon and oxygen isotope analyses in tree rings from lodgepole pine (*Pinus contorta*). The two upper graphs correspond to stable carbon isotope ratios ($\delta^{13}\text{C}$) while the two lower graphs correspond to stable oxygen isotope ratios ($\delta^{18}\text{O}$). The two graphs on the left compare isotope signatures in the entire ring versus those in the latewood only. The two graphs on the right compare isotope signatures in cellulose versus those in resin-extracted whole-wood. The shaded areas represent standard errors of the mean.

Full Design: Sample Preparation for Stable Isotope Analysis

The full study design relies on 120 trees: 1 tree/provenance \times 5 provenances/regional population \times 3 planting sites \times 2 blocks. While three trees were unsuitable for isotope analyses, 3 additional trees from the pilot study were included in the final database. Thus, we prepared 1200 samples for $\delta^{13}\text{C}$ analyses and 1200 samples for $\delta^{18}\text{O}$ analyses. We removed extractives from the wood sections by running ethanol through them in a Soxhlet apparatus for 24 hours. We then separated tree rings with a scalpel under a stereo-microscope (Leica Wild M3B, Wetzlar, Germany) and homogenized the samples with an ultra-centrifugal mill (ZM 2000, Retsch GmbH, Germany). This homogenization technique is appropriate for whole wood and poses no risk of isotopic contamination from plastic (Isaac-Renton et al., 2016). The G.G. Hatch Stable Isotope Laboratory at the University of Ottawa weighed the homogenized samples into tin (carbon) and silver (oxygen) capsules for elemental analyses and mass spectrometry. Carbon samples were flash combusted at 1800 °C while oxygen samples were pyrolysed at 1450 °C before being fed into the Isotope Ratio Mass Spectrometry interface. The resulting isotope ratios are reported in standard δ notation, which represents deviations from the reference material (Vienna Pee Dee Belemnite for Carbon; Vienna Standard Mean Ocean Oxygen for Oxygen) according to the following formula:

$$R = (R_{\text{sample}}/R_{\text{reference}} - 1)1000$$

Where R is the ratio of the heavier to lighter isotope (Farquhar et al., 1982, 1989; Francey & Farquhar, 1982; McCarroll & Loader, 2004; Seibt et al., 2008). The level of precision is $\pm 0.02\%$ for carbon and $\pm 0.4\%$ for oxygen.

Atmospheric Carbon Data and Calculation of Intrinsic Water Use Efficiency

Although isotopic signatures may change slightly due to post-assimilation fractionation and stored carbohydrate reserves can be remobilized (Helle & Schleser, 2004; Kagawa *et al.*, 2006a, 2006b; Treydte *et al.*, 2007; Offermann *et al.*, 2011; Gessler *et al.*, 2014), stable carbon isotope ratios can still provide information on stomatal behaviour and are widely used. We used tree ring $\delta^{13}\text{C}$ to estimate atmospheric and intercellular carbon concentrations (c_a and c_i , respectively), which were used to calculate intrinsic Water Use Efficiency (WUE) based on the following empirically-derived equations (Farquhar *et al.*, 1982; Francey & Farquhar, 1982; Saurer *et al.*, 1997; McCarroll & Loader, 2004; Seibt *et al.*, 2008; Tognetti *et al.*, 2014):

$$c_i/c_a = (\delta^{13}\text{C}_{\text{plant}} - \delta^{13}\text{C}_{\text{air}} + a)/(b-a)$$

$$i\text{WUE} = A/g_s = c_a[1-(c_i/c_a)](0.625)$$

Where a represents a fractionation of -4.4‰ due to kinetic diffusion discrimination at the stomata, b is the fractionation at the site of carboxylation (-27‰). The rate of carbon assimilation is denoted by A , and g_s represents stomatal conductance. Annual mean estimates for c_a were obtained from the United States Department of Commerce's National Oceanic and Atmospheric Administration Earth System Research Laboratory observations from Mauna Loa (Tans & Keeling)(available here: ftp://aftp.cmdl.noaa.gov/products/trends/co2/co2_annmean_mlo.txt). Annual estimates for $\delta^{13}\text{C}_{\text{air}}$ were downloaded from the flask analysis data for Mauna Loa, available from the Carbon Dioxide Information Analysis Center (Keeling *et al.*, 2009) (CDIAC; <http://cdiac.ornl.gov/ftp/trends/co2/iso-sio/>).

Climate Data

All climate data were derived from the ClimateWNA (version 5.4) software interface (Wang et al., 2016). This program downscales model estimates using the Parameter-Elevation Regressions on Independent Slopes Model (PRISM) method (Daly et al., 2008). We downloaded annual climate variables for planting sites. For the provenance climates (climate of seed origin), we derived average climate variables for the 1961-1990 climate normal period. For the provenance climates (climate at seed collection sites), we used average annual climate variables for the 1961-1990 normal because this period represents comprehensive climatic data and is not too dissimilar to historic climates to which trees are presumed to be adapted.

Statistical Analyses

After compiling growth, functional wood anatomical and stable isotope data, we conducted a thorough outlier analyses and found no values that warranted rejection from the database. All statistical analyses were conducted in the R statistical programming environment (R Core Team, 2017) , and graphing was implemented with various functions from the *ggplot2* package (Wickham, 2009).

Correlations to climate at the planting site were conducted at the provenance level and were calculated with the *rcorr* function from the *Hmisc* package to obtain p-values. Due to the slightly imbalanced design, provenance values for the correlations relied on least square means calculated using *lsmeansLT* and *lmerTest* (Kuznetsova et al., 2016), where provenance was specified as the fixed effect and block and a unique tree identity were specified as random effects. For testing differences among the four regional populations, the fixed effect in each model was region. Model selection was verified by confirming lower values for Akaike Information Criteria. For models based on functional wood anatomy, the distance from the apex

was included as a random effect because it affects conduit size (Mencuccini et al., 2007; Carrer et al., 2015). It is necessary to include distance to the top of the tree since conduit size is partially determined by growth patterns over time: as the tree grows higher, the new growth at the base of the tree requires larger conduits. This is therefore reflected in functional wood anatomical data in a gradient towards the pith in a stem disk or increment core (Mencuccini et al., 2007; Carrer et al., 2015). Correlations between growth and annual climate conditions at the site were based on height increments and basal area increments. Correlations of the average climate of seed origin (the long-term mean from 1961-1990) to all growth, drought, anatomical and isotopic traits are designed to show adaptation to climate. For these correlations, we used total height and DBH after 31 years of growth in real-world conditions (year 2005). All correlations were also adjusted using the Benjamini & Hochberg method (Benjamini & Hochberg, 1995) for reporting significance in tables (Tabs. 6.S3, 6.S4). Heatmaps were produced using the *pheatmap* command in the *pheatmap* package (Kolde, 2015). Post-hoc tests for regional population fixed effects were completed with the *glht* command from the *multcomp* package (Hothorn et al., 2008, 2016) and the p-values were adjusted using the Benjamini & Hochberg method as well (Benjamini & Hochberg, 1995).

6.10 Supplementary Information: Results

Tab.6.S1. Geography and average source climate variables (1961-1990 long-term average) are shown for provenances

Region	Prov	Name	Lat	Long	EI	MAR	MAT	MWMT	MCMT	TD	MAP	MSP	PAS	RH
Leading Edge (Northern provenances)	33	Ethel L.	63.30	-136.47	876	9.8	-4.2	14	-24.7	38.7	442	260	198	57
Leading Edge (Northern provenances)	30	Lower Post	59.98	-128.55	640	9.7	-2.7	14.5	-23.5	38	448	235	204	56
Leading Edge (Northern provenances)	35	Atlin	59.80	-133.78	789	9.8	-0.6	12.5	-15.5	28	374	183	181	63
Leading Edge (Northern provenances)	28	Tetsa R.	58.67	-124.17	762	10.5	-1.2	13.7	-18.2	31.9	621	419	195	58
Leading Edge (Northern provenances)	66	Stone Mt.	58.65	-124.77	1173	10.1	-1.3	11.3	-14.4	25.7	688	475	240	61
Central Interior (Provenances from center of distribution)	100	Nina Cr.	55.80	-124.82	762	10.9	0.9	13.5	-14	27.5	529	250	234	61
Central Interior (Provenances from center of distribution)	20	Collins L.	54.13	-127.23	937	11.1	2	12.5	-9.4	21.9	595	219	294	64
Central Interior (Provenances from center of distribution)	104	Nechako R.	54.02	-124.53	732	11.1	2.6	14.4	-11.4	25.7	520	238	211	58
Central Interior (Provenances from center of distribution)	107	Tweedsmuir	52.50	-125.80	1311	11.3	1.2	10.9	-8.8	19.8	1043	257	628	60
Southern Interior (Provenances from center of distribution)	14	Wentworth Cr.	50.97	-120.33	1059	12.3	4.2	14.8	-7.6	22.4	476	219	163	62
Southern Interior (Provenances from center of distribution)	72	Larch Hills	50.70	-119.18	777	11.2	5.8	17.1	-5.9	23	648	244	211	65
Southern Interior (Provenances from center of distribution)	57	Inonoaklin	49.90	-118.20	579	10.7	6.8	17.9	-4.4	22.3	685	264	162	64
Southern Interior (Provenances from center of distribution)	1	Trapping Cr.	49.58	-119.02	1006	11.1	4.5	15.7	-7.1	22.8	550	227	200	59
Southern Interior (Provenances from center of distribution)	42	Champion L.	49.18	-117.58	998	12.7	6.2	17.6	-5.2	22.8	835	269	261	67
Trailing Edge (Provenances from the far south)	111	Stevens Pass	47.78	-120.93	762	16.5	6.5	17.2	-3.5	20.7	1711	255	536	61
Trailing Edge (Provenances from the far south)	144	Missoula (a)	46.67	-113.67	1524	12.7	4.7	16.8	-6.3	23.1	568	237	199	56
Trailing Edge (Provenances from the far south)	153	Enterprise	45.63	-117.27	1311	14.5	6.7	17.7	-3.4	21.1	562	205	123	56
Trailing Edge (Provenances from the far south)	154	Prairie City	44.53	-118.57	1494	15.9	6	17.1	-4.1	21.3	639	171	206	56
Trailing Edge (Provenances from the far south)	123	Black Butte	44.38	-121.67	1006	14.3	7.3	16.6	-0.7	17.3	609	102	99	51

Region refers to the assigned population based on geographical origin. *Prov* refers to the provenance corresponding to the Illingworth provenance trial's numbering system (Illingworth, 1978). Latitude (*Lat*) and longitude (*Long*) are given decimal degrees; elevation (*Elev*) is given in meters above sea level. Mean Annual Solar Radiation (*MAR*, MJ m⁻² d⁻¹) is also provided. Climate variables include: Mean Annual Temperature (*MAT*, °C), Mean Warmest Month Temperature (*MWMT*, °C), Mean Coldest Month Temperature (*MCMT*, °C), Temperature Difference (*TD*, °C), Mean Annual Precipitation (*MAP*, mm), Mean Summer Precipitation (*MSP*, mm), Precipitation As Snow (*PAS*, mm) and Relative Humidity (*RH*, %). Climate data derived from ClimateWNA v 5.40 (Wang et al. 2016).

Tab. 6.S2. Geography and average annual climate variables (1981-2010 long-term average) of the three planting sites

Site	Lat	Long	Elev	MAR	MAT	MWMT	MCMT	TD	MAP	MSP	PAS	RH
Chuwels Lake (CHUW)	50.58	-120.62	1430	12.7	2.9	13.5	-7.2	20.7	445	214	164	63
Community Lake (COMM)	50.92	-120.07	1370	12.6	3.3	14.1	-7	21.1	664	280	262	67
Equises Creek (EQUI)	50.37	-119.60	1370	12.3	3.5	14.1	-7.1	21.2	743	270	308	66

The three planting sites are within the Illingworth lodgepole pine provenance trial (Illingworth, 1978): Chuwels Lake (*CHUW*), Community Lake (*COMM*) and Equises Creek (*EQUI*). Latitude (*Lat*) and longitude (*Long*) are given decimal degrees; elevation (*Elev*) is given in meters above sea level. Mean Annual Solar Radiation (*MAR*, MJ m⁻² d⁻¹) is also provided. Climate variables include: Mean Annual Temperature (*MAT*, °C), Mean Warmest Month Temperature (*MWMT*, °C), Mean Coldest Month Temperature (*MCMT*, °C), Temperature Difference (*TD*, °C), Mean Annual Precipitation (*MAP*, mm), Mean Summer Precipitation (*MSP*, mm), Precipitation As Snow (*PAS*, mm) and Relative Humidity (*RH*, %). Climate data derived from ClimateWNA v 5.40 (Wang et al. 2016).

Tab. 6.S3. Pearson's correlation coefficients of annual site climate to growth and physiology of four populations representing distinct climatic regions in the distribution of lodgepole pine (*Pinus contorta*)

	Growth												Physiology																		
	Basal Area Increment				Height Increment				Hydraulic Diameter				Wall Thickness				$\delta^{18}\text{O}$				$\delta^{13}\text{C}$				Water Use Efficiency (A/g)						
	LE	CI	SI	FS	LE	CI	SI	FS	LE	CI	SI	FS	LE	CI	SI	FS	LE	CI	SI	FS	LE	CI	SI	FS	LE	CI	SI	FS	LE	CI	SI
Max. temperature in spring (°C)	0.04	0.18	0.15	0.21	0.3	0.39	0.05	0.21	0.24	0.17	0.15	0.35	0.25	0.38	0.32	0.36	0.16	0.07	0.09	0.12	-0.23	-0.07	-0.05	-0.12	0.02	0.16	0.14	0.09			
Max. temperature in summer (°C)	-0.2	-0.24	-0.19	-0.34	-0.25	-0.38	-0.22	-0.31	-0.05	-0.14	-0.18	-0.18	0	0.01	0.05	-0.02	0.73	0.73	0.63	0.56	0.39	0.7	0.71	0.56	0.57	0.75	0.75	0.62			
Max. temperature in autumn (°C)	0.15	0.15	0.1	0.15	0.15	-0.03	-0.1	-0.17	-0.11	-0.03	-0.27	-0.18	0.09	0.03	0.05	0.08	-0.07	-0.16	-0.1	-0.04	0.02	0.09	0.12	0.23	0.08	0.12	0.15	0.24			
Precipitation as snow (mm)	0.44	0.48	0.53	0.35	0.4	0.4	0.58	0.5	0.56	0.51	0.32	0.54	0.43	0.42	0.4	0.16	-0.25	-0.27	-0.1	-0.29	-0.18	-0.39	-0.09	-0.32	-0.41	-0.54	-0.25	-0.46			
Precipitation in spring (mm)	0.19	0.22	0.39	-0.05	0.17	0.14	0.53	0.15	0.49	0.44	0.28	0.3	0.42	0.5	0.42	0.1	0.24	0.21	0.31	0.05	-0.1	-0.06	0.27	-0.08	-0.16	-0.1	0.19	-0.12			
Precipitation in summer (mm)	0.15	0.29	0.3	0.22	0.55	0.68	0.34	0.38	0.21	0.19	0.21	0.35	0.23	0.41	0.22	0.11	-0.46	-0.55	-0.41	-0.49	-0.59	-0.59	-0.55	-0.63	-0.59	-0.51	-0.48	-0.55			
Precipitation in autumn (mm)	0.34	0.41	0.41	0.3	0.35	0.37	0.29	0.55	0.54	0.41	0.36	0.62	0.38	0.31	0.36	-0.01	0.21	0.2	0.29	0.1	0.01	0.02	0.19	-0.11	-0.07	-0.04	0.11	-0.15			
Relative humidity in spring (%)	0.08	0.11	0.29	-0.2	0.2	0.1	0.48	0.11	0.2	0.35	0.3	0.09	0.09	0.18	0.07	-0.42	0.03	0.04	0.13	-0.15	-0.22	-0.11	0.1	-0.13	-0.37	-0.22	-0.02	-0.23			
Relative humidity in summer (%)	0.23	0.39	0.42	0.29	0.57	0.69	0.5	0.41	0.25	0.36	0.31	0.38	0.22	0.41	0.2	0.05	-0.74	-0.78	-0.61	-0.7	-0.69	-0.78	-0.68	-0.72	-0.79	-0.76	-0.67	-0.71			
Relative humidity in autumn (%)	0.13	0.33	0.41	0.15	0.5	0.62	0.57	0.57	0.53	0.56	0.54	0.66	0.3	0.59	0.4	0.15	-0.21	-0.22	-0.13	-0.33	-0.54	-0.51	-0.31	-0.61	-0.56	-0.45	-0.29	-0.55			

The four populations are represented by columns titled LE, CI, SI and TE: *LE* stands for the Leading Edge, i.e., the northern population occupying the area expected to be the leading edge of tree species migrations under climate warming; *CI* is Central Interior population, located in the central areas of the lodgepole pine distribution; *SI* is Southern Interior population, covering the mid-southern range of the lodgepole pine distribution; *TE* stands for the Trailing Edge, which represents seed sources from the far south of the lodgepole pine distribution, which is expected to see increased forest maladaptation under climate warming.

Tab. 6.S4. Pearson's correlation coefficients of provenance growth, drought tolerance and physiology to climate of seed origin (1961-1990 climate normal)

	Growth		Drought Tolerance				Physiology				
	Diameter	Height	Resistance	Recovery	Resilience	Relative Resilience	Hydraulic Diameter	Wall Thickness	$\delta^{18}O$	$\delta^{13}C$	Water Use Efficiency (A/g _s)
Latitude	-0.53	-0.53	0.39	-0.76	-0.44	-0.75	-0.1	-0.4	-0.48	0.02	0.02
Longitude	0.65	0.66	-0.24	0.43	0.21	0.41	0.3	0.49	0.74	0.18	0.18
Mean Annual Temperature (°C)	0.63	0.65	-0.26	0.7	0.49	0.71	0.25	0.45	0.56	0.04	0.04
Mean Coldest Month Temperature (°C)	0.3	0.38	-0.15	0.43	0.25	0.37	0.03	0.16	0.42	-0.1	-0.1
Mean Warmest Month Temperature (°C)	0.61	0.62	-0.34	0.76	0.5	0.78	0.22	0.47	0.51	0.05	0.05
Temperature Difference (°C)	-0.61	-0.59	0.34	-0.74	-0.5	-0.78	-0.25	-0.49	-0.44	-0.1	-0.1
Frost-free period (No. of days)	0.61	0.69	-0.05	0.26	0.17	0.22	0.38	0.41	0.59	0.13	0.13
Degree days > 5 °C (No. of days)	0.45	0.51	-0.13	0.5	0.36	0.47	0.15	0.26	0.51	-0.04	-0.04
Mean Annual Precipitation (mm)	0.16	0.17	-0.31	0.35	0.07	0.31	-0.08	0.32	0.11	-0.12	-0.12
Mean Summer Precipitation (mm)	-0.13	-0.14	0.09	-0.57	-0.51	-0.59	0.04	-0.04	0.18	0.12	0.12
Relative Humidity (%)	0.5	0.53	0.04	-0.23	-0.19	-0.22	0.51	0.43	0.32	0.32	0.32
Summer Heat:Moisture Index	0	0.02	-0.05	0.63	0.63	0.69	-0.18	-0.12	-0.06	-0.38	-0.38

Growth variables include: total diameter in centimeters in year 2005, when trees had grown in real world conditions for 31 years (*Diameter*) and height in year 2005 in meters (*Height*). Drought tolerance variables include: drought resistance, as measured by the loss of growth in a drought event (*Resistance*); drought recovery, or the speed at which a tree recovers from a drought event (*Recovery*); drought resilience, or ability to achieve pre-drought growth (*Resilience*); and relative resilience, or the ability to regain pre-drought growth with respect to growth reduction severity during the drought event (*Relative Resilience*). Physiological variables include: hydraulic diameter in μm (*Hydraulic Diameter*); mean tracheid wall thickness in μm (*Wall Thickness*); stable oxygen isotope ratio ($\delta^{18}O$); stable carbon isotope ratio ($\delta^{13}C$); and intrinsic Water Use Efficiency (A/g_s), which is derived from the carbon isotope ratio (*WUE*).

Tab. 6.S5. Least square means of growth, drought and isotope values over the 10 year study period

Regional Population	Growth		Physiology				
	Basal Area Increment	Height Increment	Hydraulic Diameter	Wall Thickness	$\delta^{18}O$	$\delta^{13}C$	Water Use Efficiency (A/g_s)
North (Leading Edge)	4.73 (0.96)	0.32 (0.03)	31.62 (0.85)	3.09 (0.17)	19.46 (0.32)	-26.76 (0.22)	79.81 (2.47)
Central Interior (Central to Distribution)	7.70 (0.97)	0.42 (0.03)	34.13 (0.88)	3.48 (0.17)	19.90 (0.32)	-26.22 (0.22)	85.57 (2.54)
Southern Interior (Central to Distribution)	8.48 (0.96)	0.43 (0.03)	34.64 (0.86)	3.44 (0.17)	20.11 (0.32)	-26.38 (0.22)	83.86 (2.47)
Far South (Trailing Edge)	6.39 (0.97)	0.38 (0.03)	31.74 (0.87)	3.38 (0.17)	19.95 (0.32)	-26.63 (0.22)	81.24 (2.51)

Least square means of four lodgepole populations from the four climatic regions LE, CI, SI and TE (standard errors are provided in brackets): *LE* stands for the Leading Edge, i.e. the northern population occupying the area expected to be the leading edge of tree species migrations under climate warming; *CI* is Central Interior population, located in the central areas of the lodgepole pine range; *SI* is the Southern Interior population covering the southern range of the central areas of the lodgepole pine range; *TE* stands for the Trailing Edge, which represents seed sources from the far south of the lodgepole pine range, which is expected to see increased forest maladaptation under climate warming. Growth variables compared include annual height increment in meters (*Height Increment*) and basal area increment in centimeters (*Basal Area Increment*). Physiological variables include: hydraulic diameter in μm (*Hydraulic Diameter*); mean tracheid wall thickness in μm (*Wall Thickness*); mean number of tracheids (*Tracheid Number*); stable oxygen isotope ratio ($\delta^{18}O$); stable carbon isotope ratio ($\delta^{13}C$); and intrinsic Water Use Efficiency, which is derived from tree-ring $\delta^{13}C$ (*WUE*).

Tab. 6.S6. Differences among the four populations tested here in terms of growth, drought and isotope responses over the 10 year study period.

Test	Growth		Drought Tolerance				Physiology				
	Basal Area Increment	Height Increment	Resistance	Recovery	Resilience	Relative Resilience	Hydraulic Diameter	Wall Thickness	$\delta^{18}O$	$\delta^{13}C$	Water Use Efficiency
N-CI=0	<0.001	<0.001	1.000	0.163	0.418	0.129	0.01134	0.002	0.029	0.006	0.006
SI-CI=0	0.659	0.924	0.988	1.000	0.998	1.000	0.927	0.985	0.580	0.761	0.764
US-CI=0	0.233	0.141	0.180	0.005	0.990	0.065	0.024	0.811	0.993	0.077	0.077
SI-N=0	<0.001	<0.001	0.994	0.203	0.321	0.157	<0.001	0.005	<0.001	0.074	0.070
US-N=0	0.063	0.006	0.157	<0.001	0.256	<0.001	0.999	0.033	0.010	0.844	0.838
US-SI=0	0.010	0.023	0.087	0.003	0.999	0.052	0.002	0.948	0.734	0.426	0.424

Multiple comparisons among four lodgepole populations from the four climatic regions LE, CI, SI and TE: *LE* stands for the Leading Edge, i.e. the northern population occupying the area expected to be the leading edge of tree species migrations under climate warming; *CI* is Central Interior population, located in the central areas of the lodgepole pine range; *SI* is the Southern Interior population covering the southern range of the central areas of the lodgepole pine range; *TE* stands for the Trailing Edge, which represents seed sources from the far south of the lodgepole pine range, which is expected to see increased forest maladaptation under climate warming. Growth variables compared include annual height increment in meters (*Height Increment*) and basal area increment in centimeters (*Basal Area Increment*). Physiological variables include: hydraulic diameter in μm (*Hydraulic Diameter*); mean tracheid wall thickness in μm (*Wall Length*); stable oxygen isotope ratio ($\delta^{18}O$); stable carbon isotope ratio ($\delta^{13}C$); and intrinsic Water Use Efficiency, derived from tree-ring $\delta^{13}C$ (*WUE*). Significance is indicated in bold and was corrected with the Benjamini & Hochberg method.

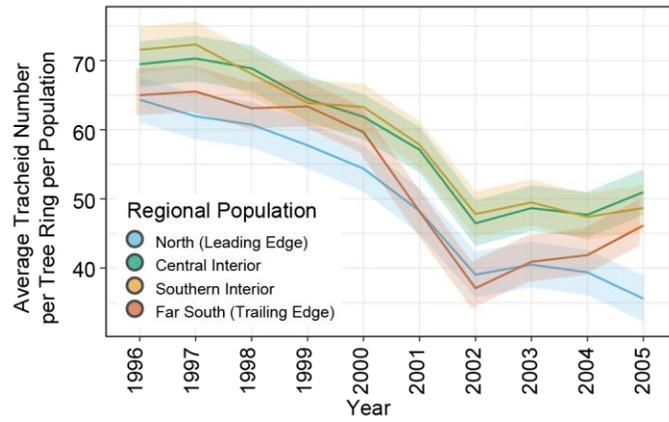


Fig. 6.S3. Change in average tracheid number per tree ring by regional population over the study period. The average values correspond strongly with annual growth rates, with decreasing trends over time (basal area increment removes the age effect and was therefore used for the primary analyses in the study).

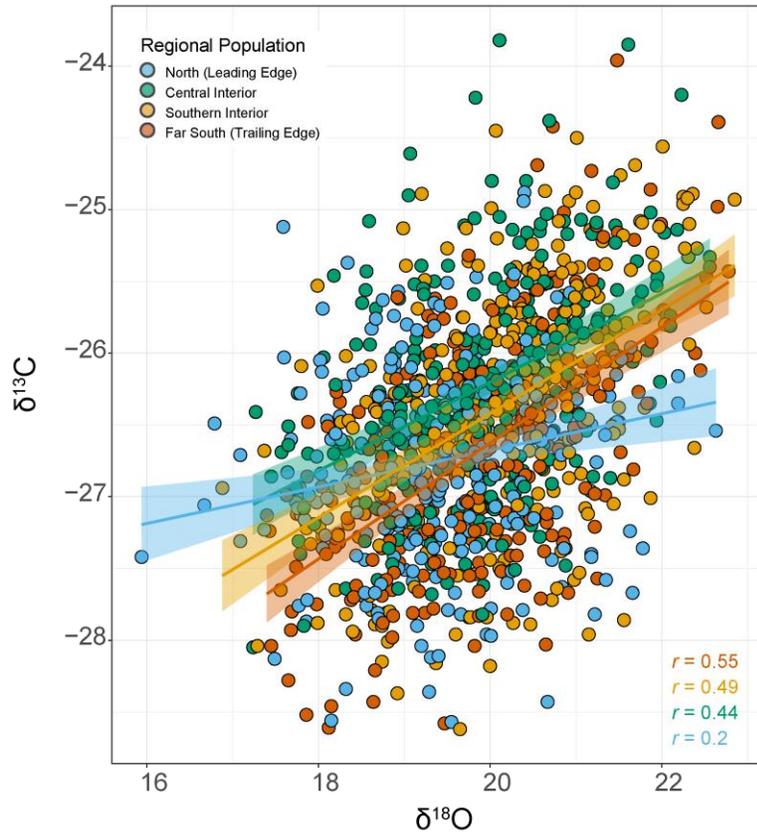


Fig. 6.S4. Relationship between stable carbon and oxygen isotope ratios in four large populations of lodgepole pine. Each dot represents the values from one tree (provenance) in one year at one block ($n = 1170$).

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7 Cold Tolerance Considerations for Assisted Gene Flow

7.1 Abstract

With lengthening growing seasons and temperature variability under climate change, frost damage to plants may remain a risk in decades to come. This may also apply to warm-adapted tree populations planted in anticipation of a warmer future climate. Here, we study cold adaptation of tree populations in a wide-ranging coniferous species in western North America to inform limits to seed transfer. We use a novel approach combining methods from tree-ring research and quantitative forest genetics. Populations from the northern extent of the species range were resistant to fall cold events but susceptible to spring frosts, implying maladaptation under projected climate warming. Central populations were productive but also sensitive to early fall frosts. Transferring the southern, warm-adapted genotypes northward caused rank-changing growth loss after a spring frost event. We conclude that cold adaptation should remain an important consideration when implementing seed transfers designed to mitigate harmful effects of climate change.

7.2 Introduction

The pace of observed climate warming, particularly in northern latitudes, implies that environmental conditions are changing faster than tree populations can adapt, acclimate or migrate (Marris, 2009; Hoffmann & Sgrò, 2011; Aitken & Bemmels, 2016). This is expected to decrease productivity while increasing mortality and susceptibility to insect attacks and diseases. This could lead to severe economic losses and reduce forest carbon sequestration potential - in

some cases even turning forests into significant sources of carbon dioxide (Kurz et al., 2008). To reduce such consequences of forest maladaptation under climate change, assisted migration as a reforestation policy has been proposed. This concept involves adjusting the genetic composition of forests by moving seed material to climate regions where they are anticipated to be well adapted in the future (Marris, 2009; Pedlar et al., 2012; Aitken & Bemmels, 2016; O'Neill et al., 2017). For example, planting seeds from southern, drought-tolerant populations may help adapt forests to drier, warmer conditions (Marris, 2009; Montwé et al., 2015a, 2016; Aitken & Bemmels, 2016). However, planting warm-adapted tree populations in anticipation of a warmer future could expose these seedlings to frost that could lead to damage and mortality as well (Benito-Garzón et al., 2013; Bansal et al., 2015). As climates warm and changing phenology (Menzel, 2000) interacts with temperature variability (Schwartz & Reiter, 2000; Marino et al., 2011), frost damage may also remain a risk in decades to come. Understanding the adaptation of tree populations to cold – as well as to heat and drought – is critical for minimizing risks under changing climates (Yeaman et al., 2016).

Adaptation and maladaptation of trees have been studied with genetic provenance trial series, also known as common garden experiments (O'Neill et al., 2008; Montwé et al., 2016). These reciprocal transplant experiments grow seeds collected from across all or part of a species range (i.e., provenances) at multiple planting sites to reveal underlying intra-specific genetic differentiation. Such trials are ideal for studying plant-climate interactions and for assessing the risks involved in seed transfer to new locations (Matyas, 1994; Schmidting, 1994; Isaac-Renton et al., 2014). Growing northern seed sources at southern planting sites involves a climate transfer that can be equivalent to projected climate change, with the growth response being predictive of future performance under realistic long-term field conditions. Conversely, moving southern

seeds northward can test the performance of locally adapted populations under currently colder environments, thereby suggesting limits to suitable seed transfer. Within these designs, genetic adaptation to cold in tree populations has been studied through the observation of tissue damage after sporadic natural frost events and by exposing collected tissue samples to artificial freezing tests in the laboratory (Rehfeldt, 1980; Hannerz et al., 1999; Anekonda et al., 2000; St. Clair, 2006; Bansal et al., 2015).

Tree rings may provide additional information for evaluating cold adaptation since they provide a record of how past climate has influenced growth. Two wood anatomical features have been linked to cold and frost. First, layers of deformed, collapsed tracheids and traumatic parenchyma cells have been described as *frost rings* (Glock & Reed, 1940; Stone, 1940; Glerum & Farrar, 1966). Frost rings have been linked to generally colder years (LaMarche & Hirschboeck, 1984) as well as specific events of air temperatures falling below freezing during a period of cambial activity (Glerum & Farrar, 1966). Second, *light rings* consist of layers of cells that are incompletely lignified (Yamaguchi et al., 1993) and have been associated with frost events that kill the cells before the lignification process is complete (Gindl & Grabner, 2000; Gindl et al., 2000). Due to the lack of lignin, light rings do not fully absorb the red Safranin dye during a double staining procedure with Astrablue. This staining procedure causes non-lignified layers of cells to appear blue, hence light rings have also been referred to as *blue rings* (Piermattei et al., 2015). These blue rings (failed lignification) and frost rings (cambium damage) therefore act as long-term records of cold damage and could be combined with provenance trial designs.

Here, we study cold adaptation of tree populations to inform limits to seed transfers designed for warmer climates. We first test whether frost imprints in tree rings can act as a

reliable record of cold maladaptation, analyzing the climate patterns linked to blue and frost rings. We further combine these responses with growth data from genetically distinct populations of lodgepole pine (*Pinus contorta* Dougl. ex. Loud.) in arguably the largest provenance trial series in existence (Illingworth, 1978). These pine populations originated from across the western US and Canada and were grown at three planting sites in British Columbia's (BC) Southern Interior region for almost three decades.

7.3 Methods

Experimental Design and Field Methods

Stem disks were cut at 1.3 m from trees of selected lodgepole pine populations in the Illingworth provenance trial. This experiment tested 153 seed sources, or provenances, at 60 planting sites in an incomplete randomized block design (Illingworth, 1978). Two-year old seedlings were planted in 1974 and most trees died in 2006 due to the mountain pine beetle epidemic. We used a representative sub-set from this trial for detailed wood anatomical analyses: Five provenances from British Columbia's North and southern Yukon (N); five provenances each from British Columbia's Central Interior (CI) and Southern Interior (SI); and five provenances from the United States (US). These 20 provenances were replicated on three experimental sites in British Columbia's Southern Interior (Fig 7.1; Tab 7.S1, Tab 7.S2). We chose these sites to have enough replications of independent samples while also representing a general area with similar climate conditions that may, given warming, become widespread throughout the central plateaus of British Columbia. Each site consists of two blocks, with provenances planted in plots of 9 trees. From each plot, we selected one tree of median height for

wood anatomical analyses, although three samples were not suitable due to early mortality or rot. Thus, a sub-sample of 117 trees was used.

Preparation of Micro Sections for Wood Anatomical Analyses

We cut micro sections of 10-20 μm thickness with a GSL-1 microtome (Gärtner et al., 2014). We applied a corn-starch solution to the sample surface to reduce detached cell walls due to pressure induced by the microtome blade (Schneider & Gärtner, 2013). We then washed the sections with distilled water (Gärtner & Schweingruber, 2014), briefly steamed them to gelatinize any remaining corn-starch granules (Montwé et al., 2015b), rinsed them with distilled water to remove the gelatinized corn-starch, and bleached them (Gärtner & Schweingruber, 2014). We then followed a standard double-staining procedure (Gärtner & Schweingruber, 2014): we applied a 1:1 mixture of Safranin and Astrablue to the micro sections for 5 min; washed them with distilled water; and then washed them with ethanol (70%, 96% and 99%) to further remove surplus stain. Canada balsam was warmed to temporarily increase viscosity and applied to the micro sections, which were then covered by a glass cover slip. We then heated the micro sections in an oven at 60 °C for a minimum of 8 hours to harden the Canada balsam. Digital photos were taken with a Nikon Eclipse Ni-E upright microscope with a resolution of 5 megapixels and 12-bit color depth (Nikon DS-Fi2). Micrographs were taken at 20 \times magnification and automatically stitched together with the NIS-Elements software (Version 4.20.01, Nikon Corporation, 2014).

Measurements of Blue and Frost Ring Occurrence and Intensity

Blue and frost ring occurrences were recorded at the tree-ring level using a light microscope at 100 \times magnification; sample labels were coded to prevent observer bias. We also

assigned an intensity score for blue and frost rings on a scale of zero to five: a zero indicates absence and a one represents a faint blue band in at least one row of cells, while a five represents a larger band of completely un-lignified cells often associated with narrower and distorted cell walls. Frost rings are characterized by deformed tracheids of varying sizes, thin cell walls, and damaged ray parenchyma (Glerum & Farrar, 1966; Kidd et al., 2014). Frost rings that occurred directly at the ring boundary in the earlywood were labeled as position 1 (Fig. 7.1a). Their position indicates cold events in winter prior to – or at the time of – cambial reactivation. Frost rings labeled as position 2 occurred after several rows of earlywood tracheids had already formed normally, thereby pointing to a spring cold event after growth had already been initiated (Fig. 7.1b).

Climate Data

We derived daily temperature minimums and averages as well as precipitation sums from the ECMWF's ERA interim data for 1979 to 2006 (Dee et al., 2011) for the grid-points closest to our site coordinates. This data is available through KNMI's Climate Explorer (climexp.knmi.nl/). To capture general temperature and moisture conditions, as well as cold and frost events on an annual level, we calculated several additional climate variables. The start of the growing season was set as the last day in spring on which minimum temperatures dropped below 0 °C (Julian date). The end of the growing season was set to the Julian day on which the first frost (<0 °C) occurred again in autumn. Growing season length was calculated as the number of Julian days between the start and the end of the growing season. Mean and minimum temperatures were calculated on annual and seasonal time scales. Growing degree days for varying base temperatures were calculated to characterize growing conditions as well as cold and frost in the fall. To investigate the relationship between blue and frost rings and the climate at the seed

source locations, were interpolated climate normal data for the 1961-1990 period with the software ClimateWNA, version 4.62 (Wang et al., 2006). This period was chosen because of wide spatial representation of climate stations and because it precedes the most recent anthropogenic climate warming signal.

Statistical Analyses

We performed all statistical analyses and rendering of graphical representations in the R programming environment (R Core Team, 2017). We used the *Hmisc*-package to calculate Spearman's correlation coefficient and p-values of blue and frost ring intensity to climate variables. Spearman's correlations were most appropriate due to heteroscedasticity. Benjamini and Hochberg's method was used for controlling the false discovery rate as implemented with the *p-adjust* function of the R-base package. Given the location of blue rings in the latewood, we tested correlations to fall cold events as well as general growing season variables, as these had been previously linked to frost imprints in the latewood (Glerum & Farrar, 1966; LaMarche & Hirschboeck, 1984). We also tested previous years' chilling units on the hypothesis that some populations could grow increasingly out-of-sync with site climate if their chilling requirements had not been met (Laube et al., 2014). To test for population differences in both blue ring and frost ring occurrences, a binomial generalized linear mixed effects model was used. The region was specified as a fixed effect; year, site, provenance, and a unique tree identifier were specified as random effects. The *lme4* and *car* packages were used to calculate the mixed model and subsequent ANOVA (Bates et al., 2015; Fox et al., 2016). Post-hoc tests were run using the *ghlt* function from the *multcomp* package (Hothorn et al., 2008). To account for the slightly imbalanced design and for variation in the length of the tree-ring series as trees reached the sampling height at different ages, the least square means of blue and frost ring intensities were

calculated with the *lsmeans* function of the *lme4* package after building a linear mixed effects model with the *lmer* function.

7.4 Results

In total, we examined 2999 tree rings. Across all populations, we recorded 689 blue ring occurrences (23%), plus 204 more severe frost ring occurrences (7%). Blue rings (Fig. 7.1a, 7.1d) occurred exclusively in the latewood, while frost ring damage occurred most often in the first rows of cells in the earlywood, labeled as *position 1* (Fig. 7.1a). The occurrence of the most severe blue rings in fall was usually followed by spring frost rings at position 1. It therefore appears that the blue ring captures a cold event at the end of the growing season that leads to cambial damage and irregular growth upon re-activation in the following spring. Occasionally, frost rings occurred in the earlywood after some tracheids had formed normally. These were labeled as frost rings in *position 2* (Figs. 7.1b, 7.1d), and we interpret them as damage caused by late spring frost events. Higher incidences in the earlier part of the experiment (Figs. 7.1d, 7.2d-f) may reflect higher susceptibility of saplings to frost. This may be due to their smaller size and lower heat absorption, higher occurrences of tender lammas growth, and their proximity to the soil surface, which is colder due to radiative cooling (Stone, 1940; Fritts, 1976; Howe et al., 2003; Gurskaya & Shiyatov, 2006; Kidd et al., 2014).

An analysis of historical climate data reveals that blue rings and frost rings in position 1 are linked to a late initiation of the growing season and low temperatures at the end of the growing season (Tab. 7.1, Fig. 7.1d, 7.1e). Cold spring conditions delay the onset of growth, pushing growth later into the fall. This increases the window of frost risk because lignification can lag behind cellular development by several weeks (LaMarche & Hirschboeck, 1984;

Yamaguchi et al., 1993; Wang et al., 2000; Tardif et al., 2011; Cuny et al., 2015). For example, the year 1999 had high blue ring intensities, and was associated with both a cool spring and summer (Fig. 7.1f) and a low accumulation of warmth measured in growing degree days (Fig. 7.1g). In contrast, no frost imprint was observed in 1998. This year had a long growing season (Fig. 7.1e), warm spring, hot summer and warm fall (Fig. 7.1f), which led to an above-average annual accumulation of growing degree days above 5 °C (Fig. 7.1g).

In contrast, frost rings in position 2 must reflect cold events in spring after growth has been initiated. Here, we found the strongest climate relationships to be with spring temperature accumulation (Tab. 7.1) and early spring temperatures. As an example, the year 1992 had a high proportion of frost rings in position 2, and shows evidence of a false spring: a short warm spell followed by cooler temperatures (Figs. 7.1e-g). However, the populations tested here appeared to respond differently to such events (Fig. 7.2c, 7.2f) and correlations were not always consistent across genotypes (Tab. 7.1). This suggests different susceptibilities of populations to cold damage under increased spring temperature variability.

Genetic differentiation of the four regional lodgepole pine populations (Fig. 7.2g) was statistically significant for blue ring occurrence ($p < 0.001$, $\chi^2 = 92.90$) and frost ring occurrence at position 1, presumed to be fall frost damage ($p = 0.008$, $\chi^2 = 11.95$). The Southern Interior population was most affected by blue rings and fall frost rings, followed by the United States (US) group. The Northern populations had the lowest levels of blue rings and fall frost rings, with the Central Interior populations showing intermediate susceptibility (Fig. 7.2a, 7.2b). In contrast, Northern populations were the most susceptible group to spring frost damage as indicated by frost rings in position 2 ($p = 0.030$, $\chi^2 = 8.91$). Similarly, correlations to climate of seed origin (Tab. 7.2) show that populations adapted to northern, colder environments with larger

temperature differences and shorter growing seasons incur less blue rings and fall frost damage, but show a higher susceptibility to spring frosts..

7.5 Discussion

The data suggest that northern populations may be most vulnerable to late spring frost events and false springs under climate change (Marino et al., 2011; Allstadt et al., 2015), especially if temperature patterns become more variable (Schwartz & Reiter, 2000; Parmesan & Yohe, 2003). The likely explanation for this counter-intuitive observation is that northern populations must adapt to a short growing season. Northern populations flush earlier than southern populations for a given chilling and heat sum measured in degree days to take full advantage of the limited northern growing season (Howe et al., 2003), but this adaptive strategy may no longer be successful under anticipated climate change. This is especially relevant since temperature has been found to be more influential for spring flushing than photoperiod in most forest trees (Zohner et al., 2016). Moreover, the resulting frost damage may be associated with forest health issues. The lack of lignification and cellular damage may facilitate the internal spread of pathogens (Diamandis & Koukos, 1992) and also reduce wood quality and value by creating weaknesses and defects in the timber (Lee et al., 2007).

Another equally important finding is that it may not always be possible to transfer genotypes northward as a management prescription to address climate change: The most productive Southern Interior populations showed the highest fall frost damage (Fig 7.2d-e). Given that we inferred a short growing season and low cumulative degree days as a likely cause of blue rings and frost rings in fall, a transfer of these populations to colder environments is likely to exacerbate this problem. Moderate transfer distances appear acceptable, however.

Central interior populations did not incur significantly higher fall frost damage than northern populations (although they showed increased blue rings, which appears to be a more sensitive measure of cold adaptation). Similarly, the US population did not incur additional fall cold damage relative to the Southern Interior population. It should be noted, however, that spring frost susceptibility may compromise growth: a substantial decline in the US population's tree-ring growth began shortly after a spring frost event in 1992 (Fig 7.2f). Prior to this rank change, these populations had shown equal productivity to the local seed sources.

We conclude that expanding allowable distances of tree seed transfer can increase the risk of frost damage and potentially reduce growth as a consequence. Cold adaptation should remain an important consideration when implementing long-distance seed transfers in temperate forests designed to mitigate harmful effects of climate change. We also demonstrate that complex cold adaptation can be retrospectively studied through frost imprints in tree rings. Independent genetic clines for spring and fall frost damage were revealed in this study, which both need to be considered to devise effective strategies to minimize risks in assisted migration prescriptions to address climate change. Provenance trials have been established for most of the major tree species of the temperate and boreal zone, and tree-ring analysis can be conducted non-destructively with increment borers to take full advantage of the information contained in these valuable transplant experiments.

7.6 References

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7.7 Figures and Tables

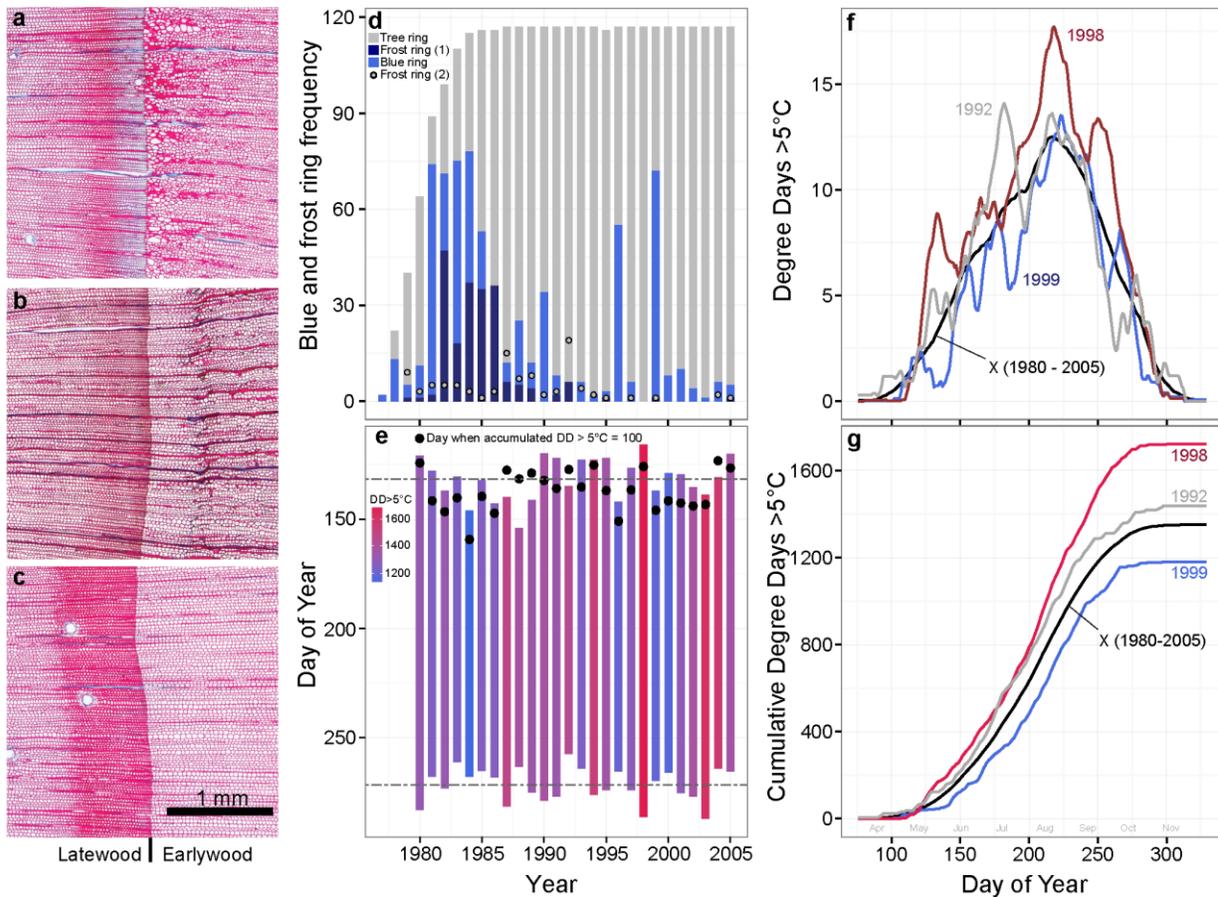


Fig. 7.1. Blue and frost rings as a function of growing degree days. Stained micro sections of tree rings are shown in panels a) to c). Safranin dyes lignin red while Astrablue dyes cellulose blue, so a dual-staining procedure results in a blue ring where a layer of cells lacks lignin. Panel a) shows a blue ring assigned to category 5 out of 5, with a subsequent frost ring in position 1 (i.e., first cells of earlywood). Panel b) shows a frost ring in position 2 (i.e., occurring later in the earlywood). Panel c) shows a normal tree ring growth pattern. Panel d) shows the frequency of blue- and frost rings by year. The grey bar indicates sample size, the light blue bar indicates the frequency of blue-rings, the dark blue bar represents the frequency of frost rings occurring in position 1, and the grey points represent frost rings occurring in position 2. In panel e), the length of the bars represents annual growing season length (days completely above 0°C) as interacting with warmth accumulation (black circle represents the day when the sum of growing degree days above 5°C exceeded 100). The top and bottom horizontal dashed lines indicate the average start and end of the growing seasons, respectively. Panel f) compares the intra-annual signature of growing degree days above 5°C among: a year with high occurrence of blue rings (1999), a year with frost rings in position 2 (1992), a year with no blue or frost rings (1998), and the mean (1980 to 2005). The lines are smoothed using a 15-day running mean. Panel g) shows the cumulative effect of the growing degree signatures shown in panel f.

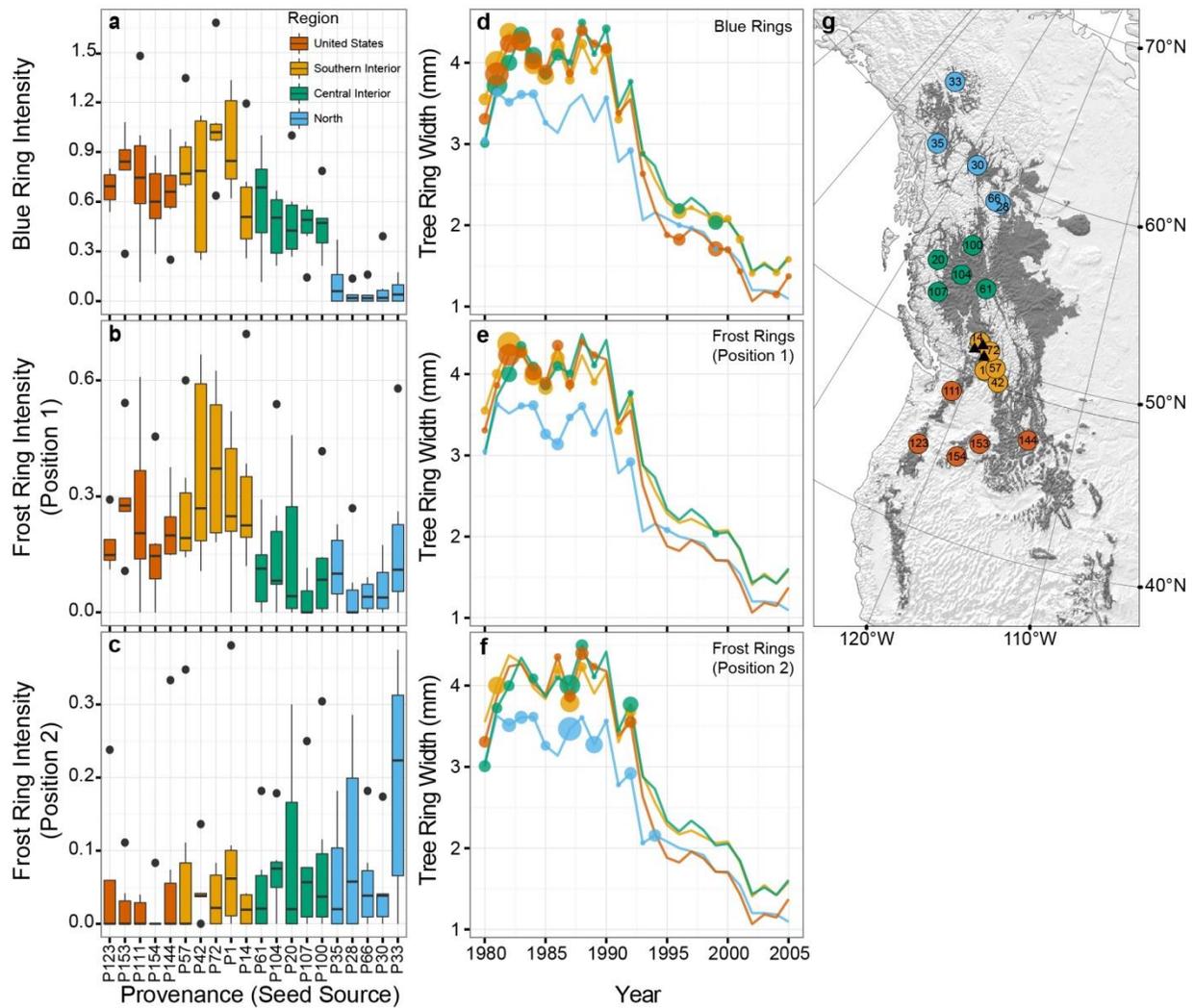


Fig. 7.2. Blue and frost ring intensities by population and growth. Panel a) is a boxplot of blue ring intensity by provenance, sorted by the mean annual temperature of their source climate (warmest on the left, $n=117$). Panels b) and c), respectively, show each provenance's average frost ring intensity in position 1 (initiating earlywood cells) and position 2 (occurring later in the earlywood). Provenances are colored according to their region and labeled according to the provenance trial's notation (see Supplementary Information and panel g). In panels d-f, annual growth in terms of tree-ring widths are shown as interacting with blue rings, frost rings (position 1) and frost rings (position 2), respectively ($n=117$). Each data point represents the average annual response of all provenances in a climatic region. Panel g) displays a map of western North America with dark grey representing the species range of lodgepole pine (*Pinus contorta* Dougl. ex. Loud.), the species used in this study. Colored circles represent seed collection sites (provenances), colored by climatic region and labeled according to the provenance trial's notation (see Supplementary Information). Black triangles show the location of the tree test sites.

Tab. 7.1. Spearman correlation coefficients of blue and frost ring intensities to annual climate variation.

Climate Variable		US	SI	CI	N
Blue Ring Intensity	Growing Season Length (days >0°C)	-0.47	-0.40	-0.45	-0.21
	Start of Growing Season (Julian date)	0.30	0.27	0.31	0.08
	End of Growing Season (Julian date)	-0.35	-0.29	-0.29	-0.22
	Annual Sum of Growing Degree Days >5°C	-0.60	-0.64	-0.50	-0.20
	Mean Annual Temperature (°C)	-0.55	-0.55	-0.44	-0.24
	Mean Summer Temperature (May - Sep, °C)	-0.41	-0.41	-0.42	-0.17
	Mean Temperature in Autumn (Sep - Dec, °C)	-0.48	-0.52	-0.51	-0.42
	Minimum Temperature in Autumn (Sep - Dec, °C)	-0.53	-0.50	-0.50	-0.38
	First Autumn Frost <-5°C (Sep - Dec, Julian date)	0.38	0.40	0.28	0.24
	Sum of Autumn Chilling Degree Days <-5°C (Sep - Dec)	-0.36	-0.37	-0.42	-0.36
	Mean Summer Precipitation (May - Sep, mm)	0.24	0.21	0.28	0.17
Frost Ring Intensity (Position 1)	Start of Growing Season (Julian date)	0.37	0.23	0.32	0.39
	Average Temperatures in April (°C)	0.01	0.04	-0.01	0.10
	Mean Summer Temperature (May - Sep, °C)	-0.34	-0.30	-0.37	-0.26
	Sum of Spring Growing Degree Days >5°C (Mar - Apr)	-0.35	-0.38	-0.26	-0.07
	Growing Season Length (GS; days >0°C) – prev. year	-0.24	-0.11	-0.27	-0.26
	End of Growing Season (Julian date) – prev. year	-0.24	-0.06	-0.27	-0.11
	Mean Annual Temperature (°C) – prev. year	-0.29	-0.31	-0.19	-0.25
	Mean Temperature in Autumn (Sep - Dec, °C) – prev. year	-0.34	-0.23	-0.29	-0.31
	Minimum Temperature in Autumn (Sep - Dec, °C) – prev. year	-0.41	-0.22	-0.37	-0.21
	Sum of Autumn Chilling Degree Days <-5°C (Sep - Dec) – prev. year	-0.13	-0.13	-0.09	-0.14
	Sum of Autumn Chilling Degree Days <-10°C (Sep - Dec) – prev. year	-0.13	-0.12	-0.10	-0.14
Frost Ring Intensity (Position 2)	Start of Growing Season (Julian date)	0.13	0.06	0.17	0.07
	Sum of Spring Growing Degree Days >5°C (Mar - Apr)	0.20	0.12	0.05	0.04
	Julian date when Accumulated Degree Days > 5°C exceeds 100	-0.21	0.10	-0.17	-0.07
	Average Temperatures in April (°C)	0.15	0.05	0.05	0.04
	Sum of Autumn Chilling Degree Days <-5°C (Sep - Dec) – prev. year	0.07	-0.01	0.01	0.00

The Benjamini-Hochberg method was used to correct p-values by the number of climate variables. Significance is indicated in bold. Cells shaded light pink highlight positive correlations greater than 0.1 while darker pink highlights cells with positive correlations greater than 0.25. Cells shaded light blue highlight negative correlations lower than -0.1 and dark blue represents correlations lower than -0.25. Variables ending with “-prev. year” indicate this is the correlation to the previous year’s autumn and cold variables.

Tab. 7.2. Spearman correlation coefficients of blue and frost ring intensities to provenance source climate (1961-1990 climate normal).

Climate variable	Frost rings		
	Blue rings	pos. 1	pos. 2
Latitude	-0.81	-0.58	0.54
Longitude	0.72	0.54	-0.58
Elevation (m.a.s.l)	0.11	0	-0.24
Mean Annual Temperature (°C)	0.89	0.70	-0.56
Mean Warmest Month Temperature (°C)	0.73	0.80	-0.33
Mean Coldest Month Temperature (°C)	0.83	0.58	-0.57
Temperature Difference (°C)	-0.70	-0.37	0.55
Mean Annual Precipitation (mm)	0.27	0.03	-0.34
Mean Summer Precipitation (mm)	-0.42	-0.42	0.09
Annual Heat-Moisture Index	0.47	0.55	-0.24
Summer Heat-Moisture Index	0.48	0.45	-0.14
Degree Days >5 °C (No. of days)	0.83	0.82	-0.42
Degree Days >8 °C (No. of days)	0.76	0.78	-0.42
Degree Days <0 °C (No. of days)	-0.84	-0.58	0.60
Degree Days <18 °C (No. of days)	-0.89	-0.70	0.57
Number of Frost Free Days	0.84	0.82	-0.52
Beginning of Frost Free Period (Julian day)	-0.67	-0.82	0.28
Frost-Free Period (No. of days)	0.76	0.83	-0.47
Precipitation As Snow	-0.02	-0.34	-0.15

Bold values indicate significance after correcting p-values for the number of climate variables with the Benjamini-Hochberg method. Cells shaded light pink highlight positive correlations greater than 0.1; dark pink signifies positive correlations greater than 0.25. Cells shaded light blue highlight correlations lower than -0.1 and dark blue cells are those with negative correlations lower than -0.25.

7.8 Supplementary Information

Tab. 7.S1. Climate of provenance origin

Region	Prov.	Lat.	Long.	Elev.	MAT	MWMT	MCMT	TD	MAP	MSP	AHM	SHM	CMD
N	33	63.30	-136.47	876	-4.3	13.8	-24.6	38.4	423	249	14	55	131
N	30	59.98	-128.55	640	-2.7	14.5	-23.5	38	449	236	16	62	197
N	35	59.80	-133.78	789	-0.4	12.6	-15	27.6	361	186	27	68	203
N	28	58.67	-124.17	762	-1.3	13.4	-15.4	28.8	689	491	13	27	7
N	66	58.65	-124.77	1173	-1.7	10.8	-13.6	24.4	675	482	12	22	0
CI	100	55.80	-124.82	762	1.3	13.6	-12	25.6	522	251	22	54	181
CI	20	54.13	-127.23	937	1.8	12.7	-10.4	23.1	551	243	21	52	208
CI	104	54.02	-124.53	732	2.7	14.3	-10.9	25.2	506	236	25	61	267
CI	61	53.87	-121.80	838	2.7	14.1	-10.1	24.2	750	331	17	43	155
CI	107	52.50	-125.80	1311	1.0	10.7	-9.4	20.1	822	209	13	51	211
SI	14	50.97	-120.33	1059	3.9	15	-7.6	22.6	466	221	30	68	304
SI	72	50.70	-119.18	777	5.7	17.1	-6.3	23.4	735	312	21	55	256
SI	57	49.90	-118.20	579	5.9	17.4	-5.8	23.2	659	258	24	67	329
SI	1	49.58	-119.02	1006	4.6	15.9	-6.5	22.4	547	226	27	70	319
SI	42	49.18	-117.58	998	5.3	16.9	-6.1	23	858	285	18	59	265
US	111	47.78	-120.93	762	7.4	17.3	-1.5	18.8	1535	237	11	73	261
US	144	46.67	-113.67	1524	4.6	16	-5.7	21.7	408	189	36	85	426
US	153	45.63	-117.27	1311	5.7	16.2	-3.9	20.1	510	217	31	75	439
US	154	44.53	-118.57	1494	6.5	17.1	-2.6	19.7	676	167	24	102	491
US	123	44.38	-121.67	1006	7.4	16.8	-1	17.8	427	73	41	230	692

Climate variables are averaged across the 1961-1990 normal period, derived from ClimateWNA, version 4.62 (Wang et al., 2006). *Region* refers to the climatic region to which each provenance is assigned, where: North is abbreviated to “N”; Central Interior is shortened to “CI”; Southern Interior is abbreviated to “SI”; and “US” indicates seed sources from the United States of America. *Prov* refers to the number assigned to each seed source from the Illingworth provenance trial¹. Latitude (*Lat.*) and longitude (*Long.*) are provided in decimal degrees, while elevation (*Elev.*) is provided in meters above sea level. Temperature variables include: mean annual temperature (*MAT*, °C), mean warmest month temperature (*MWMT*, °C), mean coldest month temperature (*MCMT*, °C), and temperature difference (*TD*, °C). Precipitation variables include: mean annual precipitation (*MAP*, mm), and mean summer precipitation (*MSP*, mm). Moisture variables include: annual heat-moisture index (*AHM*), summer heat-moisture index (*SHM*), and climate moisture deficit is given in mm (*CMD*, mm).

Tab. 7.S2. Climate of the three planting sites

Site	Lat.	Long.	Elev.	MAT	MWMT	MCMT	TD	MAP	MSP	AHM	SHM	CMD
CHUW	50.58	-120.62	1430	3.1	14	-7.1	21.1	482	241	27.2	58.1	232
COMM	50.92	-120.07	1370	3.1	13.9	-7.5	21.4	522	256	25.1	54.3	217
EQUI	50.37	-119.6	1370	3.3	14.2	-7.3	21.5	686	301	19.4	47.2	188

All climate variables are averaged across the 1961-1990 normal period. *Site* refers to the abbreviated site name from the Illingworth lodgepole pine provenance trial¹: CHUW is the abbreviation for Chuwels Lake, COMM stands for Community Lake, and EQUI stands for Equises Creek. Latitude (*Lat.*) and longitude (*Long.*) are provided in decimal degrees, while elevation (*Elev.*) is provided in meters above sea level. Temperature variables include: mean annual temperature (*MAT*, °C), mean warmest month temperature (*MWMT*, °C), mean coldest month temperature (*MCMT*, °C), and temperature difference (*TD*, °C). Precipitation variables include: mean annual precipitation (*MAP*, mm), mean summer precipitation (*MSP*, mm). Moisture variables include: annual heat-moisture index (*AHM*); summer heat-moisture index (*SHM*); and climate moisture deficit is given in mm (*CMD*, mm). Climate data derived from ClimateWNA v 4.62 (Wang et al. 2006).

8 Limits to Lodgepole Pine Seed Transfer

8.1 Abstract

Planting pre-adapted seed sources originating from warmer, drier regions within a species distribution has been proposed as a solution to help overcome the consequences of forest-climate maladaptation. To guide future reforestation work, it is essential to assess the response of locally-adapted tree populations to climate shifts and variability. We quantify maladaptation under southward seed transfers simulating warming by analyzing inter-annual and intra-specific growth responses in a multi-decade provenance trial of lodgepole pine (*Pinus contorta* Doug. Ex. Loud). We then analyze northward seed transfers to determine limits to assisted migration as a solution. When transferred southward, northern populations show higher growth compared to growth in their local environments. Although this would imply improved growth under warming, this trial underrepresented the sensitivity of these populations to climatic extremes, projected to increase in the north in the future. A low-risk intervention would involve planting a percentage of provenances from British Columbia's central interior in these northern areas, as these genotypes performed well in those areas over the duration of the study, with no undue damage or losses to cold. Assisted migration of genotypes from the far south or southern interior of British Columbia to the central interior, however, cannot be recommended due to growth losses linked to cold. The risk of implementing assisted migration in the central areas of the lodgepole pine range thus appears to currently outweigh the risks of status quo reforestation: a population-oriented approach to assisted migration is recommended for lodgepole pine, and may be suitable for other wide-ranging conifers as well.

8.2 Introduction

As climates warm and extreme climatic events occur more frequently, historic adaptation of forests to local environments may result in forest maladaptation under climate change (Davis & Shaw, 2001; Aitken et al., 2008). The current rate of climate change is causing a mismatch between the new climates materializing and the historic climates to which long-lived trees have adapted over generations (Hamann & Wang, 2006; Aitken et al., 2008; Gray & Hamann, 2013; Williams & Dumroese, 2013). While trees have developed high plasticity to cope with changing environments (Alberto et al., 2013), some of these abilities may be exceeded under relatively rapid change (Duputié et al., 2015). Furthermore, it is unlikely that the ability of trees to evolve could match the pace of current climate change given long generation times (Savolainen et al., 2004; St Clair & Howe, 2007). This is expected to be a concern throughout species ranges – not only at the species trailing edge. This is due to local adaptation of populations to historic climates – each population is projected to have its own trailing edge (O’Neill et al., 2008).

Forest maladaptation under climate change is a concern because it can reduce productivity and vigour, increase susceptibility to pests and pathogens, and ultimately lead to increased mortality and carbon feedbacks (Bonan, 2008; Kurz et al., 2008). This is a concern around the world for many tree species, especially in temperate and boreal environments (St Clair & Howe, 2007; Aitken et al., 2008; Alberto et al., 2013; Isaac-Renton et al., 2014; Bansal et al., 2015a, 2015b; Aitken & Bemmels, 2016; Montwé et al., 2016; Frank et al., 2017). One proposed solution involves expanding the historic geographic-based seed transfer guidelines to accommodate projected future climates, also known as climate-based seed transfer (O’Neill et al., 2017). This represents a type of assisted migration known as *assisted gene flow* (Aitken & Bemmels, 2016) or *assisted population migration* (Pedlar et al., 2012). Assisted gene flow

involves the transfer of “pre-adapted” seeds from warmer, drier areas within a species range are planted after harvesting in anticipation of future warming (Aitken & Bemmels, 2016). The objective is to re-align the trees with their suitable climate spaces for at least part of the forest lifespan (forest rotation) (Gray & Hamann, 2013). Climate-based seed-transfer is already being implemented to a minor degree in Canada’s western-most province, British Columbia (BC), where elevation seed-transfer limits were relaxed for western larch in 2008 (Klenk & Larson, 2015). Greater program adoption is expected in 2018, and similar programs are being considered for other Canadian provinces (Pedlar et al., 2011).

Before implementing climate-based seed transfer, it is important to quantify tree population adaptation to make accurate predictions of forest responses and minimize risks. Risks of inaction involve the potential for an increasing mismatch of tree populations with their climatic optimum, increasing susceptibility to extremes, and reducing vigour. On the opposite end of the spectrum, risks of expanding seed transfer guidelines include inadvertently introducing maladaptation by overshooting the suitable transfer distance, especially since frost-sensitive seedlings must growth through temporarily cooler climates to reach the expected future optimum. Such a misalignment of populations and their climate optima could cause cold damage and resulting growth losses (e.g., Chapter 7, Benito-Garzón et al., 2013a, 2013b; Bansal et al., 2015b).

At the same time, risks of expanding seed transfer may be minimized by incorporating only a minor percentage of such seed sources, known as composite provenancing (e.g., Aitken et al., 2008; Ukrainetz et al., 2011; Prober et al., 2015). After forest harvesting, planting a composite selection of seed sources after harvest may also have a relatively minor effect on the landscape relative to natural areas and natural regeneration. This may be especially true as it may

only be suitable for a minor number of tree species with large north-south ranges, and may be further limited by local conditions such as soils. Nevertheless, social acceptance of assisted gene flow has not always been positive: While public and policy leaders were generally accepting of some human intervention in reforestation, the level of acceptance for using non-local seed sources was not (Hajjar et al., 2014). There is a sense that forests could be made more adaptable and resilient through diversification with local seed sources only (Hajjar et al., 2014). Therefore, it is important to study the risks associated with both action and inaction – for the benefit of science and management for the benefit of the public relying on them, as well from a social acceptability perspective.

Studying climate adaptation and potential maladaptation of forest tree populations is facilitated with provenance trials. These trials are reciprocal transplant experiments that involve collecting seeds from across a species distribution and growing them in multiple common gardens that represent a range of climate conditions. These experiments can also be used to assess responses to climate warming since transferring seeds from northern environments to southern planting sites represents an increase in temperature similar to climate projections (Matyas, 1994; Schmidting, 1994). At the same time, transferring southern seeds to northern planting sites can be used to assess simulated assisted gene flow since seedlings planted under relatively cooler conditions must survive until their optimal climate conditions materialize in the future. Tree-ring methodologies can be further applied within this experimental structure to retrospectively analyze differences in tree population growth response to different environmental conditions (Montwé et al., 2016). The resulting framework can provide a means to predict response to climate warming and climate variability; better predictions provide a better foundation for which to manage these systems under such environmental change.

This study focuses on lodgepole pine (*Pinus contorta* Dougl. ex Loud.), which is one of the most widespread tree species in western North America. This species shows high ecological amplitude, covering over 1.3 million km² (Schroeder et al., 2010) in diverse climates. The more predominant interior variety (*P. contorta* var. *latifolia*) is particularly important to British Columbia (BC) as an ecological foundation species. It further sustains a large portion of the forestry industry in western Canada, which supports communities by generating hundreds of thousands of jobs (Hajjar et al., 2014). In recent years, however, BC's forests – and the communities relying on them - have been affected by an unprecedented mountain pine beetle epidemic (Kurz et al., 2008). Lodgepole pine nevertheless remains ecologically suitable for re-planting in these areas with some reconsideration of seed zones (O'Neill et al., 2008). At 125 million seedling requests for lodgepole pine in interior BC, this represents approximately half of requested seedlings for reforestation in the province (Province of British Columbia, 2016). After harvesting, planting the most suitable lodgepole pine populations for a warming climate may therefore help maintain forest health and productivity, and the people relying on these forests.

Here, we gauge the overall suitability for overcoming potential lodgepole pine maladaptation. We build on a wealth of knowledge on lodgepole pine genetic structure, adaptation to climate, and seed selection (e.g., Chapters 3, 6, 7, Illingworth, 1978; Rehfeldt, 1983, 1987, 1988, 1980, Rehfeldt et al., 1999, 2001; Guy & Holowachuk, 2001; Wang et al., 2006, 2010; Ying & Yanchuk, 2006; O'Neill et al., 2008; McLane et al., 2011a, 2011b; Yeaman et al., 2016; Liepe et al., 2016; Montwé et al., 2016; Conlisk et al., 2017; MacLachlan et al., 2017). We first evaluate the extent of possible climate maladaptation as well as the success of potential assisted gene flow transfers. We analyze annual and total growth responses of 23 seed sources under northward and southward seed transfers. This work expands on recent work testing

responses of lodgepole pine populations on three Southern Interior planting sites (Chapters 3, 6, and 7) by incorporating 6 additional planting sites across the species distribution to test assisted gene flow scenarios up to the northern extremes. We further discuss the risks of assisted gene flow and weigh these against the risks of inaction.

8.3 Methods

Experimental Design

The Illingworth lodgepole pine provenance trial was established in 1974 (Illingworth, 1978) by the British Columbia Ministry of Forests with 153 seed sources tested at 60 planting sites. Several of these planting sites incurred high mortality due to the mountain pine beetle outbreak in 2006; we were permitted to fell dead trees in 2013 and 2014. Within this large trial, we developed an experimental design that allowed us to sample 23 provenances representing four climatic regional ‘populations’ of western North America tested on 9 planting sites. The four meta-populations include 5 Northern provenances (N); 6 provenances from BC’s Southern Interior (SI); 6 provenances from BC’s Central Interior (CI); and 6 provenances from the far south (FS) of the species distribution (Fig. 8.1c, Tabs. 8.S1, 8.S2). The planting sites contained two blocks which acted as replicates, except one northern site (WATS), which contained 4 blocks. Within blocks, each provenance was planted in 9-tree plots arranged in a randomized complete block design. We aimed to cut and measure four trees per provenance per block on southern and central planting sites, although sometimes not all trees were available.

Field and Tree Ring Measurements

The analyses presented here are based on 511 trees sampled from southern interior planting sites in the 2013 summer field season, 339 trees in the central interior planting sites

sampled in the 2014 summer field season, and 218 trees measured in the Northern planting sites in 2014. On central and southern planting sites, we used an Esilon tape to measure tree height after each dead tree was felled and de-limbed. Most trees on northern sites had not died due to the mountain pine beetle epidemic, so we obtained increment cores and measured tree heights instead. On northern planting sites, tree height was measured with a Vertex hypsometer (Vertex IV, Haglof, Madiso, USA). Stem disks at diameter at breast height (1.3 m) were collected for trees on central and southern sites; increment cores were obtained on northern planting sites. All stem disks were sanded and scanned (ScanMaker 9800XLplus; Microtek, Hsinchu, Taiwan). Tree-ring widths were measured for four radii per disk using Windendro software, version 2016 (Regent Instruments Inc, 2016): Automatic measurements were manually inspected and adjusted. At the northern sites, the measurements for each tree were derived from two radii from the increment core.

Statistical Analyses

All statistical analyses were conducted in the R programming environment and *ggplot2* was used to generate all figures (Wickham, 2009; R Core Team, 2017). Statistical testing of differences among the four populations in terms of cumulative growth relied on analysis of variance as implemented with the *anova* command. This was based on a generalized linear mixed effects model developed with the *lmerTest function* of the *lmerTest* package (Kuznetsova et al., 2016). In the model, regional meta-populations and planting site regions were specified as fixed effects and random effects included site, block, and provenance. To conduct (post-hoc) multiple comparisons, the *ghlt* function from the *multcomp* package were used (Hothorn et al., 2016). The Benjamini and Hochberg method was used to reduce the false discovery rate (Benjamini & Hochberg, 1995). To investigate the relationships of provenance growth relative to

the transfer distances (climatic and geographic) of provenances to the test sites where they were grown, we calculated least square means to account for the imbalanced design. Least square means were estimated using the *lsmeansLT* function from the *lmerTest* package. To remove the age trend from tree-ring widths, we fit a spline to each tree's data across the 31-year period to express annual growth increments as anomalies from the fitted curve. This detrending procedure was carried out with the *detrend* command from the *dplR* package (Bunn, 2008). To calculate geographic transfer distances in kilometers from the provenances and sites' geographic positions, the *distVincentyEllipsoid* function within the *distm* command was used from the *geosphere* package (Hijmans et al., 2016). All climate variables for the observed study period as well as future climate projections were derived from ClimateWNA version 5.40 (Wang et al., 2016).

8.4 Results

Local Performance: Testing Historic Seed Transfer Guidelines as a Baseline

Although northern seed sources grown on northern planting sites may be considered “local seed sources”, these nevertheless represent transfer distances. Distances range from 19 km to 763 km (mean = 297 km; Figs. 8.1a, b, c, Tab. 8.1), depending on the site-provenance combination. These distances are associated with climatic differences ranging from -2°C to +3°C in mean annual temperature (mean = -0.1°C; 8.1a, b, Tab. 8.2). On northern planting sites, these northern provenances grew taller than the southern interior and far southern populations (Fig. 8.2g, Tab. 8.3), but were not significantly different from central interior populations ($p = 0.787$, Tab. 8.S3). Northern provenances also had consistently greater tree-ring growth (Fig. 8.2a) and cumulative diameter (Fig. 8.2b) over the length of the study compared to southern interior and far southern seed sources.

Central interior provenances grown in central interior planting sites also represented geographic and climatic transfer distances. These ranged geographically from 15 km to 395 km (mean = 187 km; Figs. 8.1b, c, Tab. 8.1) and climatically from -1.2°C to 1.3°C (mean = 0.05°C; Figs. 8.1a, b, Tab. 8.2). Central interior seed sources performed well (Fig. 8.2c, d, h); they were significantly better than seed sources from the north ($p < 0.01$) or far south ($p = 0.025$, Tab. 8.S3). Central interior seed sources were not, however, significantly different from southern interior seed sources on central interior planting sites ($p = 0.964$).

On southern interior planting sites, southern interior provenances perform well (Figs. 8.2e, f, i, Tab 8.4). These provenances were not significantly different from central interior seed sources ($p = 0.467$, Tab. 8.S3), but were significantly different from northern and far southern populations (both $p < 0.01$, Tab. 8.S3). The transfer distances tested here range from 19 km to 267 km (mean = 123 km; Figs. 8.1a, b, Tab. 8.1) and a difference in mean annual temperature of -4.4°C to -1.2°C (mean -2.8°C; Tab. 8.2). Although geographic transfers are small, temperature transfers are larger because provenances were sourced from lower elevations than the planting sites: the difference in elevations ranged from 303 m to 791 m, with a mean of 476 m (Tab. 8.1).

Southward Transfers: Responses under Simulated Warming

On central interior planting sites, northern seed sources are transferred southward by 317 km to 1239 km, depending on provenance and planting site combinations (mean = 698 km, Fig. 8.1c, Tab. 8.1). These distances correspond to climatic transfers of +2.4°C to +6.4°C in mean annual temperature (mean = +4.0°C, Figs. 8.1a, b, Tab. 8.2). Transfer distances on southern planting sites represent a more severe geographic- and climate-based seed transfer (range = 901 km to 1758 km, mean = 1220km; range = 3.0°C to 7.2°C, mean = 4.7°C; Figs. 8.1a, b, c, Tabs.

8.1, 8.2). Transfers to both central and southern interior planting sites simulate the level of warming expected under high-magnitude warming. These climatic transfers in fact exceed warming predictions for the year 2085 under a pessimistic warming scenario, i.e., a Representative Concentration Pathway (RCP) 8.5 scenario. The RCP 8.5 scenario projects an increase of up to 5.3°C mean annual temperature in northern areas (mean of provenances and planting site warming = 4.6°C; Tab. 8.4) by the year 2085.

Although height and diameter growth of northern seed sources is enhanced on central and southern interior planting sites relative to growth on northern sites (Figs. 8.2g, h, i), their annual growth rates show continually declining performance after some years in particular (Figs. 8.2c, d, e, f). Relative to other provenances, the northern seed sources also show low growth when planted in the central interior and southern interior planting sites (Figs. 8.1a, b, c, 8.2c, d, h, e, f, i, Tab. 8.3). At central planting sites, northern provenances were found to be significantly different in terms of height in 2005 to the provenances from the central and southern interior of BC (both $p < 0.01$, Fig. 8.2h, Tab. 8.S1), but not different from the provenances from the far south of the range ($p = 0.989$, Tab. 8.S1). The height in 2005 of these northern seed sources was not found to be significantly different from their responses on southern planting sites (Fig. 8.2h, i, Tab 8.S3). On these southern interior planting sites, however, northern provenances were found to have significantly lower height from all other populations ($p < 0.01$, Fig. 8.2i, Tab. 8.S3).

When central interior provenances are planted on southern interior planting sites, they perform well across the duration of the provenance trial, leading to competitive height and diameter growth (Figs. 8.2e, f, i, Tab. 8.3). Their height (in 2005) was not significantly different from the southern interior seed sources ($p < 0.01$, Tab. 8.S3). These provenances were transferred

geographically by 348 km to 698 km (mean = 539 km, Fig. 8.1c, Tab. 8.1), corresponding to changes in mean annual temperature of -0.6°C to 2.1°C (mean = 0.8°C, Figs, 8.1a, b, Tab. 8.2).

Northward Transfers: Growth under Simulated Assisted Gene Flow

On northern planting sites, provenances from the central interior of BC were transferred - 2.1°C to -5.6°C in mean annual temperature (mean = -3.8°C) across distances of 340 km to 864 km (mean = 623 km; Figs. 8.1a, b, c, Tabs. 8.1, 8.2). The central interior seed sources perform well on northern planting sites across the duration of the study, leading to competitive height and diameter growth with local sources (Figs. 8.2a, b, g, Tab. 8.3). The diameter growth of central interior provenances was not significantly different from northern provenances ($p = 0.787$, Tab. 8.S3). On northern planting sites, seed sources from the southern interior of BC were transferred 940 km to 1410 km (mean = 1201 km, Tab. 8.1), representing changes to mean annual temperature from -5.4°C to -9.4°C (mean = -7.4°C, Tab. 8.2). These seed sources did not perform well, and led to poor overall height and diameter growth (Fig. 8.1a, b, g, Tab. 8.3). At northern planting sites, southern interior provenances were significantly different from northern, central interior and far southern provenances ($p < 0.01$, Tab. 8.S3).

On planting sites in the central interior of BC, the southern interior seed sources performed similarly well as locally adapted seed sources. This represents a geographic transfer distance of 440 km to 848 km (mean = 628 km) and a climate transfer distance of -2°C to -5°C in mean annual temperature (mean = -3.5°C, Figs. 8.1a, b, c, Tabs. 8.1, 8.2). Southern interior populations maintained competitive growth rates over the duration of the study (Figs. 8.2c, d, h, Tab. 8.3) and height in 2005 was not significantly different from local seed sources ($p = 0.964$). Early in the provenance trial, seed sources from the far south of the range are competitive with

local sources (Fig. 8.2a, b). After almost 20 years' of growth, however, diameter growth losses mirror those observed on southern planting sites. While their growth had initially been strong, this drop led to significantly lower height growth by the end of the study compared to the central and southern interior populations ($p = 0.024$ and <0.01 , respectively, Fig. 8.2h, Tab. 8.3). The transfers of the far south population to central areas represents a northward geographic transfer distance of 761 km to 1519 km (mean = 1151 km, Tab. 8.1, Figs. 8.1a, b, c) and a climate-transfer distance of -2.5°C to -5.5°C mean annual temperature (mean = -4.3°C , Tab. 8.2). On southern interior planting sites, the far southern seed sources initially are top competitors despite being transferred northward by 304 km to 960 km and -1.7°C to -4.9°C (mean = 647 km; -3.7°C , Tabs. 8.1, 8.2).

Climate-Growth Correlations: Planting Sites Draw Out Adaptations

Correlations of height in 2005 to climate of seed origin are shown in Fig. 8.3a, as tested on the 9 planting sites. In central and southern interior planting sites, height of provenances is positively correlated to temperature and relative humidity: Generally, populations from warmer areas with more humidity perform better at these planting sites. However, when tested in the colder, northern planting sites, provenance performance is linked negatively to temperature and temperature difference. Seed sources from cold areas with high temperature differences perform best on northern planting sites. Correlations of growth, in terms of de-trended tree-ring widths, to climates of the planting sites are shown in Fig. 8.3b. These correlations are generally weak ($r < 0.2$) relative to correlations to provenance climate.

8.5 Discussion

Leading Edge of the Lodgepole Pine Range: Risks of Inaction Outweigh Risks of Assisted Gene Flow

Northern seed sources show higher growth in warmer planting environments relative to growth in northern areas. This result may at first appear to provide evidence that northern forests will benefit under warming, as predicted by other researchers (Hamann & Wang, 2006; Wang *et al.*, 2006; Bonan, 2008; Savolainen *et al.*, 2011; Pedlar & McKenney, 2017; Hember *et al.*, 2018). However, these transfers from northern to southern growing environments could represent a release from lower levels of radiation, which will not change in northern environments under climate change. Furthermore, northern provenances are more susceptible to climate variability in these environments as revealed by inter-annual growth. This may become increasingly problematic since climate change is also associated with increased climate variability (Meehl, 2004; Dai, 2011; Christidis *et al.*, 2014; IPCC, 2014). Climatic extremes can be more consequential to plants than climate warming (Reyer *et al.*, 2013).

Recent work has also shown that northern provenances are most vulnerable to climatic extremes such as drought and cold events (Chapters 3, 6, 7). These provenances show low drought tolerance (Montwé *et al.*, 2016), which could further limit regeneration under increasingly dry conditions (Hogg & Wein, 2005). In addition, our analyses are based on responses up to 2005 only; it is possible that responses here under-represent negative effects of warming to northern provenances because the study period does not capture recent warming and severe droughts that have since occurred. Climate change may also be causing a mismatch in phenology and cold hardiness. In a study on seedlings, Liepe *et al.* (2016) report that northern lodgepole pine genotypes were cold hardy but burst bud early. This early bud break could cause

a mismatch due to climate change and phenology: In Chapter 7, we found that these populations will likely experience increased frost damage as warming winters and higher variability combine with northern populations' low heat-sum requirements for flushing. Thus, increased growth in northern environments would not be expected to materialize based on warming, and would instead be limited by increasing occurrences of climatic extremes. Altogether, these areas appear to be most at-risk under climate warming.

When seed sources from the southern interior of BC are planted on northern planting sites, they perform poorly across the duration of the provenance trial, indicating limits to seed transfer. However, when populations from the central interior of British Columbia are grown on southern interior planting sites, these provenances perform just as well as the local northern provenances. No major annual growth losses are incurred relative to northern provenances; we find no evidence of any major drop after possible cold events. In an earlier study on southern interior planting sites, we also found these populations to show high physiological plasticity to changing environments (Chapter 6), likely contributing to their positive performance in temporarily cooler environments. Therefore, the provenances from the central interior of BC could be tolerant enough to cold to be transferred under assisted gene flow. Thus, in northern areas, the costs of inaction (status quo reforestation) likely outweigh the risks involved in assisted gene flow only if judiciously applied. Currently, however, forests in the far north (e.g. Yukon) do not contribute substantially to Canada's forest industry. Since assisted gene flow usually involves seed selection at the time of re-planting after harvest, assisted gene flow under a typical reforestation scenario may not in fact be realistic.

Central Interior of BC: Judicious Assisted Gene Flow Implementation Possible

At central interior planting sites, southern interior seed sources perform equally as well as their local counterparts. No undue annual growth losses are incurred; these populations appear to be sufficiently cold- and drought-adapted to tolerate moderate northward movement. Both central interior and southern interior populations were previously found to have moderate drought tolerance (Montwé et al., 2016) linked to high physiological plasticity. The present study also indicates that the risks of transferring seed sources from the southern interior of BC to the central interior of BC may be low. Thus, incorporating a percentage of southern interior populations slightly northward across the gentle geographic clines to central interior areas could be low-risk, but may not be currently necessary. However, the same is not true for provenance transfers from the far south of the lodgepole pine range. Provenances from the warm, dry areas in the far south had initially been top growth performers on central and southern interior sites. After approximately 20 years of growth, however, these provenances begin to show substantially lower growth than the other populations and continued to perform poorly until the end of the duration of the study.

8.6 Conclusions

We emphasize that there is no universally suitable seed geographic or climate transfer distance for assisted migration. Here, we show that there may be some benefits to be gained by implementing assisted gene flow in the far north, at the leading edge of the range. This could enhance forest resilience to increasing climate variability. Transferring seed from the southern interior to the central interior of BC could be a low-risk strategy to adapt to general warming, but would not increase resilience to cold or drought (Chapters 3, 6, 7). Transferring populations from

the far south of the lodgepole pine range northward to the southern or central interior of BC cannot be recommended. Therefore, a cautious and population-oriented approach is recommended under climate-based seed transfer. While it appears that assisted gene flow could be low risk under some situations and higher risk in others, the final evaluation must ultimately be made by the forest practitioners based on their professional knowledge of local site conditions.

8.7 References

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8.8 Figures

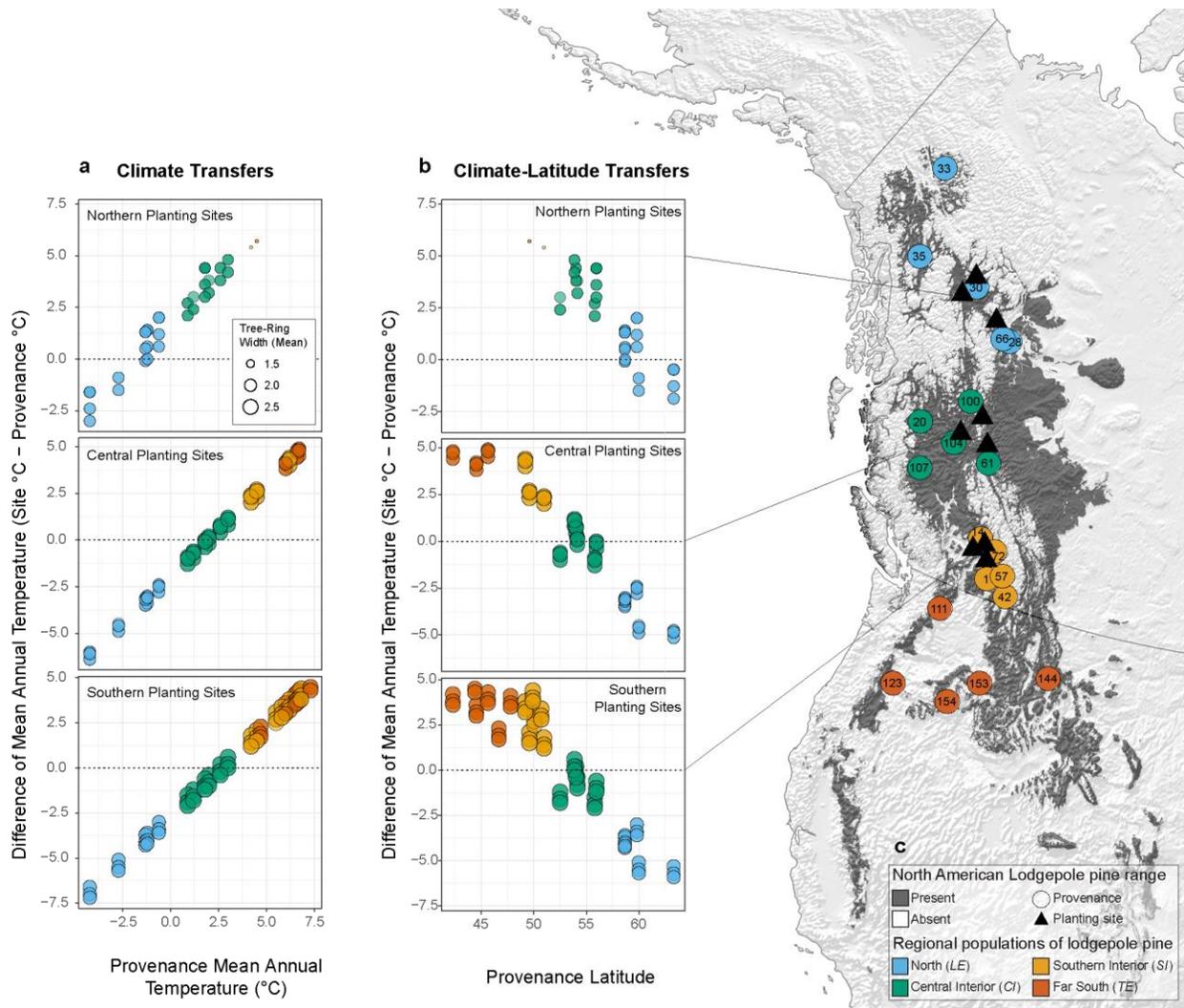


Fig. 8.1. Experimental design and transfer distances: 23 provenances tested on 9 planting sites (panel c). Provenances are numbered according to the original Illingworth provenance trial design (Illingworth, 1978). Provenances are grouped according to four regional populations, where northern seed sources (North, Leading Edge or *LE*) are shown in blue; central interior provenances (CI) are illustrated in green; southern interior (SI) seed sources are shown in yellow; and provenances from the far south of the lodgepole pine range (Far South, Trailing Edge or *TE*) are shown in orange. Climate transfer functions of provenances tested at different planting sites are shown in panel a). The temperature difference associated with latitudinal transfers is shown in panel b). Dot size corresponds to average tree ring width growth (mm).

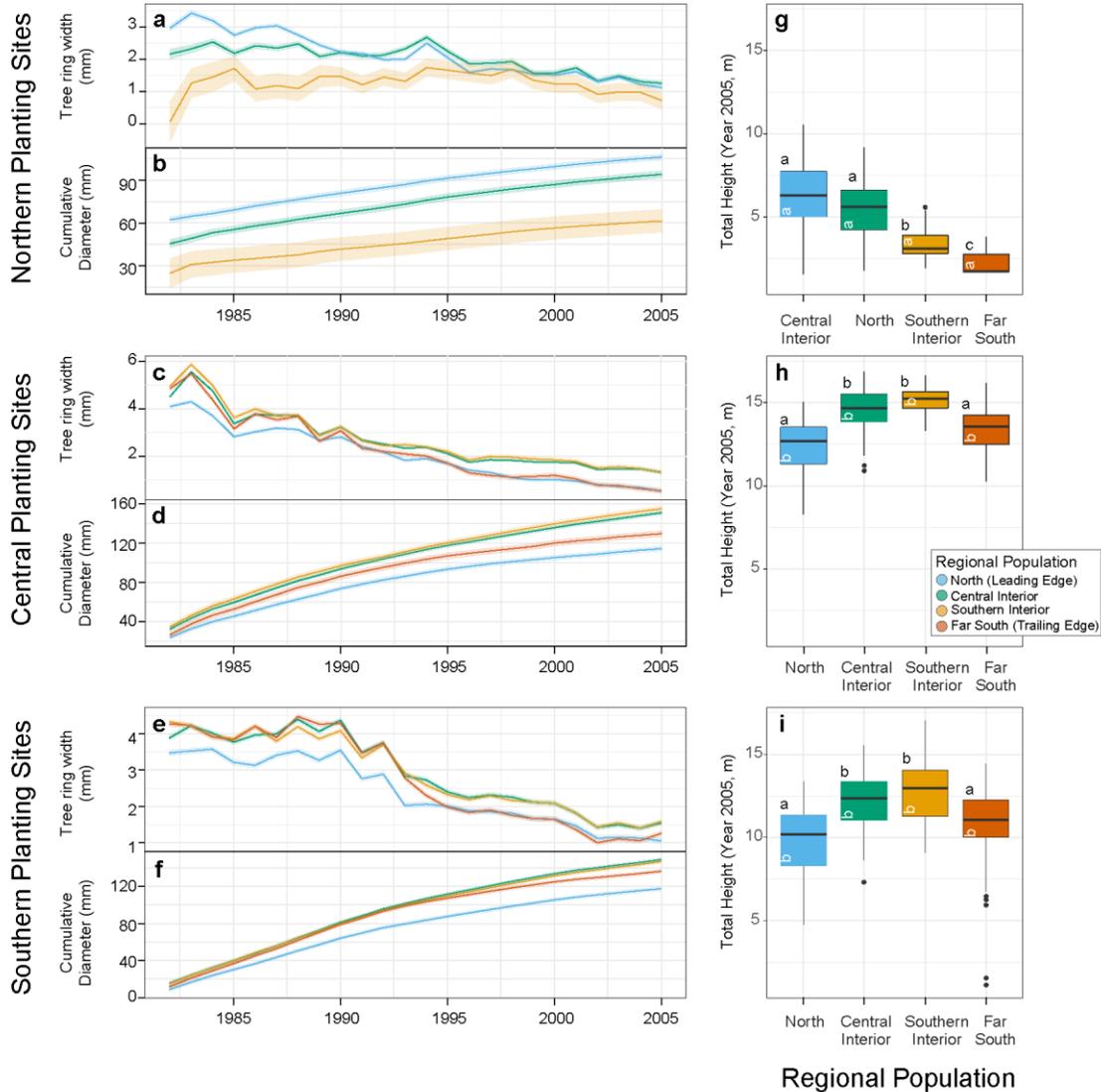


Fig. 8.2. Growth of four lodgepole pine regional populations on planting sites in three areas in the lodgepole pine distribution: The top third (panels a, b, g) represent growth on northern planting sites; the graphs in the center (panels c, d, h) represent growth in central interior planting sites; the bottom panels (e, f, i) represent growth on southern interior planting sites. Tree-ring widths (mm) over time are shown in panels a, c, e. Cumulative diameter (mm) are shown in panels b, d, f. Total height growth (m) in year 2005 (age 33) are shown in panels g, h and i: changes in black letters represent significant differences among populations within each regional planting site while white letters represent significant differences of each population among the regional planting sites.

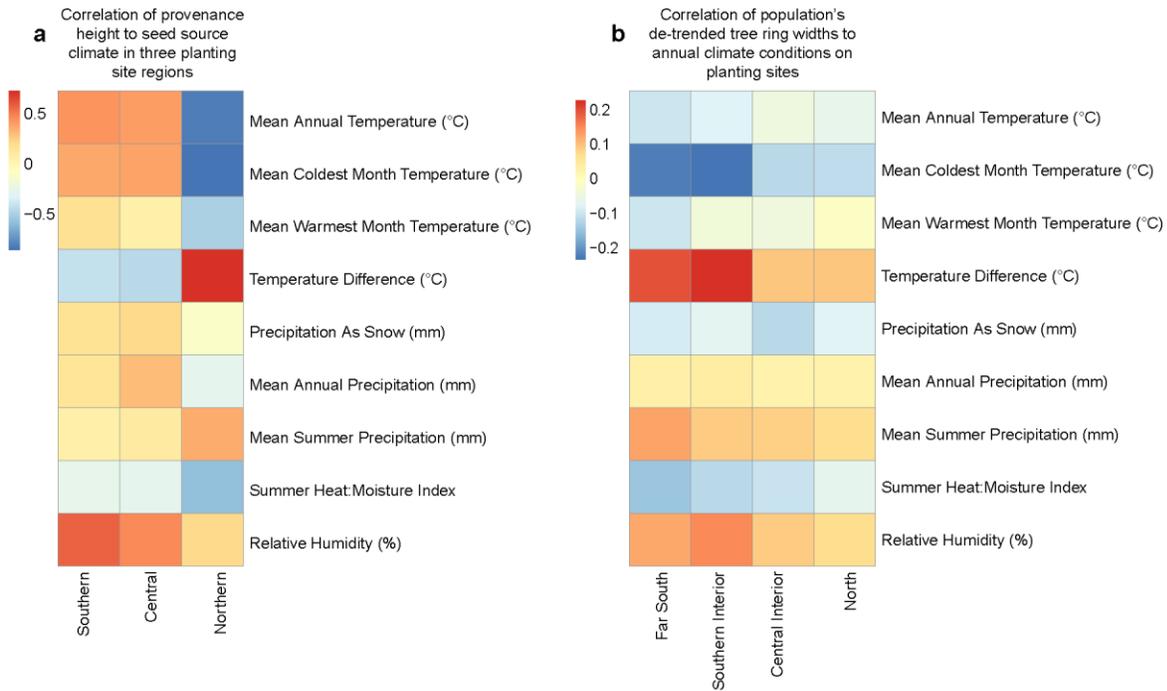


Fig. 8.3. Heatmaps showing climate-growth correlations. a) Correlations of total height in year 2005 (age 33) of provenances grown in three areas in the lodgepole pine range (Southern, Central and Northern planting sites). b) Correlations of de-trended tree-ring widths of regional populations across all planting sites.

8.9 Tables

Tab 8.1: Elevation and geographic transfer distances of lodgepole pine (*Pinus contorta* var. *latifolia*) provenances to planting sites within the Illingworth lodgepole pine provenance trial (Illingworth, 1978).

Population	Provenance	Elevation Difference (m)									Geographic Distance (km)								
		Northern Sites			Central Interior Sites			Southern Interior Sites			Northern Sites			Central Interior Sites			Southern Interior Sites		
		BLUE	M451	WATS	CARP	DOGC	WEST	CHUW	COMM	EQUI	BLUE	M451	WATS	CARP	DOGC	WEST	CHUW	COMM	EQUI
Northern	P28	-32	338	-62	28	188	-92	668	608	608	309	92	308	459	490	317	929	901	969
Northern	P30	90	460	60	150	310	30	790	730	730	40	206	19	688	684	544	1160	1141	1210
Northern	P33	-146	224	-176	-86	74	-206	554	494	494	553	763	540	1239	1220	1098	1703	1689	1758
Northern	P35	-59	311	-89	1	161	-119	641	581	581	261	471	279	874	837	744	1320	1312	1381
Northern	P66	-443	-73	-473	-383	-223	-503	257	197	197	279	59	281	465	488	321	937	911	980
Central Interior	P100	-32	338	-62	28	188	-92	668	608	608	512	342	532	180	172	70	645	628	698
Central Interior	P104	-2	368	-32	58	218	-62	698	638	638	700	541	723	123	30	209	466	459	527
Central Interior	P107	-581	-211	-611	-521	-361	-641	119	59	59	836	705	864	302	218	395	418	433	492
Central Interior	P20	-207	163	-237	-147	13	-267	493	433	433	639	531	669	285	186	295	599	603	668
Central Interior	P24	44	414	14	104	264	-16	744	684	684	531	341	547	160	191	15	634	612	681
Central Interior	P61	-108	262	-138	-48	112	-168	592	532	532	795	603	812	110	176	250	374	348	417
Southern Interior	P1	-276	94	-306	-216	-56	-336	424	364	364	1306	1117	1325	619	639	763	160	167	97
Southern Interior	P14	-329	41	-359	-269	-109	-389	371	311	311	1126	940	1146	440	459	586	48	19	84
Southern Interior	P15	-337	33	-367	-277	-117	-397	363	303	303	1239	1053	1259	553	571	698	91	101	36
Southern Interior	P42	-268	102	-298	-208	-48	-328	432	372	372	1393	1197	1409	705	736	847	268	263	196
Southern Interior	P57	151	521	121	211	371	91	851	791	791	1301	1106	1318	614	645	756	188	175	113
Southern Interior	P72	-47	323	-77	13	173	-107	653	593	593	1190	997	1207	502	532	645	103	67	47
Far Southern	P111	-32	338	-62	28	188	-92	668	608	608	1438	1269	1461	771	761	915	312	354	304
Far Southern	P123	-276	94	-306	-216	-56	-336	424	364	364	1785	1631	1811	1140	1117	1281	693	737	684
Far Southern	P144	-794	-424	-824	-734	-574	-854	-94	-154	-154	1778	1574	1793	1097	1136	1234	672	667	601
Far Southern	P153	-581	-211	-611	-521	-361	-641	119	59	59	1761	1575	1780	1075	1087	1221	604	623	555
Far Southern	P154	-764	-394	-794	-704	-544	-824	-64	-124	-124	1839	1664	1861	1162	1162	1308	690	719	654
Far Southern	P156	-885	-515	-915	-825	-665	-945	-185	-245	-245	2027	1870	2053	1377	1357	1519	920	960	902

Elevation differences are reported in (m), where negative numbers indicate a transfer from higher collection sites to lower planting sites. Geographic transfer distances are reported in kilometers (km). These are calculated as the simple distances between the provenance and planting site, as implemented with the *distVincentyEllipsoid* function within the *distm* command was used from the *geosphere* package in the R programming environment (Hijmans et al., 2016; R Core Team, 2017). The abbreviations of the planting sites correspond to the Illingworth lodgepole pine provenance trial (Illingworth, 1978), where: *BLUE* stands for Blue River, *M451* stands for Mile 451 along the Alaska Highway, *WATS* stands for Watson Lake, *CARP* stands for Carp Lake, *DOGC* stands for Dog Creek, *WEST* stands for Weston Lake, *CHUW* stands for Chuwels Lake, *COMM* stands for Community Lake, and *EQUI* stands for Equises Creek. Climate data derived from ClimateWNA v 5.40 (Wang et al. 2016).

Tab 8.2: Climate transfer distances in terms of mean annual temperature (°C) of all lodgepole pine provenances (*Pinus contorta* var. *latifolia*) to planting sites within the Illingworth lodgepole pine provenance trial (Illingworth, 1978).

Population	Provenance	Northern Sites			Central Interior Sites			Southern Interior Sites		
		BLUE	M451	WATS	CARP	DOGC	WEST	CHUW	COMM	EQUI
Northern	P28	-0.6	0	-1.4	3.4	3	3.1	3.6	4	4.2
Northern	P30	0.9	1.5	0.1	4.9	4.5	4.6	5.1	5.5	5.7
Northern	P33	2.4	3	1.6	6.4	6	6.1	6.6	7	7.2
Northern	P35	-1.2	-0.6	-2	2.8	2.4	2.5	3	3.4	3.6
Northern	P66	-0.5	0.1	-1.3	3.5	3.1	3.2	3.7	4.1	4.3
Central Interior	P100	-2.7	-2.1	-3.5	1.3	0.9	1	1.5	1.9	2.1
Central Interior	P104	-4.4	-3.8	-5.2	-0.4	-0.8	-0.7	-0.2	0.2	0.4
Central Interior	P107	-3	-2.4	-3.8	1	0.6	0.7	1.2	1.6	1.8
Central Interior	P20	-3.8	-3.2	-4.6	0.2	-0.2	-0.1	0.4	0.8	1
Central Interior	P24	-3.6	-3	-4.4	0.4	0	0.1	0.6	1	1.2
Central Interior	P61	-4.8	-4.2	-5.6	-0.8	-1.2	-1.1	-0.6	-0.2	0
Southern Interior	P1	-6.3	-5.7	-7.1	-2.3	-2.7	-2.6	-2.1	-1.7	-1.5
Southern Interior	P14	-6	-5.4	-6.8	-2	-2.4	-2.3	-1.8	-1.4	-1.2
Southern Interior	P15	-7.3	-6.7	-8.1	-3.3	-3.7	-3.6	-3.1	-2.7	-2.5
Southern Interior	P42	-8	-7.4	-8.8	-4	-4.4	-4.3	-3.8	-3.4	-3.2
Southern Interior	P57	-8.6	-8	-9.4	-4.6	-5	-4.9	-4.4	-4	-3.8
Southern Interior	P72	-7.6	-7	-8.4	-3.6	-4	-3.9	-3.4	-3	-2.8
Far South	P111	-8.3	-7.7	-9.1	-4.3	-4.7	-4.6	-4.1	-3.7	-3.5
Far South	P123	-9.1	-8.5	-9.9	-5.1	-5.5	-5.4	-4.9	-4.5	-4.3
Far South	P144	-6.5	-5.9	-7.3	-2.5	-2.9	-2.8	-2.3	-1.9	-1.7
Far South	P153	-8.5	-7.9	-9.3	-4.5	-4.9	-4.8	-4.3	-3.9	-3.7
Far South	P154	-7.8	-7.2	-8.6	-3.8	-4.2	-4.1	-3.6	-3.2	-3
Far South	P156	-8.4	-7.8	-9.2	-4.4	-4.8	-4.7	-4.2	-3.8	-3.6

Negative numbers indicate provenances originating from warmer environments transferred to cooler planting sites.

Tab. 8.3: Least square means of height and diameter growth in year 2005 (age 32) of four lodgepole pine regional populations tested across three major climatic zones in the lodgepole pine distribution.

Regional Population	Planting Site Region	Diameter (cm)	Diameter Standard Error (cm)	Height (m)	Height Standard Error (m)
North	Northern	11.0	4.8	6.1	0.5
Central Interior	Northern	9.7	5.0	5.3	0.5
Southern Interior	Northern	7.1	8.5	4.1	0.7
North	Central	11.7	5.5	12.0	0.7
Central Interior	Central	15.2	5.3	14.4	0.7
Southern Interior	Central	15.3	5.6	15.0	0.7
Far South	Central	13.4	5.8	12.5	0.8
North	Southern	11.8	5.4	9.8	0.6
Central Interior	Southern	15.0	5.2	12.3	0.6
Southern Interior	Southern	14.9	5.3	12.9	0.6
Far South	Southern	13.6	5.3	10.7	0.6

Tab 8.4: Climate warming expected by 2085 at all seed-collection and planting sites under two Intergovernmental Panel on Climate Change emissions' scenarios

Population	Provenance or Site	Observed	Projected Temperatures (°C)		Difference (°C)	
		Temperatures (°C) Climate normal 1961-1990	RCP 4.5 projections to 2085	RCP 8.5 projections to 2085	RCP 4.5 Warming	RCP 8.5 Warming
Northern	P28	-1.2	2.3	4.6	3.5	5.8
Northern	P30	-2.7	0.8	3.2	3.5	5.9
Northern	P33	-4.2	-0.5	2.0	3.7	6.2
Northern	P35	-0.6	2.9	5.3	3.5	5.9
Northern	P66	-1.3	2.1	4.5	3.4	5.8
Northern	BLUE	-1.8	1.7	4.1	3.5	5.9
Northern	M451	-1.2	2.2	4.6	3.4	5.8
Northern	WATS	-2.6	1.0	3.4	3.6	6.0
Central Interior	P100	0.9	4.3	6.6	3.4	5.7
Central Interior	P104	2.6	5.8	8.0	3.2	5.4
Central Interior	P107	1.2	4.4	6.4	3.2	5.2
Central Interior	P20	2.0	5.2	7.3	3.2	5.3
Central Interior	P24	1.8	5.2	7.5	3.4	5.7
Central Interior	P61	3.0	6.3	8.5	3.3	5.5
Central Interior	CARP	2.2	5.5	7.8	3.3	5.6
Central Interior	DOGC	1.8	5.1	7.3	3.3	5.5
Central Interior	WEST	1.9	5.2	7.5	3.3	5.6
Southern Interior	P1	4.5	7.8	10.0	3.3	5.5
Southern Interior	P14	4.2	7.5	9.7	3.3	5.5
Southern Interior	P15	5.5	8.8	11.0	3.3	5.5
Southern Interior	P42	6.2	9.5	11.7	3.3	5.5
Southern Interior	P57	6.8	10.1	12.4	3.3	5.6
Southern Interior	P72	5.8	9.1	11.4	3.3	5.6
Southern Interior	CHUW	2.4	5.6	7.8	3.2	5.4
Southern Interior	COMM	2.8	6.1	8.4	3.3	5.6
Southern Interior	EQUI	3.0	6.4	8.6	3.4	5.6
Far South	P111	6.5	9.6	11.6	3.1	5.1
Far South	P123	7.3	10.1	12.0	2.8	4.7
Far South	P144	4.7	8.1	10.3	3.4	5.6
Far South	P153	6.7	9.9	12.0	3.2	5.3
Far South	P154	6.0	9.1	11.2	3.1	5.2
Far South	P156	6.6	9.8	11.8	3.2	5.2

RCP 4.5 represents a more optimistic and moderate emissions scenario while RCP 8.5 represents a more realistic and higher-magnitude emissions scenario (Sanford et al., 2014). Climate data and climate projections derived from ClimateWNA v5.40 (Wang et al., 2016).

8.10 Supplementary Information

Tab. 8.S1. Average source climate variables from the 1961-1990 normal period are shown for provenances

Region	Prov. Number	Prov. Name	Latitude	Longitude	Elevation	MAR	MAT	MCMT	MWMT	TD	MAP	MSP	PAS	SHM	RH
Northern	28	Tetsa R.	58.67	-124.17	762	10.5	-1.2	-18.2	13.7	31.9	621	419	195	32.6	58
Northern	30	Lower Post	59.98	-128.55	640	9.7	-2.7	-23.5	14.5	38	448	235	204	61.8	56
Northern	33	Ethel L.	63.30	-136.47	876	9.8	-4.2	-24.7	14	38.7	442	260	198	53.9	57
Northern	35	Atlin	59.80	-133.78	789	9.8	-0.6	-15.5	12.5	28	374	183	181	68.3	63
Northern	66	Stone Mt.	58.65	-124.77	1173	10.1	-1.3	-14.4	11.3	25.7	688	475	240	23.8	61
Central Interior	20	Collins L.	54.13	-127.23	937	11.1	2	-9.4	12.5	21.9	595	219	294	57.2	64
Central Interior	24	Finlay Forks	55.95	-123.80	686	11.1	1.8	-12.9	14.2	27.1	525	249	212	57.2	65
Central Interior	61	Purden	53.87	-121.80	838	11.5	3	-9.9	14.3	24.2	1050	402	457	35.6	62
Central Interior	100	Nina Cr.	55.80	-124.82	762	10.9	0.9	-14	13.5	27.5	529	250	234	54	61
Central Interior	104	Nechako R.	54.02	-124.53	732	11.1	2.6	-11.4	14.4	25.7	520	238	211	60.4	58
Central Interior	107	Tweedsmuir	52.50	-125.80	1311	11.3	1.2	-8.8	10.9	19.8	1043	257	628	42.7	60
Southern Interior	1	Trapping Cr.	49.58	-119.02	1006	11.1	4.5	-7.1	15.7	22.8	550	227	200	69.2	59
Southern Interior	14	Wentworth Cr.	50.97	-120.33	1059	12.3	4.2	-7.6	14.8	22.4	476	219	163	67.8	62
Southern Interior	15	Esperon L.	50.05	-119.65	1067	12.4	5.5	-5.5	16.3	21.8	650	205	231	79.5	67
Southern Interior	42	Champion L.	49.18	-117.58	998	12.7	6.2	-5.2	17.6	22.8	835	269	261	65.4	67
Southern Interior	57	Inonoaklin	49.90	-118.20	579	10.7	6.8	-4.4	17.9	22.3	685	264	162	67.7	64
Southern Interior	72	Larch Hills	50.70	-119.18	777	11.2	5.8	-5.9	17.1	23	648	244	211	70.2	65
Far Southern	111	Stevens Pass	47.78	-120.93	762	16.5	6.5	-3.5	17.2	20.7	1711	255	536	67.4	61
Far Southern	123	Black Butte	44.38	-121.67	1006	14.3	7.3	-0.7	16.6	17.3	609	102	99	163.4	51
Far Southern	144	Missoula (a)	46.67	-113.67	1524	12.7	4.7	-6.3	16.8	23.1	568	237	199	70.9	56
Far Southern	153	Enterprise	45.63	-117.27	1311	14.5	6.7	-3.4	17.7	21.1	562	205	123	86.5	56
Far Southern	154	Prairie City	44.53	-118.57	1494	15.9	6	-4.1	17.1	21.3	639	171	206	99.8	56
Far Southern	156	Quartz Pass	42.30	-120.78	1615	16.7	6.6	-2	17.2	19.2	534	118	128	146	54

Region refers to the assigned population based on geographical origin. *Prov. Number* and *Prov. Name* refer to the provenance corresponding to the Illingworth provenance trial's numbering system (Illingworth 1978). Latitude is given in decimal degrees (*Lat.*), longitude is given decimal degrees (*Long.*); and elevation is given in meters above sea level (*Elev.*). Mean annual solar radiation (*MAR*) is provided in MJ m⁻² d⁻¹. Climate variables include: Mean annual temperature in °C (*MAT*); mean coldest month temperature in °C (*MCMT*); mean warmest month temperature in °C (*MWMT*); temperature difference is a measure of continentality and is given in °C (*TD*); mean annual precipitation is given in mm (*MAP*); mean summer precipitation is given in mm (*MSP*); Precipitation as Snow in mm (*PAS*); summer heat:moisture index (*SHM*); and relative humidity (%). Climate data derived from ClimateWNA v 5.40 (Wang et al. 2016).

Tab. 8.S2. Average source climate variables from the 1961-1990 normal are shown for all planting sites.

Region	Site Name Abbreviation	Latitude	Longitude	Elevation	MAR	MAT	MCMT	MWMT	TD	MAP	MSP	PAS	SHM	RH
Northern	BLUE	59.78	-129.13	730	9.8	-1.8	-19.6	13.9	33.5	463	229	224	60.9	57
Northern	M451	58.83	-125.72	1100	10.7	-1.2	-15.3	12.1	27.4	592	348	249	34.7	59
Northern	WATS	60.08	-128.83	700	9.9	-2.6	-22.6	14.7	37.3	411	236	171	62.2	56
Central Interior	CARP	54.60	-122.92	790	11	2.2	-11.8	14.3	26.1	806	285	403	50.1	63
Central Interior	DOGC	54.27	-124.40	950	11.3	1.8	-11.3	13.3	24.6	602	244	285	54.7	61
Central Interior	WEST	55.83	-123.70	670	11.1	1.9	-12.9	14.3	27.2	550	249	233	57.4	64
Southern Interior	CHUW	50.58	-120.62	1430	12.7	2.4	-8.3	13	21.3	430	197	184	65.8	62
Southern Interior	COMM	50.92	-120.07	1370	12.6	2.8	-8.2	13.6	21.8	639	255	292	53.3	66
Southern Interior	EQUI	50.37	-119.60	1370	12.4	3	-7.9	13.6	21.6	699	244	330	55.9	65

Region refers to the general area within the lodgepole pine range in which the planting site occurs. *Site Name Abbreviation* refers to the abbreviated site name from the Illingworth lodgepole pine provenance trial (Illingworth 1978). Latitude is given in decimal degrees (*Lat.*), longitude is given decimal degrees (*Long.*); and elevation is given in meters above sea level (*Elev.*). Mean annual solar radiation (*MAR*) is provided in MJ m⁻² d⁻¹. Climate variables include: Mean annual temperature in °C (*MAT*); mean coldest month temperature in °C (*MCMT*); mean warmest month temperature in °C (*MWMT*); temperature difference is a measure of continentality and is given in °C (*TD*); mean annual precipitation is given in mm (*MAP*); mean summer precipitation is given in mm (*MSP*); Precipitation as Snow in mm (*PAS*); summer heat:moisture index (*SHM*); and relative humidity (%). Climate data derived from ClimateWNA v 5.40 (Wang et al. 2016).

Tab. 8.S3. Multiple comparisons among planting site regions and their tested regional populations

Test	Diameter p-value	Height p-value
Northern planting sites testing N provenances - Northern planting sites testing CI provenances == 0	1	0.787
Northern planting sites testing SI provenances - Northern planting sites testing N provenances == 0	<0.01	<0.01
Northern planting sites testing SI provenances - Northern planting sites testing CI provenances == 0	<0.01	<0.01
Northern planting sites testing FS provenances - Northern planting sites testing N provenances == 0	<0.01	<0.01
Northern planting sites testing FS provenances - Northern planting sites testing CI provenances == 0	<0.01	<0.01
Northern planting sites testing FS provenances - Northern planting sites testing SI provenances == 0	<0.01	<0.01
Northern planting sites testing N provenances - Central planting sites testing N provenances == 0	<0.01	<0.01
Northern planting sites testing N provenances - Central planting sites testing CI provenances == 0	<0.01	<0.01
Northern planting sites testing N provenances - Central planting sites testing SI provenances == 0	<0.01	<0.01
Northern planting sites testing N provenances - Central planting sites testing FS provenances == 0	1	0.0218
Northern planting sites testing CI provenances - Central planting sites testing N provenances == 0	1	0.4152
Northern planting sites testing CI provenances - Central planting sites testing CI provenances == 0	<0.01	<0.01
Northern planting sites testing CI provenances - Central planting sites testing SI provenances == 0	<0.01	<0.01
Northern planting sites testing CI provenances - Central planting sites testing FS provenances == 0	<0.01	<0.01
Northern planting sites testing SI provenances - Central planting sites testing N provenances == 0	<0.01	<0.01
Northern planting sites testing SI provenances - Central planting sites testing CI provenances == 0	1	<0.01
Northern planting sites testing SI provenances - Central planting sites testing SI provenances == 0	1	<0.01
Northern planting sites testing SI provenances - Central planting sites testing FS provenances == 0	1	0.5005
Northern planting sites testing FS provenances - Central planting sites testing N provenances == 0	<0.01	<0.01
Northern planting sites testing FS provenances - Central planting sites testing CI provenances == 0	<0.01	<0.01
Northern planting sites testing FS provenances - Central planting sites testing SI provenances == 0	<0.01	<0.01
Northern planting sites testing FS provenances - Central planting sites testing FS provenances == 0	<0.01	<0.01
Central planting sites testing N provenances - Central planting sites testing CI provenances == 0	<0.01	<0.01
Central planting sites testing SI provenances - Central planting sites testing N provenances == 0	<0.01	<0.01
Central planting sites testing SI provenances - Central planting sites testing CI provenances == 0	1	0.9642
Central planting sites testing FS provenances - Central planting sites testing N provenances == 0	0.0939	0.9892
Central planting sites testing FS provenances - Central planting sites testing CI provenances == 0	<0.01	0.0246
Central planting sites testing FS provenances - Central planting sites testing SI provenances == 0	<0.01	<0.01
Southern planting sites testing N provenances - Northern planting sites testing N provenances == 0	<0.01	<0.01
Southern planting sites testing N provenances - Northern planting sites testing CI provenances == 0	<0.01	<0.01
Southern planting sites testing N provenances - Northern planting sites testing SI provenances == 0	<0.01	<0.01
Southern planting sites testing N provenances - Northern planting sites testing FS provenances == 0	<0.01	<0.01
Southern planting sites testing CI provenances - Northern planting sites testing N provenances == 0	1	0.2072
Southern planting sites testing CI provenances - Northern planting sites testing CI provenances == 0	<0.01	<0.01
Southern planting sites testing CI provenances - Northern planting sites testing SI provenances == 0	<0.01	<0.01
Southern planting sites testing CI provenances - Northern planting sites testing FS provenances == 0	0.8905	0.1246
Southern planting sites testing SI provenances - Northern planting sites testing N provenances == 0	<0.01	<0.01
Southern planting sites testing SI provenances - Northern planting sites testing CI provenances == 0	<0.01	<0.01
Southern planting sites testing SI provenances - Northern planting sites testing SI provenances == 0	<0.01	<0.01
Southern planting sites testing SI provenances - Northern planting sites testing FS provenances == 0	<0.01	<0.01
Southern planting sites testing FS provenances - Northern planting sites testing N provenances == 0	<0.01	<0.01
Southern planting sites testing FS provenances - Northern planting sites testing CI provenances == 0	<0.01	1
Southern planting sites testing FS provenances - Northern planting sites testing SI provenances == 0	1	0.1802
Southern planting sites testing FS provenances - Northern planting sites testing FS provenances == 0	1	0.0842
Southern planting sites testing N provenances - Central planting sites testing N provenances == 0	0.2396	1
Southern planting sites testing N provenances - Central planting sites testing CI provenances == 0	<0.01	<0.01
Southern planting sites testing N provenances - Central planting sites testing SI provenances == 0	<0.01	<0.01
Southern planting sites testing N provenances - Central planting sites testing FS provenances == 0	<0.01	<0.01
Southern planting sites testing CI provenances - Central planting sites testing N provenances == 0	<0.01	<0.01
Southern planting sites testing CI provenances - Central planting sites testing CI provenances == 0	<0.01	0.9853
Southern planting sites testing CI provenances - Central planting sites testing SI provenances == 0	1	0.776
Southern planting sites testing CI provenances - Central planting sites testing FS provenances == 0	0.9999	0.2248
Southern planting sites testing SI provenances - Central planting sites testing N provenances == 0	0.3564	1
Southern planting sites testing SI provenances - Central planting sites testing CI provenances == 0	<0.01	<0.01
Southern planting sites testing SI provenances - Central planting sites testing SI provenances == 0	1	0.8957
Southern planting sites testing SI provenances - Central planting sites testing FS provenances == 0	<0.01	<0.01
Southern planting sites testing FS provenances - Central planting sites testing N provenances == 0	<0.01	<0.01
Southern planting sites testing FS provenances - Central planting sites testing CI provenances == 0	<0.01	<0.01
Southern planting sites testing FS provenances - Central planting sites testing SI provenances == 0	<0.01	<0.01
Southern planting sites testing FS provenances - Central planting sites testing FS provenances == 0	0.2325	0.924
Southern planting sites testing N provenances - Southern planting sites testing CI provenances == 0	0.5274	<0.01
Southern planting sites testing SI provenances - Southern planting sites testing N provenances == 0	0.4251	<0.01
Southern planting sites testing SI provenances - Southern planting sites testing CI provenances == 0	0.9993	0.4672
Southern planting sites testing FS provenances - Southern planting sites testing N provenances == 0	<0.01	0.6339
Southern planting sites testing FS provenances - Southern planting sites testing CI provenances == 0	<0.01	0.0123
Southern planting sites testing FS provenances - Southern planting sites testing SI provenances == 0	0.0162	<0.01

The four populations are represented by columns titled LE, CI, SI and TE: LE stands for the Leading Edge, i.e., the northern population; CI is Central Interior population, located in the central areas of the lodgepole pine distribution; SI is Southern Interior population, covering the mid-southern range of the lodgepole pine distribution; TE stands for the Trailing Edge, which represents seed sources from the far south of the lodgepole pine distribution, which is expected to see increased forest maladaptation under climate warming. Bold values indicate significant relationships, as corrected for with the Bonferroni p-adjustment method.

9 Conclusions

9.1 Northern Forests at Risk: Possible Candidates for Assisted Gene Flow

Much of the research presented here is based on northern populations tested at southern planting sites. As indicated in Chapter 8, the provenance-site transfer combinations represent climate warming ranging from 3°C to 7.2°C, with a mean of 4.7°C. This range is suitable for testing climate change scenarios projected to 2085: Warming at these locations under an RCP 4.5 is predicted to be approximately 3.5°C, while warming under a RCP 8.5 emissions scenario is projected to be up to 6.2 °C (mean = 5.9°C) (Chapter 8). Under these experimental conditions, three chapters presented here indicate upcoming forest issues at the leading edge of the lodgepole pine range under warming and drought in less than seven decades.

As described in Chapter 8, the northern population showed higher levels of growth in southern planting environments compared to when they were planted in local, northern planting environments. Initially, this may appear to provide evidence that northern forests will benefit under warming, as predicted by other researchers (Hamann & Wang, 2006; Wang et al., 2006; Bonan, 2008; Savolainen et al., 2011; Pedlar & McKenney, 2017). However, this seed transfer from northern environments to southern growing environments could represent a release from lower levels of radiation, which will not change in northern environments under climate change. Furthermore, northern populations appear to be vulnerable to climatic extremes such as drought (Chapters 3, 6) and cold events (Chapter 7). Thus, increased growth in northern environments would not be expected to materialize based on warming, and would instead be limited by increasing occurrences of climatic extremes.

In Chapter 3, for example, northern population were observed to show the lowest drought tolerance under simulated high-magnitude climate change. The northern provenances were the least tolerant to a strong spring drought event occurring in 2002. Although their drought *resistance* was higher, this was partially due to their overall growth which was low to begin with. These populations also never regained pre-drought growth levels before succumbing to the mountain pine beetle epidemic in 2006. This shows that northern populations had the least drought tolerance, as measured by *relative resilience*. This drought response further challenges expectations that populations adapted to cold would be better suited for tolerating drought (Bansal et al., 2015, 2016; Montwé et al., 2015).

In Chapter 6, we found that the low drought tolerance of northern provenances was linked to a suite of physiological maladaptations. Trees grown from seed from the leading edge of the species range appeared to show lower rates of transpiration, yet sustained this transpiration even under drier conditions, as evidenced through the oxygen isotope proxy. These trees also showed a lower ability to discriminate against the disfavoured heavier carbon isotope. This indicates that, under drought, their stomata remained open. While this would allow them to continue photosynthesizing to maintain respiration and growth, it would also increase xylem tension substantially. Since the thickness of the tracheid walls were surprisingly low, this would implicate low ability to withstand the increasing xylem tension. Therefore, these populations would be susceptible to cavitation. Such embolisms can block the transport of water to the crown, thereby reducing the ability to photosynthesize and sustain tree metabolism.

In addition to drought, northern populations were found to be susceptible to spring frost damage, as reported in Chapter 7. Since northern populations are adapted to short growing seasons, they tend to flush (burst bud) under lower degree day accumulations. Similar patterns

have been reported for other conifers: For example, in black spruce, northern populations flushed earlier but set bud earlier as well (Perrin et al., 2017). This may confer some level of safety against fall frost, but that may not be relevant if spring frosts cause damage in seedlings. Under climate change, higher latitudes are expected to experience greater than average warming, much of which will occur in winter. Warmer winters combined with an expected increase in climatic variability would lead to a higher probability of “false springs” to occur in northern latitudes, to which northern boreal forests are highly maladapted.

The low tolerance of northern populations to the expected increased probability of extreme events has significant implications for expectations surrounding the lodgepole pine species distribution. Although bioclimatic envelope models for lodgepole pine generally project major losses in habitat, new suitable habitats in northern latitudes have been projected (Hamann & Wang, 2006; Wang et al., 2006; Coops & Waring, 2011). However, warming and drought, especially recurrent droughts, can limit regeneration in high-latitude and high-elevation areas, reducing recruitment and range shifting abilities (Hogg & Wein, 2005; Barros et al., 2017; Conlisk et al., 2017). Given the high probability of severe warming materializing by the end of the century (Sanford et al., 2014) and the consequence of physiological maladaptations to warming and climatic extremes, these populations appear to be most at-risk. Contrary to the leading-edge hypotheses, range contraction could occur in northern areas.

These findings contradict assumptions that forest growth in northern latitudes and survival of northern pine populations could be enhanced (Bonan, 2008; Lindner et al., 2010; Savolainen et al., 2011; Pedlar & McKenney, 2017), as predicted by modeling and response functions (Rehfeldt et al., 2001; Hamann & Wang, 2006; Wang et al., 2006), by climate-growth correlations (McLane et al., 2011a, 2011b; Berner et al., 2013), by longer growing seasons or by

more optimal temperature ranges for photosynthesis (Sage & Kubien, 2007). While increased net primary productivity may have been temporarily true historically or may be true for far northern tundra vegetation (Keeling et al., 1996; Myneni et al., 1997; Goetz et al., 2005), our findings instead align with more recent research reporting warming and drought-induced growth decline in high-latitudes and elevations (Barber *et al.*, 2000; D'Arrigo *et al.*, 2004; Peng *et al.*, 2011; Charney *et al.*, 2016; Girardin *et al.*, 2016; Montwé *et al.*, 2016; Rehfeldt *et al.*, 2017). Understanding the physiological mechanisms behind the low tolerances to climatic extremes helps frame, for the first time, *why* earlier predictions of boreal greening are not occurring as predicted and instead showing declining growth patterns.

A possible solution to the developing maladaptation is discussed in Chapter 8. This study tests the response of seed sources from the central interior of British Columbia at northern planting sites. This simulates a realistic assisted gene flow scenario involving climate transfers of -2.1°C to -5.6°C . These provenances were top performers over the entire experiment (32 years), indicating no undue annual responses to changing environmental conditions. They also showed suitable phenological adaptation to tolerate both fall and spring cold events, as found in Chapter 7. Given the high-risks of a status-quo reforestation scenario that would involve planting local seed sources, these areas appear to be good candidates for assisted gene flow. Judicious transfers could present possible benefits to these northern areas if implemented with a percentage of southern seed sources; such “composite” provenancing is generally recommended (Aitken et al., 2008; Ukrainetz et al., 2011; Prober et al., 2015). Should there be an opportunity to re-plant in these areas after harvest, fire or other disturbance, the climate-based seed-transfer system in British Columbia could facilitate fast acting implementation within its jurisdiction

9.2 Central Interior Areas: Resilient and Unsuitable for Assisted Gene Flow

In direct contrast to the northern population, the cumulative work here indicates that the seed sources from the central interior of British Columbia show high resilience to warming and climatic extremes. In Chapter 3, we noted that these populations performed well under a modest warming scenario. This is evidenced by good growth under simulated warming of up to 2.1°C in mean annual temperature, although generally the climatic distance was not large: the mean warming was approximately 0.5°C due to the high elevation of the southern planting sites. However, more southern planting sites were not available, nor did our experiment capture recent warming, so the tolerance of these seed sources to warming is not known. Given the rate of change, the warming experienced may have already exceeded what we could test within the experimental design.

Although we were not able to test growth under higher-magnitude warming, the responses of central interior seed sources under mild warming nevertheless indicate that these provenances could sustain their high performance for at least a short time. These seed sources also showed a high ability to cope with changing conditions. In Chapter 6, we report that their good growth was linked to an ability to maximize growth under optimal conditions through larger xylem conduits, while their moderate drought tolerance was attributed to stomatal responsiveness. We also found in Chapter 6 that they showed high plasticity to changing environments, which could further indicate resilience to changing climates. In Chapter 3, we conclude that these seed sources had moderate drought tolerance. Furthermore, these seed sources showed low damage due to both spring and fall frosts, even under a mild degree of

warming, which could alter phenological cues (see Chapter 7). Taken together, these populations appeared to be the most capable of tolerating a modest degree of warming.

While it may not be urgent to implement assisted gene flow to these areas, proactive forest practitioners may be interested in planting a proportion of seed sources from lower latitudes and elevations. This could be seen as a precautionary measure given the expected warming in these areas ($>5^{\circ}\text{C}$ by 2085 under an RCP 8.5 scenario). When southern interior seed sources are transferred to these central interior planting sites, they show growth that is competitive with local seed sources (Chapter 8). However, such transfers may not confer additional drought tolerance, as the drought tolerances of central and southern interior seed sources were not significantly different (Chapter 3). Furthermore, the risks associated with such transfers would involve increased probability of damage due to cold (Chapter 7): Southern interior seed sources incurred the highest fall frost damage under an elevation transfer that is more climatically mild (mean = -2.8°C) than such a latitudinal transfer (mean = -3.5°C). Long-distance seed transfers from drought-tolerant populations at the trailing-edge of the species range can also not be recommended; these seed sources showed poor performance under the temporarily cooler conditions on the central interior planting sites. Altogether, it appears that the risks of assisted gene flow in the central interior outweigh the risks of inaction.

9.3 Southern Interior Areas: Maladaptation to Cold Limits Assisted Gene Flow

Seed sources from the southern interior of British Columbia, when planted on southern interior planting sites, generally perform well and show moderate drought tolerance (Chapter 3). Given the physiological plasticity of the southern interior seed sources and ability to cope with

changing environments (Chapter 6), the risks of inaction in the southern interior of British Columbia appear to be more moderate than would otherwise be anticipated. However, since there were no available planting sites lower in latitude or elevation, these seed sources were not tested under warming. We therefore cannot estimate the time-frame when these populations may become maladapted due to warming. There may be risks under such warming, but we could not quantify them given our experimental design (no common gardens in the far south) and study period (missing warming and droughts after 2005).

These areas are expected to increase in temperature of approximately 5.5°C by 2085 under the RCP 8.5 scenario (Wang et al., 2016). These projections may make the concept of incorporating assisted gene flow more attractive in these areas. Assisted gene flow of seed sources from the far south to the central parts of the species distribution may increase drought tolerance due to the cavitation-resistant xylem of trailing edge populations, as noted in Chapter 6. Opportunity costs, however, include reduced growth, as shown in Chapter 3.

Long-distance assisted gene flow may also cause cold damage in these southern interior forests: The far southern populations had generally high levels of fall frost damage when transferred to southern interior planting sites, as discussed in Chapter 7. Although their response to cold was not significantly different from “local” seed sources, it is important to note that it is not valid to interpret these data as suggesting that latitudinal transfers under assisted gene flow would not increase fall frost damage (Chapter 7). This relates to the lower-elevation origins of the “local” seed sources compared to the relatively higher elevation southern interior planting sites. These transfer distances represented elevation gains of, on average, 475 m. These transfers thus emulate the current maximum allowed upward elevation seed transfer of 500 m (O’Neill et al., 2017). Since lodgepole pine is known to have steep clines in cold adaptation along an

elevation gradient but more gentle clines across latitude (Rehfeldt, 1987), both “local” and “southern” seed sources represent assisted gene flow transfers; one across latitude and one across elevation. Although our data cannot be used to compare cold response between northward-transferred provenances and seed sources from similar elevations as the planting sites, it seems probable that long-distance (approximately 625 km) latitudinal transfers would increase cold damage relative to seed sources chosen under more restrictive historic transfer guidelines regulating upward seed transfer.

Without the ability to compare the risks of assisted gene flow against the risks of the inaction, it may not be prudent to recommend relatively long-distance latitudinal transfers under assisted gene flow at this time. On average, the populations from the far south were transferred approximately 625 km to the southern interior planting sites. A more conservative approach would involve sourcing planting stock from half this distance; as combined with composite provenancing, this could reduce the risks of undue cold damage relative to local sources. Another alternative would be to move seed sources upward in elevation, but more conservatively than the 500 m allowed maximum. As with latitudinal transfers, cautious implementation is recommended because, with increasing gains in elevation and latitude, there is a higher probability of cold damage. This has been shown with our data, where we test elevation transfers of approximately 475 m.

In general, frost risks are increasing due to increased climate variability combined with growing seasons extending into the riskier shoulder seasons. Damage due to cold could reduce regeneration survival since smaller trees are more susceptible to frost (Kidd et al., 2014). Although older trees can survive cold events, cold-induced damage could cause defects in the timber and increase susceptibility to pathogens (Diamandis & Koukos, 1992). Caution may be

further required as the far southern population experienced rank-changing growth losses after a spring frost event on the southern interior planting sites. Assisted gene flow to the southern or central interior of British Columbia could thus introduce cold maladaptation from populations adjusted to different phenological cues, further exacerbating the probability of damage to cold. Thus, the risks of assisted gene flow are also moderate, rivalling the risks of inaction. By incorporating a small percentage of southern seed sources (representing shorter transfer distances), forest managers may, however, be able to increase diversity and avoid over-committing to one reforestation strategy. The lodgepole pine forests of the southern interior of British Columbia could also be priority areas for monitoring. As combined with the adaptive capacity now afforded by the new climate-based seed-transfer system, this facilitates relatively rapid changes to reforestation as it becomes necessary.

An additional aspect to consider regarding the late-occurring rank-change in growth loss in the trailing edge population is the age of the provenance trial itself. A longer-term provenance trial dataset may be more likely to capture the extreme events to which populations are truly adapted. Studying longer-term datasets would thus enable a more accurate assessment of differences among genotypes. Combining longer-term provenance trials with tree ring analyses is a particularly useful approach for studying responses to extreme events. This is illustrated by the fact that the work herein contradicts earlier findings from the Illingworth lodgepole pine provenance trial (i.e., Rehfeldt et al., 1999). Earlier analyses relied on cumulative height growth and therefore did not fully capture the response to the spring cold event in 1992. With that dataset, it would not have been possible to detect the rank-change in growth loss because little time had elapsed since the cold event; the competitive growth in the first 20 years prior to the cold event would have dominated the few years' data after the spring frost event that showed the

beginning of the decline. Thus, this earlier study concluded that realized niches of lodgepole pine populations are smaller than their fundamental niches, and that a whole-sale redistribution of genotypes across the landscape would be possible. Our more recent findings based on a longer dataset combined with annually resolved growth data directly contradict this conclusion. This difference underscores the importance of interpreting younger provenance trials with caution and further highlights the importance of analyzing annually-resolved response information that can be derived from tree rings.

9.4 Uncertainties for Trailing-Edge Lodgepole Pine Populations

Since this lodgepole pine provenance trial includes no planting sites south of the Canadian border, the response of trailing-edge lodgepole pine populations to warming, drought, and cold could not be tested in their natural habitat. Although it may be difficult to predict their responses to warming and drought in the far south, we found them to exhibit high drought tolerance under a transfer to the southern interior of British Columbia (Chapter 3). Their drought tolerance was found to be linked to morphology, relying on cavitation-resistant xylem conduits (Chapter 6). A dual-isotope approach was, in turn, used to infer low stomatal responsiveness under changing environmental conditions (Chapter 6). Together, these characteristics likely contribute to an anisohydric physiological strategy (Chapter 6). Anisohydry may be a successful adaptation under some level of warming and drought: these trees can tolerate less moisture and continue to assimilate carbon, thereby avoiding slow death through carbon starvation. When transferred northward, however, these populations show consistently low adaptation to cold (Chapter 7, Chapter 8). These populations may therefore become increasingly susceptible to cold-induced damage as climates become warmer and more variable.

Given these strong reactions to cold, and the steep adaptive clines to cold in the mountainous regions of the southern lodgepole pine range (Rehfeldt, 1980, 1983, 1987, 1988; Rehfeldt et al., 1999), assisted migration may not be a suitable option. To increase adaptive capacity as problems arise, it could be beneficial to allow flexibility within public policy, facilitating rapid reactive measures as necessary. It may be possible to, for example, transfer seed sources over very small elevation distances. Other methods of promoting drought tolerance, thinning for example, could be a temporary measure to enhance resilience in the forests that are already present (D'Amato et al., 2013; Diaconu et al., 2015, 2017; Elkin et al., 2015; Sohn et al., 2016). This could also be a large component of planning for climate change. While any results-interpretation for far-southern locations comes with high uncertainty given projections beyond what was tested here, the responses from these populations on northern sites can be used to compliment the knowledge of practitioners of their local forests in southern locations. It also indicates that these populations are unsuited to long-distance seed transfers to Canada (e.g. ~625 km) due to cold maladaptation.

9.5 Proactive or Reactive: Population-Specific Risks in Assisted Gene Flow

Risk can be defined as the interaction between probability and consequence. “Can we afford to wait to begin implementing assisted gene flow?” is a risk analysis question that assesses the likelihood of different impacts under action and no-action scenarios.

At the scientific level, the work presented herein suggests that risk analysis questions are best answered by considering specific populations of tree species. For instance, we found that northern populations were most at risk. Although it is uncertain how exactly these

maladaptations will manifest across the landscape due to the complexity of the system, the data here all suggest that the northern lodgepole pine population will be severely affected: probability of forest maladaptation in northern areas is high. The consequences of such maladaptation are also high, as they could range from reduced forest ecosystem resilience including increased susceptibility to disease and higher mortality, to altered carbon feedbacks, to negative socioeconomic impacts on nearby communities. In contrast, populations from the central part of the range show high resilience to some warming and climatic extremes. This indicates that they may remain productive under mild warming and, therefore, that intervention may not be immediately necessary. Thus, the current probability of maladaptation with their associated consequences is low. If assisted gene flow were to be implemented in these regions nevertheless, it would be associated with higher risks due to high probability of reduced growth and cold damage, both with forest health and socioeconomic consequences. Thus, the risks of action (assisted gene flow) outweigh those of inaction (“local is best”) in central areas at this time.

Adaptive capacity and flexible policies are additional aspects relating to the question: “Can we afford to wait?”. The degree to which population-specific risk analyses are accounted for in forest management is also determined by the preferences of policy makers and forest practitioners for either a reactive or proactive approach. When managing long-lived species, having a long-term, proactive approach may be most suitable for some populations, especially where policies are more rigid – forward planning by planting “pre-adapted” seed sources could help reduce negative consequences of anticipated problems. Such proactive approaches lead to higher potential consequences, as populations may be maladapted to current conditions. Reactive approaches may be suitable where there are few barriers to public policy changes that allow rapid implementation of alternative reforestation strategies as negative consequences begin to

emerge (indicating high probability of continued problems). While providing more flexibility to forest managers, this approach may be less suitable for populations that appear to require more urgent intervention (i.e., in the north). In the far southern populations, where uncertainty is high, reactionary approaches may be most suitable. For example, assisted gene flow could be implemented where forest problems in those areas become evident through increased mortality, forest health issues, or lost productivity. When problems are not entirely evident yet, carefully monitoring forests could provide an indication of when changes might be necessary. The climate-based seed transfer system being adopted in British Columbia could be an excellent way to allow local forest practitioners to react to problems as they present themselves.

9.6 Refined Methodologies to Support Sound Tree-Ring Research

In addition to the contributions to science and management, this dissertation also made two contributions regarding methodologies to facilitate tree-ring research. A simple addition to the preparation of micro sections for wood anatomical analyses involves a one-minute steaming treatment to gelatinize corn starch granules that remain after standard micro section preparations. This treatment serves to reduce artefacts in micro graphs, resulting in more efficient image analysis. Such a treatment therefore facilitates the analysis of a large number of samples, as required by long chronologies and for the work presented here.

Preparing tree rings for stable isotope analyses can also be laborious for large sample sizes. An efficient and common method of homogenization during the process of preparing plant tissues for isotope analyses involves adding steel balls to plastic sample tubes, shaking the tubes, and then removing the steel balls with a magnet. While this method successfully homogenizes the samples, it also causes abrasion to the inside of the plastic tube, which risks contamination of

plastic from the sample tube. Clear evidence of contamination from plastic, and resulting changes in stable carbon isotope signatures, suggest this method should be avoided in the preparation of samples for stable carbon isotope analyses..

9.7 Thesis Contributions to Science and Management

For a wide-ranging coniferous tree species in western North America, this work provides new insights into responses of tree populations to warmer temperatures, drought, cold and seed transfer. This dissertation also evaluated intra-specific differences in physiology, and, in the process, refined methodologies to support sound tree-ring research. While isohydry and anisohydry are two contrasting physiology behaviours often used to compare species (e.g., McDowell et al., 2008; Altieri et al., 2015), this framework has rarely been used for studying intra-specific differences (Anderegg, 2015). This work helps fill that knowledge gap by showing the full range across this gradient, with associated implications for management in a warmer world.

Additionally, this dissertation estimates the limits to seed transfer as a strategy to reduce maladaptation to climate change. This was tested first by analyzing genetic differences in cold damage and, second, by analyzing growth responses over multiple combinations of transfer distances using annual growth increments to explain cumulative growth. The strong genetic differences, the clinal gradient in cold adaptation with temperature, and the relationship with climate indicate that the analysis of frost imprints in tree rings is an effective method for revealing intra-specific cold adaptation in trees. Blue rings appear to provide a more sensitive parameter than the related light rings or frost rings, enabling studies of milder cold events and their responses. If combined with provenance trials, blue and frost rings provide an annual

resolution of genotype by environmental interactions. Provenance trials have been established for most of the major tree species of the temperate and boreal zone, and tree-ring analysis is a common approach that can also be conducted non-destructively with increment borers.

By analyzing the functional traits of a foundation tree species (morphology, physiology and phenology), this work indicates sensitivity and adaptations essential for good vulnerability assessments and predictions of forest response under climate change (Aubin et al., 2016). It thus provides a basis for effective management of forest ecosystems as new climate-based seed-transfer policies begin to unfold.

9.8 Concluding Statement

The intra-specific responses and strategies reported here would suggest that generalizations should be avoided: Managing forest ecosystems under environmental change requires an understanding of the response of locally adapted tree populations to climate shifts and variability. Cautious approaches are also advisable for sound implementation of assisted gene flow where it has been shown to be necessary. Furthermore, it should be emphasized that these must be at the discretion of practicing forest professionals, who have knowledge and experience working in local forest ecosystems. In the Canadian context, seed source choice is likely the most effective and efficient means of adapting our public resources to general warming and increasing probability of extremes. The local forest practitioners, who will ultimately make such science-based decisions, leave a lasting legacy on these forests and the Canadians relying on them.

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