“We’ll walk this road together, through the storm.

Whatever weather, cold or warm.”

- Eminem

Quantifying the effects of frost anomalies and cold hardiness on the growth of red spruce (*Picea rubens* Sarg.) populations

by

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Bachelor of Science in Forestry, University of New Brunswick, 2020

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of:

Master of Science in Forestry

in the Graduate Academic Unit of Forestry and Environmental Management

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This thesis is accepted by the

Dean of Graduate Studies

THE UNIVERSITY OF NEW BRUNSWICK

December 2022

ABSTRACT

Climate change adaptation strategies such as assisted migration require a better knowledge of the genetic variations in climate sensitivity, including cold hardiness. In this thesis, I compared the cold hardiness of seven mature provenances of red spruce (*Picea rubens* Sarg.). under controlled conditions and detected significant differences in autumn acclimation between the most cold-adapted and warm-adapted seed sources. Looking at tree rings of the past 50 years in the same provenances, I found no significant impact of frost on growth since the 1990s, but a large drop in growth occurred in 1987 in synchrony with a severe frost event. The growth decline was significantly higher in southern seedlots and covaried with their experimental levels of cold hardiness. My results suggest limited evidence for recent frost damage in southern provenances and support red spruce as a good candidate for assisted migration.

ACKNOWLEDGEMENTS

Thank you to Loïc for inspiring me and helping me to create a unique and novel project and being there every single step of the way, and to Neil Pederson of Harvard Forest for his advice and enthusiasm, and to Charles Bourque for sitting on my committee. Thanks to Canada's Natural Sciences and Engineering Research Council (NSERC) for supporting this research. Thank you to my field partners Jesse and Jeneya, no matter the sleepless nights or your own projects to worry about, I could always count on you. Thank you to Susan for being my parallel, and Renee for being my best friend and supporter. Shout out to my parents for helping pay for Tyler's goalie gear when I ran out of money (even though you remind me it's coming out of my inheritance), I thank you. Thanks to Daisy, my chihuahua for keeping me company and keeping me warm whether working in the lab, at home, at Picaroons or the Grad House. And especially to my loves Mike and Tyler, thank you for sticking with me through my moods and constant crises, you two make it all worth it ♡.

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List of Symbols and Abbreviations

CO_2 – Carbon dioxide

US – United States

LT_{50} – Lethal killing value for 50% of the samples

EC – Electrical conductivity

CFS – Canadian Forest Service

ID or ProvID – Provenance Identification number

GDD – growing degree days

MAT – mean annual temperature

FFP – frost free period

MWT – mean winter temperature

mASL – elevation in meters

m - meters

g – grams

ml – millilitres

°C – degrees Celsius

h – hours

± - plus or minus, indicating standard deviation above and below the mean

EC_{max} – conductivity of cooked samples

EC_{ctrl} – conductivity of non-frozen samples

EC_{test} – conductivity of frozen samples

k – electrolyte leakage rate coefficient

DD – degree days

RGC – relative growth change method of pointer year detection

NMW – normalization in a moving window method of pointer year detection

FSS – friedman super smoother

RWI – ring width index

BAI – basal area increment

ANOVA – analysis of variance

UNB – University of New Brunswick

CMI – climate moisture index

DBH – diameter at breast height

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Chapter 1 : General introduction

1.1 Climate change

Mean annual temperature in Canada is expected to increase by 2.0 – 6.0 °C by 2100, with the greatest warming occurring in winter (Zhang et al. 2019). Such warming should also lead to more frequent spring rain over snow, along with reductions in days with snow cover, and a lower accumulation of hardiness-inducing cold temperatures (Zhang et al. 2019). Overall, these changes are expected to affect the occurrence, distribution, and severity of late-spring frost, winter thaws, and early-autumn frosts (herein referred to as frost anomalies) (Gusta et al. 2009b, Gu et al. 2008). An increase in temperature lengthens the growing seasons, which can increase the period during which plants are exposed to damaging frost in the autumn and spring. The interacting effects of changes in temperature, precipitation, and CO_2 concentrations make frost a complex issue in the context of climate change, but the increase in the length of the growing season is one indication of a change that may result in more frequent late-spring frost damage to plants (Liu et al. 2018, Gu et al. 2008, Rigby & Porporato 2008). This damage includes foliar injury and cellular damage in both coniferous and deciduous trees. Atlantic Canada saw an increase of approximately 1.5 growing season frost days per year within the last three decades (Liu et al. 2018). The regions with the biggest increases in growing season length have experienced increasing frost events during the growing season, and this trend should continue in the future (Liu et al. 2018). Delayed timing of bud set and delayed development of frost hardiness will both be possible in the new climate.

1.2 Climatic adaptation

The capacity of long-lived trees to keep up with today's rapidly changing climate is still under intense debate (Richter et al. 2012). This rate of change challenges the natural genetic adaptability of forest populations, leading to a phenomenon known as adaptation lag (Matyas, 1996, Leites et al., 2012). The adaptive capacity of trees to changes in climate such as increasing frost anomalies hinges on the slow, evolutionary adaptation of population genetics, along with the capacity of individuals to quickly acclimate using phenotypical plasticity (Geburek et al. 2008, Gratani, 2014). Phenotypic plasticity is considered a key mechanism for a tree's adaptation to rapid environmental change. For example, the genetics of red spruce may determine the level of frost hardiness the population can attain, but individual phenotypic plasticity of the individual tree may determine the degree to which the tree is resistant or resilient to frost injury. Species with large geographic ranges usually have more intraspecific genetic variation in phenology, physiology, and growth rate (Gratani, 2014), but differences in environmental stress response can still be observed in species with less genetic variation, suggesting phenotypic plasticity also plays a role (Nahum et al., 2008).

Assessing adaptive capacity to frost events among populations is important to better inform management decisions such as assisted migration (human-assisted movement of species in response to climate change). To do so, it is important to examine traits that might vary by geographic clines. Genetic studies have confirmed the importance of allelic and individual genetic diversity in woody plants (Leites et al., 2012). Variation in growth characteristics is evident across geographic clines, indicating a plastic growth response to local environmental conditions (Gratani, 2014). However,

most studies on tree climatic sensitivity focus on species-level responses, discarding the large amount of intraspecific variability that reflects the population's geographic distribution (Matyas, 1996).

Species with large intraspecific genetic differentiation have shown geographic differences for important traits such as cold hardiness (Leites et al., 2012). Variation in both growth and cold hardiness is clinal, that is, differences between populations change gradually along a latitudinal gradient, but the slopes of cold hardiness and growth gradients are opposite in many species (Leites et al. 2019). For example, populations of Western white pine (*Pinus monticola* Douglas. ex D.Don.) from the north that were planted further south (warming) generally had high growth potential and low cold hardiness, and southern populations moved north (cooling) exhibited low growth potential and high hardiness (Rehfeldt, 1984). This pattern has also been found in species such as black cherry (*Prunus serotina* Ehrh.), black walnut (*Juglans nigra* L.), and more (Leites et al. 2019).

The development and degree of cold hardiness in trees depends on the timing of cold acclimation in the autumn (bud set or hardening), the rate of acclimation, the degree and maintenance of frost hardiness during winter, and the rate of deacclimation during winter thaws and spring (bud break or dehardening; Wisnewski et al. 2003). Many studies have explored the measurement and comparisons of cold hardiness in woody plants, and a multitude of methods have been developed (Burr et al. 1990, Burr et al. 2001). Temperature stress in trees results in symptoms of injury that can range from visual damage such as necrosis of leaves, or less obvious cellular symptoms that can be detected with methods that measure cell health. For example, a visual assessment is

widely implemented to assess frost damage by assessing visual signs of leaf damage like discoloration following freezing but is usually used in combination with other methods (Sutinen et al. 1992, Repo et al. 1992, Lim et al. 1998). Impedance analysis (electrical impedance spectroscopy) measures the amount of extracellular resistance in plant tissues, which demonstrates cellular breakdown (Burr et al. 2001). Chlorophyll fluorescence detects small changes in the photosynthetic system resulting from freezing injury, and finally, the electrolyte leakage method measures the cellular breakdown that occurs after freezing damage, where cold-induced weakening of the plasma membrane results in the release of intracellular electrolytes. When samples are placed in water, the electrolytes can be measured by way of relative conductivity (Murray et al. 1989, Kovaleski and Grossman, 2021). Each of these methods have benefits, shortcomings, and issues of standardization highlighted in Chapter 2.

Photoperiod and temperature determine the timing and rate of bud set in the autumn and this will induce and perpetuate the attainment of maximum cold tolerance levels in the winter (Schaberg and DeHayes, 2000). Early work highlighted the importance of photoperiod for budbreak, bud formation, and dormancy, but in most cases, there is a strong confounding effect between photoperiod and temperature that is difficult to disentangle (Matyas, 1996). Strong clinal patterns in the timing of bud set and growth cessation were found in white spruce (*Picea glauca*) and Siberian spruce (*Picea obovata* Ledeb.) A general increase in the expression of genes and regulators that control their phenology was found with an increase in night length (Chen et al. 2014). The phenology of white pine (*Pinus strobus* L.) is also clinal, especially during autumn, and the genetic control of acclimation appeared to be triggered by a short photoperiod in

northern populations, as well as an extended growing season in southern seed lots (Fréchette et al. 2020). Nevertheless, temperature is currently considered the most important environmental control that regulates cold hardiness in coniferous trees, but under species migration, photoperiodic constraints may also affect the success of populations (Fréchette et al. 2020).

Leinonan et al. (1997) found that deacclimation in spring is dependent on temperature in two ways; it has a direct, short-term effect on the cold hardiness, such that fluctuating temperatures cause fluctuation in the level of cold hardiness. Secondly, high temperatures during deacclimation have a long-term effect on cold hardiness through the life of the tree. This has also been described as a reduction in hardening competence (Leinonen et al. 1997). Thus, an increase in temperature in autumn and spring results in an extension of the growing season causing plants to delay cold acclimation in the fall and accelerate deacclimation in early spring (Ball and Hill, 2009). Considering growing season frost days are expected to increase, this will increase the risk of frost damage (Leinonen et al. 1997).

1.3 The red spruce decline

All tree species are not equally vulnerable to frost events, and red spruce (*Picea rubens* Sarg.) is known to be especially susceptible to frost injuries (DeHayes et al. 1990, Schaberg et al. 2008) due to its high sensitivity to temperature fluctuations (Strimbeck et al. 2015). The proximity of red spruce glacial refugia to the ocean, which likely buffered temperature variations, has been hypothesized to explain the lower species capacity to develop deep cold hardiness (Schaberg and DeHayes, 2000).

An important species of the Acadian Forest Region, red spruce is a late-successional, shade-tolerant conifer that grows naturally in the temperate forest regions of northeastern North America (Dumais and Prevost, 2007). In addition to its sensitivity to frost, it is a species of particular concern due to its narrow range, low abundance, and low genetic diversity (Capblancq, 2020). The natural range of the species extends from southeastern Ontario to the Maritime provinces in Canada, to the New England region of the United States. Pockets of red spruce can also be found further south, but only at higher elevations along the Appalachian Mountains (Little, 1999).

Red spruce experienced a historic growth decline between the mid-1960's and2000's in many parts of eastern North America (Schaberg, 2000). Dendrochronology studies showed an abrupt change after 1960 in the relationship between climate and red spruce tree-ring width (Johnson, 1992). This decline coincided with a reduction in growth and the beginning of reports of winter injury and unusually high august temperatures, and low winter temperatures (Johnson, 1992, Johnson et al. 1988, Cook and Johnson, 1989, DeHayes, 1992, DeHayes et al. 1999, Auclair et al. 2010).

Until recently, predicting climate change impact on frost injuries was complicated by the amplifying effects of acid rain on the frost vulnerability of red spruce. In the first years of compliance after Phase I of the 1990 Clean Air Act, implemented in 1995 and 1996, emissions dropped dramatically resulting in less acid deposition (Lynch et al. 1996). Red spruce had displayed an upward trend in growth in the Northeastern US ever since, even through a major winter injury event in 2003 (Kosiba et al. 2013, Kosiba et al. 2018). This change had been attributed to changes in climate such as an extended growing season as well as the changes in acid deposition (Kosiba et al. 2018). Kosiba et

al. (2019) suggest that future warming may be beneficial for red spruce. However, for a northern temperate species, there may be a limit to increases in growth due to warming, and we are still unsure if any potential gain in growth will maintain over time or be cancelled out by the projected increases in climate anomalies such as winter thaws, late-spring frost, and early-autumn frost. Considering temperature fluctuations have been linked with such strong declines of this species, such knowledge is crucial to better inform its future management.

1.4 Red spruce vulnerability to frost events

The vulnerability of red spruce to freezing injury likely involves an impairment in the timing or rate of cold acclimation in autumn, the depth of cold tolerance attained in the winter, or the timing and rate of dehardening in spring (DeHayes, 1992). Specifically, red spruce hardens faster than its associate balsam fir in autumn, and tends to remain hardy longer in the spring, but it does not reach the same level of cold hardiness in the winter (DeHayes, 1992). The predicted increase of frost anomalies in all three of these seasons, suggests the importance of understanding the mechanisms of red spruce vulnerability in the spring and autumn, as well as the winter.

Prolonged winter thaws followed by freezing have been recognized as an important mechanism to incite winter injury in some woody plants (Bourque et al. 2005). Strimbeck et al. (2015) found that only three days after the onset of a winter thaw, mature red spruce had dehardened by an average of 8.6°C, reaching 14°C in some individuals. These partially dehardened conditions lasted 10-19 days after subfreezing temperatures resumed. Compared to balsam fir (*Abies balsamea* L.), red spruce breaks dormancy

sooner during mid-winter thaws (Major et al. 2003). In fact, multiple studies indicate that such rapid dehardening response to winter thaws may be unique to red spruce. For example, Strimbeck et al (2015) found no evidence of dehardening occurring in balsam fir trees from the exact same stand, and DeHayes (1992) found that same response for seedlings of balsam fir and red spruce exposed to simulated thaw treatments (Schaberg and DeHayes, 2000).

Although it was clear in the past that red spruce's susceptibility to frost in the winter had the highest importance, the extension of the growing season presents new concerns over the growth potential of red spruce in the autumn and spring seasons. Comparisons of red spruce provenances have shown that warm-adapted provenances had a two-week extension in autumn growth during the growing season over cold-adapted provenances (McLeod, 1961). This delay in completion of growth is likely to put southern population more at risk from early autumn frosts (McLeod, 1961). Indeed, cold-adapted red spruce provenances have a 4-5 ° C higher cold tolerance than warm-adapted provenances in the autumn (DeHayes et al. 2001).

Freezes that occur late in the spring, after trees have dehardened, have been largely reported to damage established trees, destroy seed crops, and kill regeneration (Hadad et al. 2020). Frost occurring in the spring during the opening of buds is a key predictor of altitudinal and latitudinal range limits for many tree species (Körner et al. 2016, Kollas et al. 2014). We currently lack knowledge on the historical prevalence of spring frost injuries in red spruce. However, an ecological study revealed strong associations between warmer temperatures and increasing frost injury in black spruce (*Picea mariana* Miller), a close relative of red spruce known to be a late spring frost

avoider (Hadad et al. 2020). Frost-rings were reported to increase under warm spring temperature, to be more frequent at warm locations, and to occur in years with an early growing season start (Hadad et al. 2020). If red spruce behaves similarly, an earlier dormancy release in spring with warming could put the species at risk from late spring frost.

1.5 Future risks to red spruce

The potential extension of the growing season influences the autumn and spring phenology, which we know influences the cold hardiness of red spruce (Schaberg and DeHayes, 2000). Thus, we expect variations in seasonal cold hardiness to impact red spruce in ways that have not yet fully been tested. If episodes of frost or prolonged drought become more frequent, the predicted growth of red spruce populations may become limited (Kosiba et al. 2018). Frost events have the most deleterious effect on the growth of trees during episodes of late-spring frost, midwinter thaws, and early-autumn frosts. The predicted increase of these anomalies (Bourque et al. 2005) and the high vulnerability of red spruce to temperature fluctuations and freeze-thaw events (Strimbeck et al. 2015), present a risk to the future growth and recovery of the species (Kosiba et al. 2018).

Compared with other species, red spruce has a lower population size and genetic diversity which could further reduce its ability to cope with climate change (Capblanq, 2020). Specifically, Capblanq et al. (2020) identified three distinct red spruce ancestry groups that occupy different geographic regions; a low latitude trailing edge population growing in the southern Appalachians, a transitional or marginal region in Pennsylvania,

and a core population at the northern part of the species range. However, there may be more than three genetic groups, as Capblanq et al. (2020) only measured one population in all of Canada, and very few in Maine, US, assuming low genetic diversity in northern populations. In fact, DeHayes and Hawley (1988) concluded that northern red spruce populations showed the highest levels of diversity and Rajora et al. (2000) found that there were three major genetic groupings just within northern populations from Ontario and the Maritimes. Testing the full range of the species will help us to understand the potential for phenotypic plasticity in adaptive traits such as cold hardiness (Prakash et al. 2022).

This thesis aims to quantify the effects of cold hardiness on the growth of red spruce. In Chapter 2, I compared and improved upon current methods of cold hardiness testing. I optimized electrolyte leakage methods to quantify the rate of frost damage incurred on red spruce provenances while controlling for the natural decay rate. I hypothesized that i) the time at which leakage is measured (incubation time) can have as much impact on the results as the test temperature; ii) accounting for time provides a standardized and consistent estimate of cold hardiness and can be used to compare cold hardiness between species or populations; iii) warm-adapted provenances incur more cold damage than cold-adapted provenances. For Chapter 3, I assessed the impact of historical frost events in New Brunswick (1976-2017) on the radial growth of red spruce populations. Thus, I compared the cold tolerance of red spruce provenances with their average annual growth as well as their response to historical climate anomalies. I hypothesized that i) frost events will have a deleterious effect on red spruce growth, ii)

the growth of the species covaried with frost anomalies and that iii) warm-adapted populations are more sensitive to frost anomalies than cold-adapted populations.

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Chapter 2 : Comparing cold hardiness of mature red spruce (*Picea rubens* Sarg.) provenances while accounting for time sensitivity of electrolyte leakage testing.

2.1 Abstract

One of the most precise and objective approaches developed to assess cold hardiness in woody plants is to measure levels of electrolyte leakage. However, protocols to measure electrolyte leakage are highly variable between studies and often do not account for the time-dependency of electrolyte leakage, where conductivity increases with time after freezing (incubation). Here, using latest year's growth of seven mature, range-wide provenances of red spruce (*Picea rubens* Sarg.), we measured the rate of electrolyte leakage over one year. We then fit two versions of an asymptotic model to the time-varying electrolyte leakage rates to compare seasonal differences in cold hardiness between provenances. Using the simpler model (1), the electrolyte leakage rate coefficient was derived to compare the frost damage of each provenance. The most warm-adapted provenances (North Carolina and New Hampshire) are both significantly different from the cold-adapted New Brunswick, Quebec, and Maine provenances during acclimation. In the spring, the provenance from Massachusetts incurred significantly higher rates of damage than the one from Maine and North Carolina but there was no apparent pattern or ranking of frost damage that correlates with the mean annual temperature of the seed source, as it did during acclimation. Overall, red spruce is a frost hardy species that may be a good candidate for assisted migration if managers ensure the selection of optimal seed sources, but future increases in frost risk should still be considered..

2.2 Introduction

Woody plants have evolved the ability to cold acclimate at the onset of sub-freezing temperatures in the autumn. They do this by extracellular freezing of apoplast water and deep supercooling when cells withstand freezing temperatures without actually freezing (Marchand, 2014). All boreal and temperate species experience extracellular freezing when exposed to cold enough temperatures, but only certain species experience supercooling. Concentrations of solutes such as sugars in a cell may play a direct role in the development and maintenance of cold hardiness, and this contributes to the variation in hardiness between trees, needle age classes, and seasons (DeHayes et al 2001). Temperature stress in trees results in symptoms of injury that can range from obvious visual damage such as necrosis of leaves, or less obvious cellular symptoms that can be detected with methods that measure cell health.

A variety of methods exist for measuring cold hardiness. They all have in common the controlled freezing of plant tissue but vary in their assessments of frost damage. (1) Visual assessment is a widely implemented method used to assess frost damage by assessing visual signs of leaf damage like discoloration following freezing but is usually used in combination with other methods (Sutinen et al. 1992, Repo et al. 1992, Lim et al. 1998). Issues with visual assessment are obvious in that colour is open to interpretation, and the method can take weeks to produce significant results. It is also true that while this method can confirm whether tissues are alive or dead, it does not identify non-lethal damage (Deans et al. 1995). (2) Differential thermal analysis is based on the principle that tissues release heat when freezing. By comparing the temperature inside cooling buds with external temperature, freezing points are detected with the rise in

temperature associated with release of heat of fusion; first during extracellular freezing, then during intracellular freezing, associated with cell death (Tinus et al. 1985, Marchand, 2014). This method is limited in its application to species where deep supercooling occurs, but results can be obtained the same day (Burr et al. 1990). (3) Impedance analysis (electrical impedance spectroscopy) measures the amount of extracellular resistance in plant tissues (Burr et al. 2001). The plasma membrane acts as an electrical insulator that controls the movement of ions and electrolytes across the cells. A voltage is applied to the plant, and the current flows through cell walls, which produces an electrical impedance, and the magnitude of the impedance can be measured at multiple frequencies using an analyzer (Hussain et al. 2021). Like differential thermal analysis, electrical impedance can be measured the same day as freezing (Burr et al. 2001), although potential lag effects have caused some underestimation of hardiness in the winter (Burr et al. 1990). (4) Chlorophyll fluorescence detects small changes in the photosynthetic system resulting from freezing injury. This is accomplished by flashing green tissue with pulses of light in a specific wavelength and recording the amount of red light emitted back by the photosynthetic system (Burr et al 2001). Green tissue must be used for this method, so this may become difficult in the winter for certain species. The results for chlorophyll fluorescence take 24 - 42 hours to obtain. (5) The electrolyte leakage method measures the cellular breakdown that occurs after freezing damage, where cold-induced weakening of the plasma membrane results in the release of intracellular electrolytes (Burr et al. 1990, Kovaleski and Grossman, 2021). When the apoplastic water has frozen, the vapour pressure difference of extracellular ice and intracellular water results in dehydration (DeHayes et al. 2001) and eventually leads to a

collapse of the cell wall if the plant does not have sufficient cold hardiness (Wisnewski et al. 2003). This method has several advantages: i) changes in leakage follow the seasonal progression of acclimation and deacclimation; ii) it can be done with small amounts of tissue and from various tissues in the tree; iii) it is easily replicated; iv) it can be inexpensive with access to a freezer; and v) it has been determined as a precise, sensitive, and objective method of cold hardiness testing (Burr et al. 2001). However, it can take between five and ten days to obtain results, depending on the electrolyte leakage methods chosen.

The aim of electrolyte leakage measurements is to determine a precise lethal killing temperature for a given species, usually represented by an LT_{50} value that represents 50% of the samples determined dead or fully damaged (Kovaleski and Grossman, 2021; Fowler et al. 1989; Lim et al, 1998). Since 1930, a variety of protocols have been developed to measure electrolyte leakage (Kovaleski and Grossman, 2021). Most studies apply a controlled freezing and thawing that mimic natural rates, but they vary in target temperatures, where some studies use one test temperature, and others use a series. The freezer used by different studies usually result in different cooling rates and the time at which the samples are held at the test temperature varies between studies as well. The time post-freezing when electrical conductivity (EC) rates are measured (incubation time) has a large impact on the conductivity results (Kovaleski and Grossman, 2021), but only few studies have integrated this time sensitivity into electrolyte leakage methods (Murray et al. 1989, Cape et al. 1990, Deans et al. 1995). There are no standardized incubation times, or maximum and minimum control protocols. Further, many studies simply do not include sufficient detail on these critical

parameters in their work, making it very difficult to develop a standardized electrolyte leakage protocol that would allow the comparison of results across studies and the development of a species cold hardiness trait database. Murray et al. (1989) were the first to provide experimental evidence that electrolyte leakage rates post-freezing increase hourly, then saturate following a Michaelis-Menten saturation curve. The authors concluded that rates of electrolyte leakage, instead of electrolytes released after an arbitrary incubation time, resulted in a more reliable estimate of LT_{50} (Murray et al. 1989, Deans et al. 1995). Given the importance of cold hardiness assessments in estimating a species tolerance to freeze-thaw cycles, the time sensitivity of electrolyte leakage must be better explored and understood, especially in the context of climate change. Changes in the frequency, duration, and intensity of frost events are expected, and they have an increasing probability of occurring under a changing climate (Bourque et al. 2005, Strimbeck et al. 2015, Gusta et al. 2009b, Gu et al. 2008).

In this study, we measured the cold hardening process in 54 to 67 year-old red spruce trees, from seven different provenances, grown in common garden experiments, in order to 1) quantify the decay rate in electrolyte leakage and 2) assess if time-sensitive electrolyte leakage models can be used to detect variations in cold hardiness between provenances. Red spruce was historically at risk of frost injury (DeHayes et al. 1990) due to its high sensitivity to temperature fluctuations (Strimbeck et al. 2015). Many cold hardiness tests have been performed on red spruce (Cape et al. 1991, Fowler et al, 1989), but none since the implementation of the Clean Air Act, and the expected recovery of the species (Kosiba et al. 2018).We hypothesize that the time at which leakage is measured has as much of an impact in evaluating differences between provenances as the test

temperature (H1); using a cold hardiness model that accounts for the time-sensitivity of electrolyte leakage provides a consistent good estimate of cold hardiness across time and can be used to compare cold hardiness between populations (H2); and that warm-adapted red spruce provenances will experience higher rates of damage than cold-adapted provenances (H3).

2.3 Methods

2.3.1 Study area

Throughout this paper, we use the term ‘provenance’ when referring to the geographic and climatic origin of a population, and the term ‘population’ when referring to the trees grown from seeds sampled at the provenance. From 1960 to 1964, the Canadian Forest Service (CFS) established two different red spruce provenance studies. The first, called series 14-95, but commonly known as the “Rangewide" study, included 16 provenances covering the entire species range and planted at seven locations. The number of trees per plot, number of replicates, and spacing differed between trials (Figure 2-1, Table A 1). The second study, named series 18 and known as the "Maritimes” study, was planted a few years after, and it included 30 provenances from the Atlantic provinces of Canada planted in 19 trials (Morgenstern et al., 1980). Unlike the Rangewide study, all trials had the same spacing and received the same number of provenances and replications. Each provenance was planted using a 4-tree square plot replicated within ten blocks at each trial using a randomized design. Characteristics of the two red spruce provenance studies, including trial identification code, establishment year and location, tree spacing, average climate (1975-2018) and soil information collected in

2013 by the Canadian Forest Service can be found in Table A 1. The Maritimes and Rangewide studies have a total of 46 provenances and 12 trials.

Six red spruce provenances were chosen for this chapter from Rangewide trial 14A, and one provenance from Maritime trial 18B2, both located at the Acadia Research Forest in New Brunswick. To determine an attainable number of samples to collect (and therefore an appropriate number of provenances and replicates), we calculated available space in the lab fridge, freezer, and also incorporated travel time and access to the provenance trials. We also selected provenances that tree cores were collected for in the tree-ring dataset used for Chapter 3 (Table 2-1). The seven provenances we selected were chosen to cover the full range of the species, a 5°C gradient in mean annual temperature (MAT) and showed low hybridization with black spruce.

Table 2-1 - Provenances selected for this red spruce (*Picea rubens* Sarg.) study showing location of seed source, provenance ID (ID), growing degree days (GDD), mean annual temperature (MAT), frost free period (FFP), mean winter temperature (MWT), and elevation (mASL) in order of coldest to warmest.

Location of Origin	**Provenance ID**	**Growing degree days (days)**	**Mean annual temperature (°C)**	**Frost free period (days)**	**Mean winter temperature (°C)**	**Elevation (meters above sea level)**
Rocky Brook, NB	15	1575	3.8	117	-9.9	339
Valcartier, PQ	2032	1749	5.7	129	-7.4	167
Amherst, ME	2030	1860	6.5	135	-5.5	123
Corberrie, NS	2101	1605	6.5	124	-9.8	67
October Mountain State Forest, MA	2022	1926	6.6	145	-4.4	514
Andorra Forest, NH	2031	2151	7.6	146	-2.3	444
Indian Gap, NC	2019	2205	9.1	149	0.2	1187

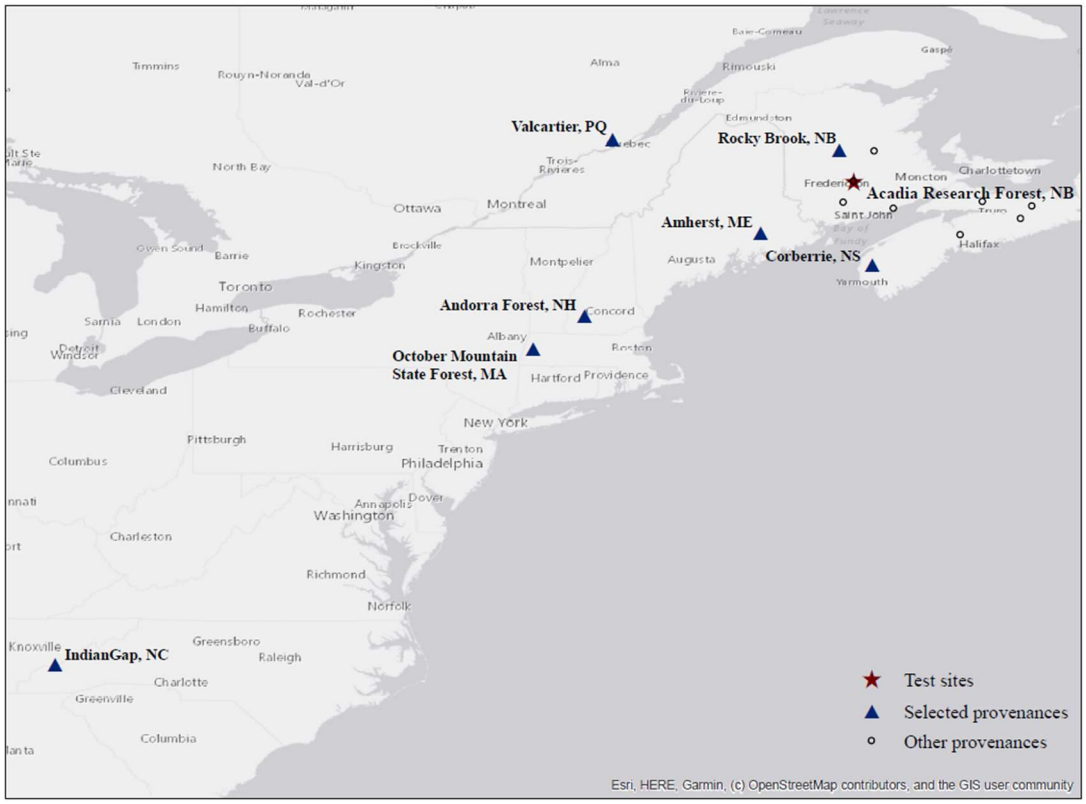

Figure 2-1 - Seed origins (blue triangle) of the seven provenances selected for cold hardiness testing from trials at Acadia Research Forest, NB (red star).

2.3.2 Sampling methods

Lateral shoots of the current year's growth in the upper canopy (12-14 m) were harvested from mature trees (16-18 m in height) with a pole pruner in each of the seven provenances every two weeks in spring 2021, fall 2021 and spring 2022, for a total of 17 collections, to capture the acclimation and deacclimation periods. We had observed multiple winter thaws that year, but road conditions and complications due to the pandemic made many of our planned mid- winter collections impossible. We collected two non-shaded branches from each tree, and two trees were sampled per provenance. Our aim was to collect from the southern aspect of each tree, but realized many branches

were shaded on different aspects of each tree at 14m, and so we determined any branch in a gap that received sunlight would be more beneficial than maintaining a similar aspect. All the samples trees and branches looked very healthy in the stand, so presence of insect damage was considered but was not of major concern. Harvested shoots of latest years growth (including needles, stems, and buds) were sealed in polyethylene bags and stored at 4°C (Sheppard et al. 1993). The same trees were harvested throughout each season, but a new set of trees was sampled the following season to limit tree damage. Bud break was assessed visually using a high resolution SeBaView® camera for each provenance on each harvest date in the spring.

2.3.3 Cold hardiness testing

On each date, we weighed two replicated 1 g samples per tree, per provenance and placed each sample in 100 ml vials. We repeated this for each of the three test temperatures for a total of 84 samples (two replicates per tree, two trees per provenance, seven provenances, three treatments). All samples were filled with distilled water and left to soak for 20 minutes, before shaking vigorously to wash any external electrolytes from the samples. Of the 84 samples, two replicates per tree per provenance (total=28) remained unfrozen and were kept at 4°C as a control, while two replicates were frozen at -10°C and two other replicates at -30°C (mild and extreme, respectively) with a CryoMed 7452 controlled-rate liquid nitrogen freezer. Shoots were cooled at $6°C \cdot h^{-1}$ (minimum for the freezer is $0.1°\ C\ min^{-1}$), maintained at the test temperature for three hours, then thawed at $12°C \cdot h^{-1}$ ($0.2°C \cdot min^{-1}$) up to 4°C and then transferred to the fridge (4.0 ± 0.5 °C) overnight.

Following the freezing treatment, 25 ml of deionized water was added to each vial and the base electrical conductivity (EC at 0 hours) of the bathing solution was measured using a benchtop conductivity meter (Orion Star A122; Fisher Scientific). The samples were measured again 24, 48, 72, 120, and 168 hours after the initial measurement and placed back in the fridge between measurements to minimize microbial growth, for a total of six measurements per sample. Maximum EC (EC_{max}), i.e. when all membranes are disrupted and solutes released, was determined by cooking the samples in solution for 4 hours at 95° C and remeasuring their conductivity after cooling.

Non-frozen (EC_{ctrl}) and cooked conductivity values (EC_{max}) were used to standardize the frozen conductivity values (EC_{test}). Non-frozen control values represent excess conductivity from natural cell decay and microbial growth (Kovaleski and Grossman, 2021) and were subtracted from frozen electrolyte leakage values. Non-frozen conductivity values remained stable over storage time, showing that storage at 4°C between measurements was sufficient to prevent microbial growth (Figure 2-2). The 20% increase in damage seen after budbreak is accounted for by standardization.

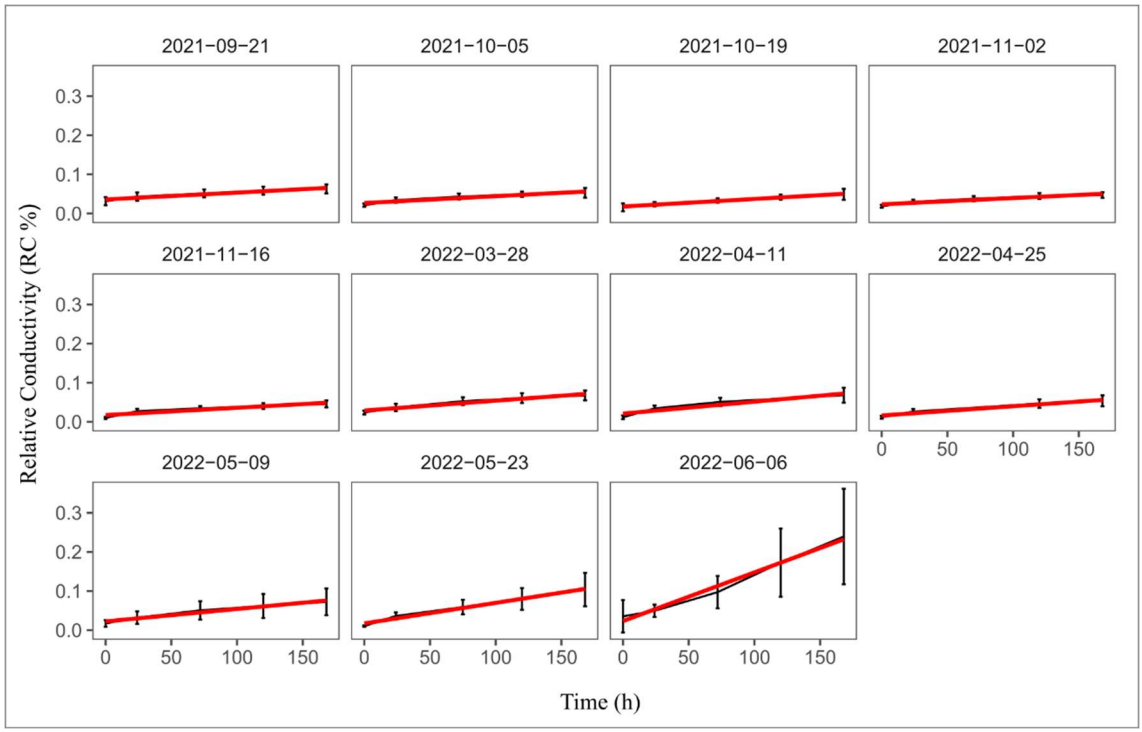

Figure 2-2 – The 4°C raw conductivity values showed a linear relationship with incubation time. The values remained stable throughout most of the experiment, increasing damage only occurred after budbreak (June 6). The red line is the linear model predicted conductivity and the black line is the average measured conductivity. Error bars represent standard deviation of the mean.

The EC_{max} represent the maximum attainable leakage and was used to scale the observed EC values. Relative conductivity (EC_{rel} in %) for each sample was calculated as the ratio of the EC of a freeze-stressed sample, minus the EC value of the control, non-frozen sample (Kovaleski and Grossman 2021), over EC_{max}:

$$EC_{rel} = \frac{EC_{test} - EC_{ctrl}}{EC_{max}} * 100$$

We then estimated the electrolyte leakage rate coefficient (κ in %·hour*)* of each sample by fitting a non-linear regression model on the calculated RC values at each time step after freezing. Two variations of the model were tested. The first model derived from Murray et al. (1989), uses a single parameter (κ), to determine EC_{rel}:

$$EC_{rel} = 1 - e^{-\kappa t} \qquad (1)$$

where *t* is the number of hours since freezing. A second, two-parameter asymptotic exponential model was tested which included a modifier for the asymptote value:

$$EC_{rel} = \alpha(1 - e^{-\kappa t}) \ (2)$$

where α represents the asymptote of the curve. The nls function in R (Version 4.2.0) was used to determine the non-linear least-squares estimates of the parameters of the models (α, κ).

2.3.4 Statistical analyses

The variation explained by each model was calculated by comparing the residual variation with the total variation (Crawley et al. 2012). Differences in electrolyte leakage rates (k) between provenances were determined for each frost acclimation period. The tests were divided into acclimation (September 21 – November 2), winter (November 16 and March 28: the start and end of the season), and deacclimation (April 11 – June 6). Each period was statistically analyzed for differences with a two-way ANOVA using provenance and harvest date as fixed factors, followed by a Tukey HSD post hoc test in the package DescTools (Version 0.99.45). Tests for homoscedasticity, linearity, and normal distribution were performed before analysis (Figure A 1). Results of the studentized Breusch-Pagan tests were significant at $p < 0.05$ for both acclimation and

deacclimation datasets and therefore the data was heteroscedastic. This was expected as southern provenances have been known to have greater variance. We then added a Welch correction to the denominator degrees of freedom in the F-test and the results were still significant. All analyses were run in R software (Version 4.2.0).

2.4 Results

2.4.1 Electrolyte leakage must account for time sensitivity

Relative conductivity values displayed an asymptotic relationship, starting by increasing with incubation time, followed by saturation. The higher the incubation time after freezing samples, the higher the conductivity reading. Many clear differences can be seen in conductivity values depending on the time after freezing that the readings are conducted (Table 2-2). and we observed that the curve and point of saturation changed dependent on harvest date (Figure 2-3).

Table 2-2 – Differences in average relative conductivity (EC_{rel} %) between incubation times. Compares 0 - 24 hours, 24 - 72 hours, 72 - 120 hours, and 24- 120 hours.

Hours	Sep 21	Oct 5	Oct 19	Nov 2	Nov 16	Mar 28	Apr 11	Apr 25	May 9	May 23	Jun 6
0/24	8	8	9	4	1	1	8	9	18	9	22
24/72	21	18	16	9	2	1	14	20	20	25	22
72/120	11	14	15	9	2	1	10	3	11	14	6
24/120	32	33	31	18	3	1	24	13	31	39	28

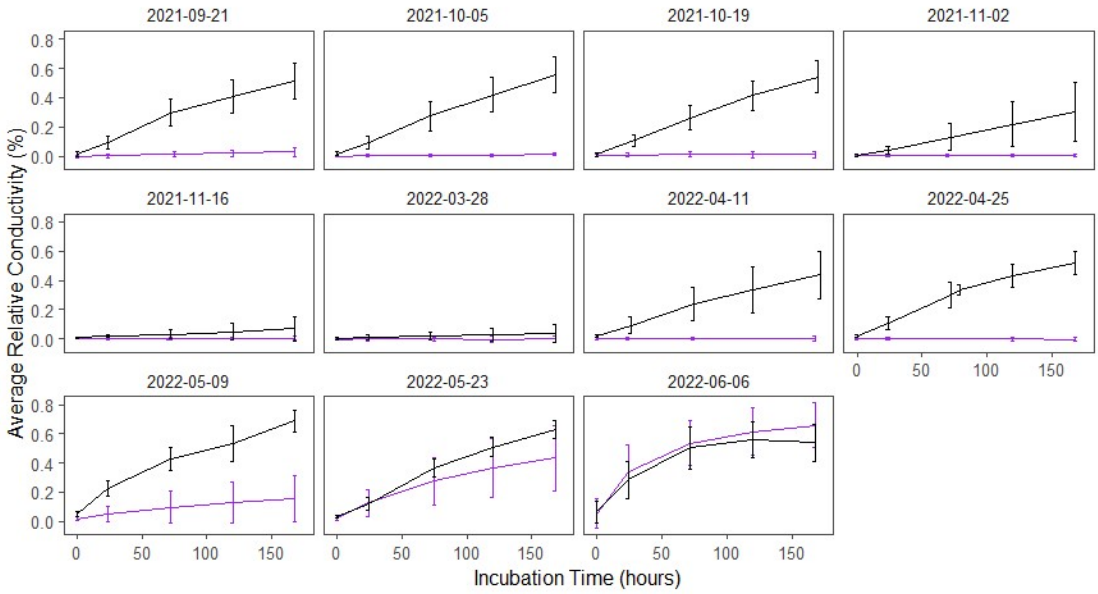

Figure 2-3 – Average conductivity readings (standardized) by harvest date showing the importance of incubation time when measuring electrolyte leakage. The black line is the -30°C treatment and the purple is the -10°C treatment. Error bars represent standard deviation above and below the mean.

The seasonal progression of cold hardiness in red spruce can be seen by viewing the average electrolyte leakage (Figure 2-4). In September, the trees experienced high damage, and as they progressed through the fall they began cold acclimation, which is fully reached by mid-November. The spruce trees were frost hardy at the end of March and began deacclimation in early April. Deacclimation was a slower process in red spruce than acclimation. On May 19th, 2022, there was a late spring frost in Fredericton, NB, and this may explain the dip in frost damage to the samples harvested on May 23, 2022. Bud burst occurred within the two weeks between May 23, 2022, and June 6, 2022. The 24-hour averages are mostly indistinguishable between the -10°C and -30°C treatments,

supporting our hypothesis that the incubation time, and also the time of year, are just as important as the treatment temperatures to determine a representative cold hardiness value (H1).

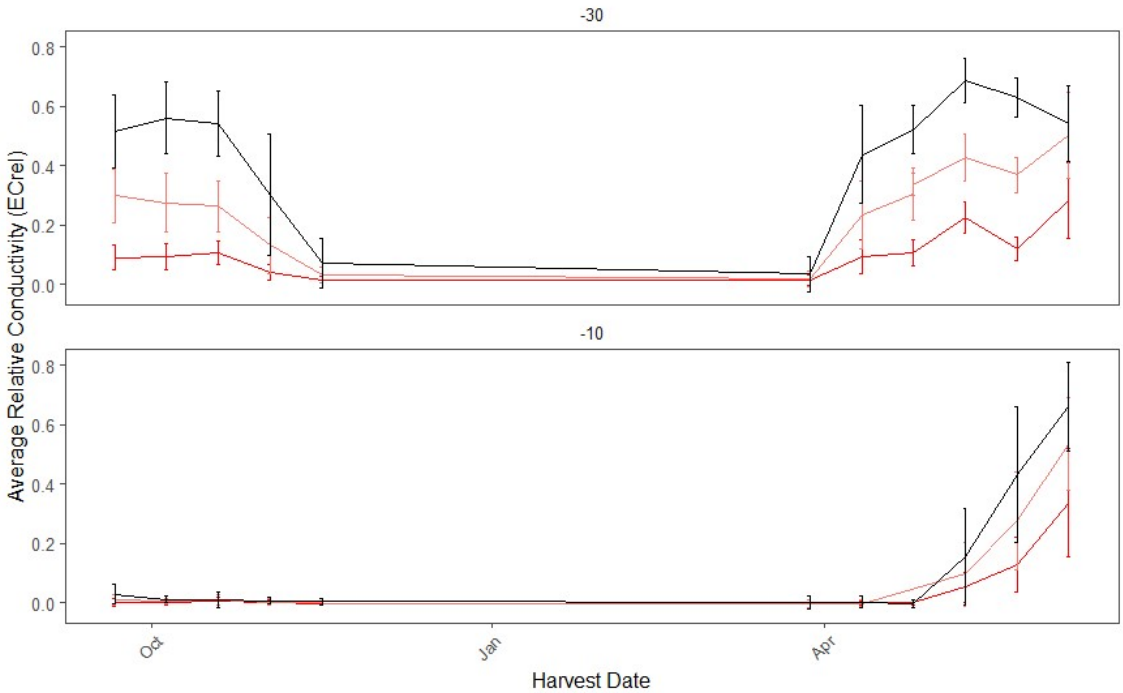

Figure 2-4 – The seasonal progression of cold hardiness in our red spruce study at both the -30 and -10 test temperatures. Each line represents average ECrel values at a different incubation time. Black = 168 hours, orange = 120 hours, red = 24 hours. Error bars represent standard deviation above and below the mean.

The red spruce in this study were frost hardy to below -10°C on all dates except during pre and post budbreak (May 23 - June 6th). For this reason, the remainder of the analyses and the case study were done with the standardized -30°C treatment.

2.4.2 A model to represent cold hardiness

The one-parameter model (model 1) converged with 99.3% of EC_{rel} time series by provenance, date, and test temperature. Convergence issues were observed when fitting model 1 to the dates during dormancy, given their near-zero EC_{rel} values. The 2-parameter model (model 2) successfully converged for only 78% of the time series. Model convergence issues were mostly associated with late fall and early spring (late acclimation and dormancy) harvest dates, when EC_{rel} values are low and remain stable over time. Despite the reduced ability of model 2 to fit low EC_{rel} values, model 2 was better able to track the time sensitivity of EC_{rel} values, as measured by comparing the explained variation between models. The simpler model 1 appears more appropriate than model 2 in the fall and spring because a simple model is best, and the difference between models was not significantly better, with the notable exception of late spring as the trees deacclimate and progress toward budbreak, displaying a stronger saturation signal in EC_{rel} values. Once budbreak occurs, the two-parameter asymptotic model (2) fits better for most provenances (Table 2-3).

Table 2-3 - Explained variance (in %) for the standardized Murray model (1) and the standardized modified model (2) by provenance on each harvest date, with significant differences highlighted and with an asterisk. Significance codes: p value < 0.001 ***; < 0.01 **; < 0.05 *.

		Acclimation				Dormancy		Deacclimation				
Date > Model		Sep 21	Oct 5	Oct 19	Nov 2	Nov 16	Mar 28	Apr 11	Apr 25	May 9	May 23	Jun 6
NB	1	59.2	66.4	84.3	54.2	15.9	28.1	92.9	97.2	98.0	96.9	53.1 ***
	2	59.2	NA	84.8	NA	19.2	29.9	92.9	97.5	98.3	96.9	**82.4**
NS	1	98.2	85.6	66.8	25.9	27.8	NA	85.7	92.3	88.4	98.2	72.2 ***
	2	98.6	85.6	66.8	26.1	27.8	NA	86.0	92.9	89.6	98.2	**88.5**
QC	1	95.3	97.6	95.9	53.9	NA	46.3	72.7	97.8	94.3 *	85.8	34.3 ***
	2	95.5	98.0	96.5	55.2	42.4	46.3	73.1	98.2	**96.1**	85.9	**72.6**
ME	1	75.1	91.7	94.4	55.2	40.7	18.7	83.6	75.3	81.5	93.1	55.9 *
	2	81.9	NA	NA	NA	NA	NA	83.9	75.3	82.4	93.1	**67.8**
MA	1	91.4	98.2	98.3	90.9	21.3	12.1	97.3 *	96.9 *	96.1 *	99.3	46.3 ***
	2	91.4	98.4	98.7	NA	24.3	12.3	**98.2**	**97.5**	**97.0**	99.3	**83.6**
NH	1	97.3	97.3	99.3	50.0	50.0	NA	46.9	95.8	96.7 *	98.5	69.0
	2	97.7	97.4	99.4	50.0	50.0	NA	NA	96.4	**97.4**	98.5	71.6
NC	1	96.9	98.5	95.5	83.6	61.5	NA	41.0	88.9	69.5	97.3 *	0.8 **
	2	97.0	98.7	95.5	NA	66.3	NA	41.1	89.5	70.9	**98.0**	**38.2**

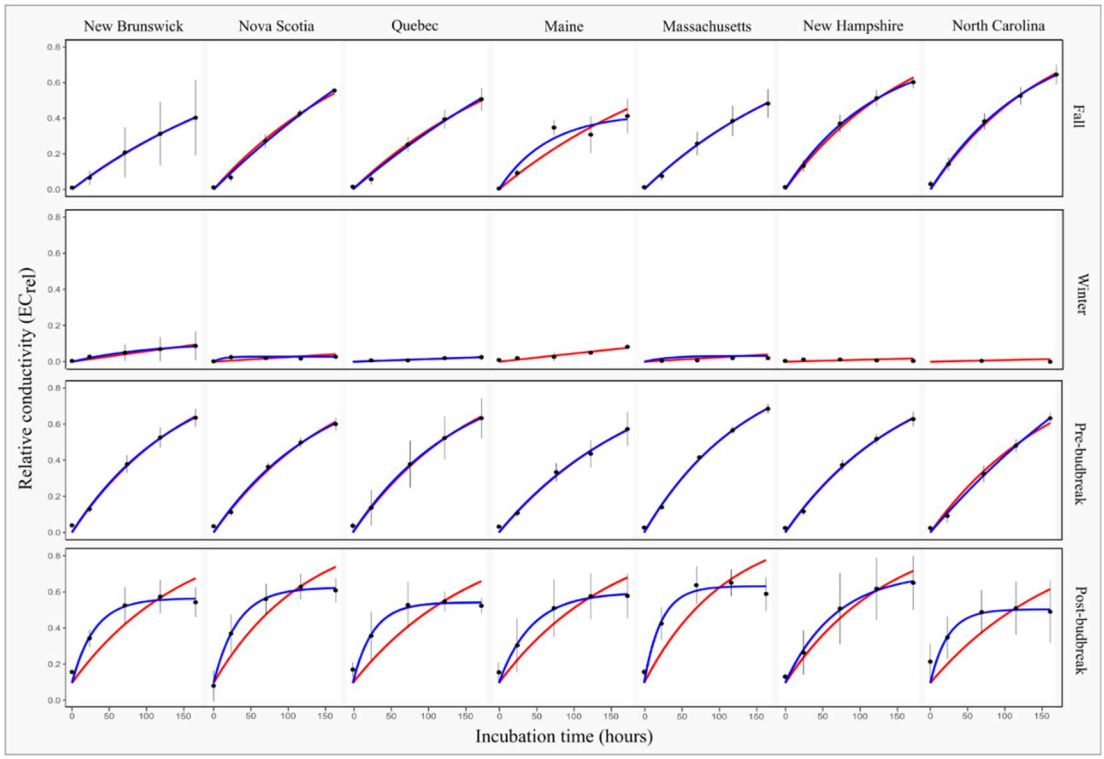

Figure 2-5 - Comparison of models 1 (red) and 2 (blue) fitted on standardized relative conductivity values at the -30°C test temperature, during fall (September 21, 2021), dormancy (March 28, 2022), and spring pre-budbreak (May 23, 2022) and post-budbreak (June 6, 2022). The error bars represent standard deviation above and below the mean.

2.4.3 Cold acclimation shows clinal variation among red spruce provenances

Using the simpler model for the data, recommended by Murray et al (1989) (model 1), the electrolyte leakage rate coefficient was derived to compare the frost damage of each provenance, throughout the seasons. The results of all provenances followed the typical pattern of cold acclimation in the fall, starting with high rates of damage in early September, then lower rates through October and into November. This is particularly apparent in the warm-adapted provenances from North Carolina and New

Hampshire, and not as apparent in the coldest provenance, New Brunswick, which acclimates at a steadier rate through October. All provenances showed almost zero damage in mid-November and the end of March as they remain dormant for winter. Deacclimation in springtime followed the same seasonal progression found in the raw electrolyte leakage data, including the rise and fall in rates of damage from May 9 to May 23 (Figure 2-6).

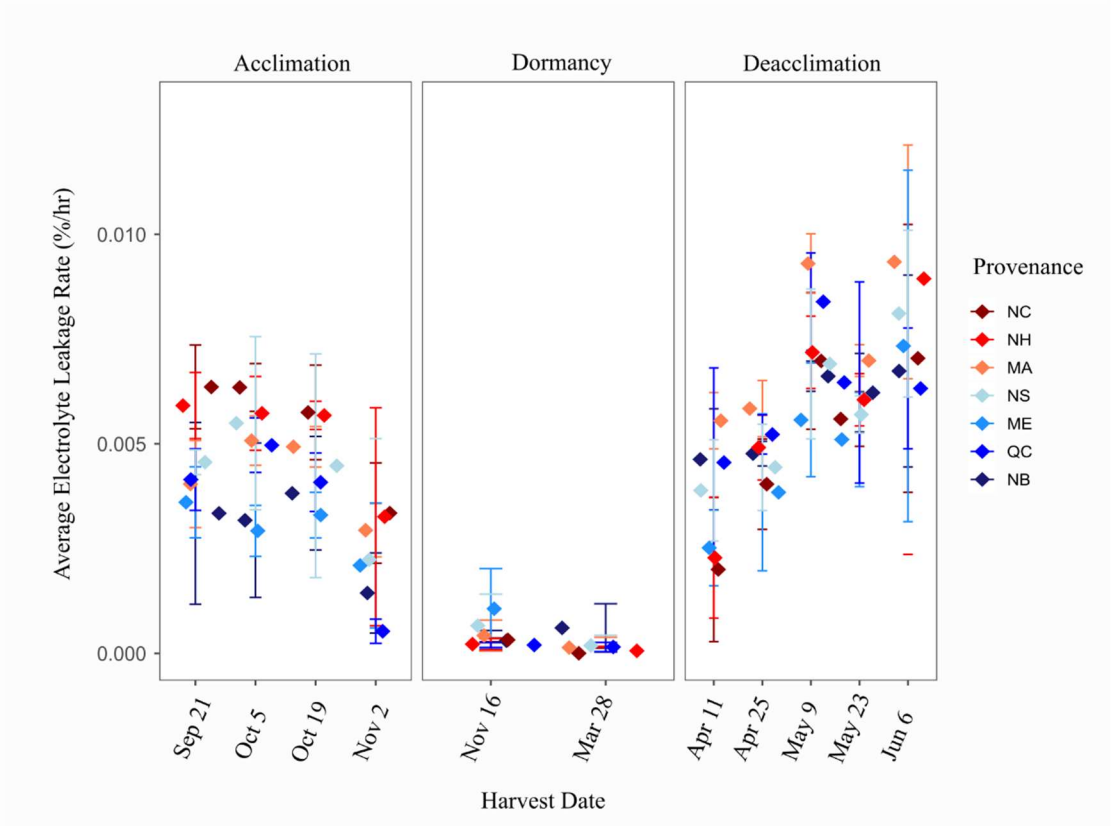

Figure 2-6 – Electrolyte leakage rates (k) showing the seasonal progression of cold hardiness by provenance. Error bars represent standard deviation above and below the mean.

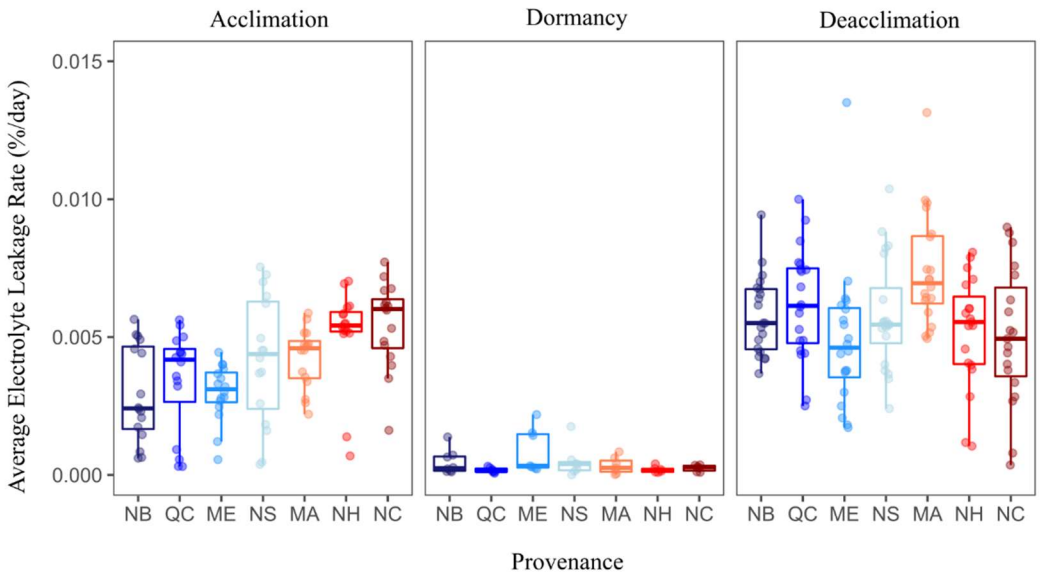

Figure 2-7 -A summary of each season's electrolyte leakage rates, showing observed differences in cold hardiness between provenances. The provenances are arranged by mean annual temperature of the seed source from left to right and also from coldest (blue) to warmest (red). The letters indicate groupings of significant differences from a to b ($p < 0.05$)

The results from November 16 to March 28 (winter) showed no significant differences between provenances, as expected because they are all frost hardy, and so they were removed from the analysis. We found significant differences between provenances in the fall season while the trees were experiencing acclimation. The most warm-adapted provenances (North Carolina and New Hampshire) are both significantly different from the cold-adapted New Brunswick, Quebec, and Maine provenances (Figure 2-7).

In the spring during deacclimation and budbreak, the provenance from Massachusetts incurred significantly higher rates of damage than the ones from Maine

and North Carolina, and there was no apparent pattern or ranking of frost damage that correlates with the mean annual temperature of the seed source, as it did during acclimation (Figure 2-7).

2.5 Discussion

2.5.1 Time sensitivity of electrolyte leakage

In this study, we quantified the decay rate in electrolyte leakage by standardizing the maximum and minimum controls and producing an electrolyte leakage rate. The seasonal progression of cold hardiness can be observed with the raw electrolyte leakage data, but the raw conductivity values at each incubation time produced a different seasonal cold hardiness curve. Temperature curves would be difficult to accurately produce using only a single arbitrary incubation time. Thus, different LT_{50} values would be produced dependent on the incubation time selected, making comparisons between studies difficult. The time sensitivity is important to consider on all harvest dates during acclimation, deacclimation, and budbreak, Contrary to some other studies that recommend a 24-hour or 48-hour (Kovaleski and Grossman, 2021) incubation time, in our study, the seasonal progression of cold hardiness is best seen after 5 – 7 days, in fact in most cases the red spruce did not see 50% damage until they had incubated for over 5 days after freezing. Even with this observation, the values at these incubation times would not be able to be compared between studies due to differences in maximum control methods (Kovaleski and Grossman, 2021). Many studies still debate the most appropriate controls to use in electrolyte leakage methods. We used a maximum damage control that included cooking in the oven for 95°C for 240 minutes, then waiting 24 hours before

measurements. This compared with other studies that use autoclaving at 121°C for 120 minutes (Deans et al. 1995), or submersion in liquid nitrogen (Kovaleski and Grossman, 2021). Whether using heat killing or liquid nitrogen as a maximum leakage control, the time sensitivity of electrolyte leakage must be considered.

We hypothesized that the time at which leakage is measured has as much of an impact in evaluating differences between provenances as the test temperature (H1). We found this to be true when observing the 24-hour incubation EC_{rel} which followed the same seasonal progression of cold hardiness in both the -10°C and -30°C treatment. We recommend taking the time-sensitivity of the leakage into account and considering the asymptotic curve when evaluating electrolyte leakage. We agree that rates of electrolyte leakage, instead of electrolytes released after an arbitrary incubation time, result in a more reliable estimate of cold hardiness. While this method does not require multiple test temperatures, its use in producing temperature curves would produce a much more reliable estimate of LT_{50} (Murray et al. 1989, Deans et al. 1995). We also suggest that an electrolyte leakage rate is much more accurate for comparison between provenances, but that the model proposed by Murray et al. (1989) represents the data best during test temperatures or seasons where the samples are mostly frost hardy. In the fall and spring, model (1) is more appropriate, but model (2) is better as the trees deacclimate and progress toward budbreak. Then once budbreak occurs, the two-parameter asymptotic model (2) fits best. Previous studies have not observed the asymptotic curve as it is observed here after budbreak, and the consequence of selecting the wrong model is an underestimation of the rate of damage to the samples, or an overestimation of the cold hardiness. The first order rate constant (k) derived from electrolyte leakage rate differed

dependent on the model selected. Without considering the shape of the curve that best fits the data, comparisons between populations and studies would be incorrect and inconsistent, respectively.

2.5.2 Case study of red spruce provenances

We hypothesized that deriving electrolyte leakage rates from a cold hardiness model that accounts for the time-sensitivity of electrolyte leakage would provide a consistent good estimate of cold hardiness and could be used to compare populations (H2). In our study, we assessed if time-sensitive electrolyte leakage models could be used to detect variations in cold hardiness between provenances of red spruce. Red spruce was historically at risk of frost injury (DeHayes et al. 1990) due to its high sensitivity to temperature fluctuations (Strimbeck et al. 2015). Many cold hardiness tests have been performed on red spruce (Cape et al. 1991, Fowler et al, 1989), but none since the implementation of the Clean Air Act, and the expected recovery of the species (Kosiba et al. 2018).

In support of our hypothesis (H3), we did find significant differences between provenances in the fall season while the trees were experiencing acclimation. The most warm-adapted provenances (North Carolina and New Hampshire) are both significantly different from the cold-adapted New Brunswick, Quebec, and Maine provenances (H3). The coldest provenances (besides Nova Scotia) consistently had the lowest electrolyte leakage rate (deepest cold hardiness), and the warm-adapted provenances consistently had the highest rate (least cold hardiness). Differences in cold hardiness between red spruce provenances have been detected in the past (DeHayes, 1990, DeHayes et al.

2001), In a similar study, DeHayes et al. (2001) found that a tradeoff occurred between fall and spring. In their study, they found the same pattern in fall cold hardiness as our study, but in the spring the cold-adapted provenance from Quebec was the least cold hardy, and the warm-adapted provenance North Carolina was the most cold hardy. This may suggest that the cold-adapted provenances invest their resources into cold hardiness during acclimation, but deacclimate sooner than warm-adapted provenances in the spring, putting their investment in growth. This is in support of a phenomenon known as a growth-cold hardiness tradeoff (Leites, 2019, Schaberg, 2000).

We did not find significant differences between provenances in the spring that correlated with the mean annual temperature of the seed source. In the spring during deacclimation and budbreak, the provenance from Massachusetts incurs significantly higher damage than the ones from Maine and North Carolina. The variance in the MA results was much smaller than the other provenances, suggesting there may have been less variation between sampled branches or trees on those dates. In a study by DeHayes et al. (1990) the Massachusetts provenance also consistently suffered the most freezing injury. Otherwise, the provenances seem to deacclimate with no specific pattern, suggesting the trees at these sites respond more individually to the environmental, temperature, and light cues required for budbreak (Zeng et al. 2022). Like our study, Sebastien-Azcona et al. (2019) found that in white spruce, the fall cold hardiness was directly influenced by latitude, where the spring cold hardiness results were not. The environmental cue for bud set in fall is usually influenced more by daylength, where temperature in the form of growing degree days is more influential in springtime bud break. It is also possible the foliar sugar concentrations during acclimation were different

than they were during deacclimation, as we know the differences in sugar concentrations of current-year red spruce needles (specifically sucrose and raffinose (Schaberg et al. 2000)), follow the seasonal pattern of cold hardiness (DeHayes et al. 2001). The way individual trees respond to these variables in the spring may outweigh the importance of light and temperature cues that typically signal the trees to start bud break. We observed a certain degree of within provenance variation (asynchronous breaking of buds within and between trees) and consequently, a potential avoidance of frost damage (Benomar et al. 2022). This would help to explain the much higher variation and outliers seen across provenances in the spring.

2.6 Conclusion

In this study, we measured the cold hardening process in 54 to 67 year-old red spruce trees from seven different provenances, grown in common garden experiments, in order to 1) quantify the decay rate in electrolyte leakage and 2) assess if time-sensitive electrolyte leakage models can be used to detect variations in cold hardiness between provenances. We found that the time at which leakage is measured has as much importance in evaluating differences between provenances than test temperatures. Electrolyte leakage time sensitivity varies according to level of cold hardiness, and this should be considered in future studies to ensure the electrolyte leakage rate is not underestimated, especially near budbreak. Regardless of the method chosen, the time-sensitivity and standardization of electrolyte leakage must be adapted and fully described in future cold hardiness studies if we are to move toward standardization.

Using a cold hardiness model that accounts for the time-sensitivity of electrolyte leakage provides a consistent good estimate of cold hardiness across time and can be used to compare cold hardiness between species or populations. We were able to detect significant differences between the two most warm-adapted provenances and three cold-adapted provenances in the fall, supporting our third hypothesis.

Future red spruce studies should attempt to incorporate winter sampling for cold hardiness, especially during and after winter thaws, as we know red spruce is especially susceptible to winter temperature fluctuations and winter injury. Our study was limited to sampling in the fall and spring months due to unforeseen complications due to the covid 19 pandemic, including limited access to the two red spruce stands in the snow.

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Chapter 3 : Limited frost damage in mature red spruce provenance trials, but evidence for clinal variation

3.1 Abstract

Assisted migration is a silvicultural tool in which warm-adapted trees are moved to colder climates to reduce future global warming impacts on our forests. However, large uncertainties remain on the capacity of warm-adapted trees to adjust to today's colder climate. Notably, red spruce is an important species of the Acadian Forest, and a potential candidate for assisted migration, but the species has been historically sensitive to spring frosts and winter warm spells. In this study, we analyzed ~50 years of annual growth among range-wide populations of red spruce planted in a network of provenance trials in New Brunswick to compare past impacts from frost events between populations. We found no significant impact of frost anomalies on growth since the 1990s, which track the historical reduction in atmospheric sulfur emissions (i.e. acid rain) and its documented effect on red spruce cold hardiness, but report a large drop in growth in 1987 synchronous with a severe spring frost event. The level of growth decline during that event was significantly higher in southern seedlots and covaried with their experimental levels of cold hardiness. Although we find no recent spring frost impact on growth, such clinal response to frost may help inform future assisted migration strategies as frost anomalies are projected to increase with climate change.

3.2 Introduction

Warmer temperatures can pose a threat to trees because of their physiology during acclimation and deacclimation. Photoperiod and temperature determine the timing and rate of bud set in the autumn (acclimation). Temperature has a direct, short-term effect on the cold hardiness during deacclimation in spring and winter such that fluctuating temperatures cause fluctuation in the level of cold hardiness. Secondly, high temperatures during deacclimation have a long-term effect on cold hardiness described as a reduction in hardening competence (Leinonen et al. 1997). Thus, an increase in temperature in autumn and spring results in an extension of the growing season causing plants to delay cold acclimation in the fall and accelerate deacclimation in early spring (Ball and Hill, 2009).

As the growing season extends, the capabilities for the trees to develop a deeper frost tolerance throughout the autumn, winter, and spring are reduced and the result of this is an increased risk of frost damage (Leinonen et al. 1997, Kramer et al. 2000, Marquis et al. 2020), and this may decrease growth (Li et al 2020, Kramer et al. 2000). The consequences of these climate anomalies on growth depends on the level of cold hardiness attained throughout acclimation, dormancy, and deacclimation. Trees acclimate by extracellular freezing of apoplast water and deep supercooling when cells withstand freezing temperatures without freezing (Marchand, 2014). Sugars and other concentrations of solutes in a cell may play a direct role in acclimation to cold, and this contributes to the genetic variation in hardiness between trees, needle age classes, and seasons (DeHayes et al 2001). Considering the strong relationship between temperature, cold hardiness, and the phenology of trees (Fréchette et al. 2020), a further understanding

of the relationship between seasonal growth, cold hardiness, and adaptation to temperature is essential to assess the impact of climate change in the Acadian Forest.

Testing differences between geographic populations of trees, or provenances, can be traced back to the first comparative trials as early as 1745 and 1755 in France (Matyas, 1996). In provenance trials, seeds are collected from a range of naturally occurring seed sources and are planted into common gardens (trials) to compare the performance of tree populations. Originally established to maximize volume growth for silviculture, provenance trials (also known as common garden experiments) now offer excellent opportunities to study intraspecific genetic variation in climatic adaptation in trees (Matyas, 1996; Leites, 2012; Capblancq et al. 2022). By comparing how different seed sources, which have adapted to their climate of origin, perform under different climates, the differences between source and trial site can be treated as an experimental change in climate. Up until recently, the adaptive capacity of trees to environmental gradients was assessed from height or diameter measurements (Matyas, 1996, Leites, 2012, Leites 2019). The analysis of annual growth from tree-rings provides a finer resolution of climate sensitivity, which could reveal past impacts from climatic anomalies (Vitasse et al. 2019, Housset et al 2018) and quantify legacies of climate extremes (Pederson et al. 2014; Anderegg et al. 2015).

Some tree-ring evidence even points to rare, historic frosts that pre-date modern climate records, such as the 1774 spring frost that may have been the trigger for a large-scale red spruce mortality event in the southern US (Pederson et al. 2014). Tree-ring analysis also played a large role in the discovery and documentation of the 1960s red spruce decline in the Appalachian Mountains, but there was much difficulty in

disentangling effects of climatic factors and stand dynamics on the observed growth patterns (Cook and Zedaker, 1992). The major conclusions from the multitude of studies of the red spruce decline were that red spruce had the lowest genetic diversity and least winter hardiness of all common northern conifers, resulting in widespread, episodic winter injury in high elevation forests, made worse by high sulfur content in acid rain in the 1960s (Johnson et al. 1992). Tree rings can provide information on whether these factors led to radial growth reductions in areas that have not yet been studied, or if a recovery of the species has occurred since.

Recent comparison of diameter and height measurements revealed superior growth in cold red spruce populations moved to warmer locations, suggesting limited growth decline from moderate warming in the near future (Li et al. 2020). These results indicate sufficient phenotypic plasticity in the growth of cold-adapted populations to cope with increasing temperatures but did not assess the adaptation capacity of moving warm-adapted populations north under assisted migration management strategies. Applying a tree-ring approach to provenance trials should provide more recent information on population adaptation to climate anomalies and help to support or reject recommendations for seed transfer in a changing climate.

Frost events have the most deleterious effect on the growth of trees during episodes of late-spring frost, midwinter thaws, and early-autumn frosts. The predicted increase of these anomalies (Bourque et al. 2005) and the high vulnerability of red spruce to temperature fluctuations and freeze-thaw events (Strimbeck et al. 2015), present a risk to the future growth and recovery of the species (Kosiba et al. 2018). It is also of concern that forest simulation models project the distribution and abundance of red spruce in this

region will decline towards the end of the century (Ashraf et al. 2015, Taylor et al. 2017). In this chapter, we assessed the impact of frost events over the last 50 years (1976 – 2017) with low growth anomalies in range-wide red spruce populations from four provenance trials across New Brunswick, Canada. We first tested the hypothesis that interannual growth variations were related to the level of frost anomaly across years (H1), then tested the hypothesis that only low growth anomalies were associated with frost anomalies (H2), then tested the hypothesis that warm-adapted populations are more sensitive to frost anomalies than cold-adapted populations (H3).

3.3 Methods

3.3.1 Study area

From 1960 to 1964, the Canadian Forest Service (CFS) established a red spruce provenance trial named 14-95 which included eight provenances planted at four locations. The number of trees per plot, number of replicates, and spacing differed between trials (Table B 1 in supplementary material). The four trials will herein be referred to as sites. 14b from Grimmer Parish, NB is the coldest site, with a mean annual temperature (1991-2020) of 2.6 °C, compared to 4.9-5.7 °C at the three other sites. Precipitation is high at all sites, although 14c is wetter due to its proximity to the Fundy Bay, receiving 1410 mm annually compared to 1059-1182 mm at the three other sites (Table B 1). Eight red spruce provenances were chosen from the four sites for this study (Table 3-1, Figure 3-1), and provenance 15 from the previous chapter is also mentioned in the results for comparison, for a total of nine provenances.

Table 3-1 - Location, climate of origin and experimental cold hardiness of the seedlots under study

Provenance	Location	Growing degree days (GDD)	Mean annual temperature (°C)	Mean winter temperature (°C)	Fall cold hardiness (electrolyte leakage rate (%/day)	Spring cold hardiness (electrolyte leakage rate (%/day)
2019	Indian Gap, NC	2205	9.1	0.2	0.13	0.12
2031	Andorra Forest, NH	2151	7.6	-4.4	0.12	0.14
2022	October Mountain State Forest, MA	1926	6.6	-5.5	0.10	0.18
2101	Corberrie, NS	1605	6.5	-2.3	0.10	0.14
2030	Amherst, ME	1860	5.7	-7.4	0.07	0.12
2100	Sheet Harbour, NS	1621	5.7	-5	NA	NA
2103	Acadia Research Forest, NB	1831	5.6	-7.4	NA	NA
2032	Valcartier, PQ	1749	5.7	-9.8	0.08	0.15
15 *	Rocky Brook, NB	1575	3.8	-9.9	0.07	0.14

*the provenance from Rocky Brook, NB was used for the cold hardiness testing only

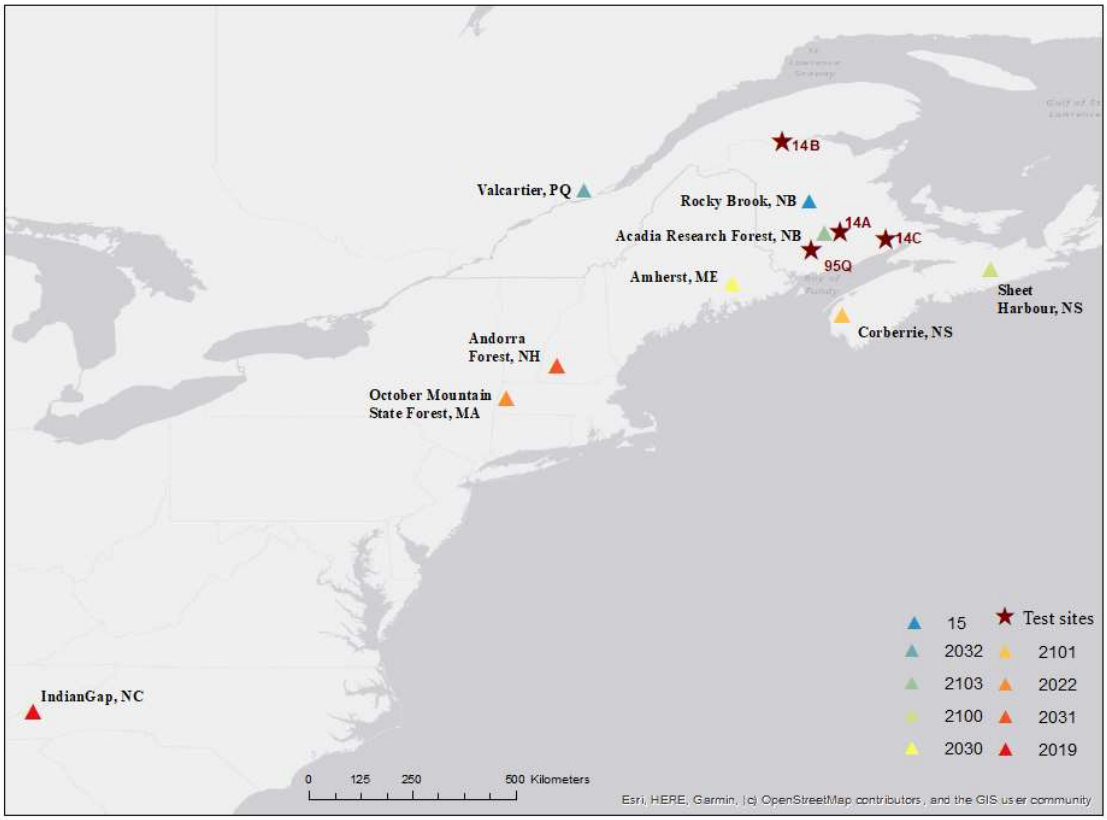

Figure 3-1 - Location of the four trial sites and nine provenances used throughout this chapter.

3.3.2 Climate data

Daily minimum and maximum temperatures for four of the red spruce sites were obtained from Natural Resources Canada (McKenney et al., 2013), covering the period 1980 - 2019. We identified the start of the growing season as the fifth consecutive day during which the daily mean temperature was > 4°, and the end of the growing season defined as the fifth consecutive day after August 1st for which the daily minimum temperature was < 0°C (Huang et al. 2010, Moreau et al. 2020). We defined a midwinter thaw as a period between December and March when the temperature rises above 0 for at least one day, followed by the temperature dropping below -16°C sometime during the

next three days (Heilmen et al. 2000). The use of these temperature thresholds is based on tree dehardening and subsequent tissue damage in the northern Great Lakes region. A biologically significant thaw for yellow birch (*Betula alleghaniensis* Britton) has been defined as a period of maximum temperatures above 4°C for > 4 days, ending when daily minimum temperatures reach -4°C (Bourque et al. 2005). This same definition will be used to define a late-spring frost and an early autumn frost in the Acadian Forest Region because temperatures > 4°C followed by freezes < -4°C influence the dehardening of plant tissue (Cox and Zhu, 2003).

3.3.3 Frost event quantification

To quantify frost events, we ran frost event detection on all years (including non-pointer or regular growth years). We calculated the heat accumulation in degree days (DD) leading up to a spring or fall frost or during a winter thaw. For fall frost, a lower value would represent an earlier frost, and for spring, a higher value would indicate a later spring frost. We also extracted six frost anomaly criteria that indicate thawing and freezing intensity: (1) the number of winter thaw-freeze events, (2) temperature minimum during a spring frost, (3) cumulative DD before a spring frost, (4) cumulative DD during a winter thaw, and (5) cumulative DD before a fall frost, using the ClimatRends package (Version 0.4) and late_frost function in R (Version 4.2.0).

3.3.4 Growth and tree-ring analysis

Ten increment growth cores were collected in 2020 for each of the eight provenances for a total of 80 samples from each of the four sites. They were each dried, sanded at 400 grit and scanned using a SeBaView® camera with a 3000 dots per inch

(DPI) resolution. Annual ring widths were measured and cross-dated using CDendro and CooRecorder. The interseries.cor function in the package dplR (Version 1.7.4) calculates correlation serially between each tree-ring series and a master chronology built from all the other series in the dataset (leave-one-out principle). The mean inter-series correlation was 0.69 for site 14a, 0.52 for site 14b, 0.73 for 14c, and 0.68 for 95q.

To precisely quantify the effects of extreme events on tree growth, it is essential to correctly identify the anomalous growth years (Buras et al. 2020). To do so, pointer year analyses leverage tree-ring data by detecting the years in which most of the trees show growth values that deviate largely from the average, corresponding to unusually narrow or wide growth rings (Jetschke et al. 2019). These years are known in dendrochronology as pointer years and are commonly used to identify climate anomalies (Hadad et al. 2020). Three different methods of pointer year analysis were used in this study. RGC (relative growth change) identifies negative pointer years as years when more than 75% of trees display a 40% reduction in growth over a 7-year moving window (Schweingruber et al. 1990), while NMW (normalization in a moving window, "Neuwirth"; van der Maaten-Theunissen et al. 2015), and ZChron (z-chronology; Jetschke et al. 2019) standardize growth before converting it to standard deviation from the mean, and identify pointer years as years where at least 75% of trees display a standard deviation of at least one. Here, standardization was done using two different approaches: a smoothing spline where the frequency response is 0.50 at a wavelength of 15, and FSS (Friedman super smoother) which is a running lines smoother which chooses between three spans for the lines detrending (Friedman,1984). The NMW method converts growth to standard deviation from the mean along a 13-year moving window,

while the Zchron method converts growth to standard deviation of the mean over the entire growth period in the analysis. In total, the five pointer year approaches were applied to the 1971-2017 growth data, separately for each provenance and site, excluding earlier growth due to lower sample depth, using the packages dplR (Bunn, 2008) and pointRes (van der Maaten-Theunissen et al. 2015, van der Maaten-Theunissen et al. 2021). Years identified by at least three out of five methods were identified as pointer years.

To compare growth response between provenances during pointer years, we derived indices of growth resistance, resilience, and recovery from each individual tree, after Lloret et al. (2011; i.e. resistance, recovery, resilience), Thurm et al. (2016; recovery period, total growth reduction) and Schwarz et al. (2020; average growth reduction, average recovery rate). These indices were calculated for every tree ring-width time series standardized with a smoothing spline (ten trees per provenance) using the following equations:

$$Resistance = \frac{RWI_e}{RWI_{pre^e}}$$

$$Recovery = \frac{RWI_{post^e}}{RWI_e}$$

$$Resilience = \frac{RWI_{post^e}}{RWI_{pre^e}}$$

where RWI_e is the ring width index during the corresponding event year, RWI_{pre^e} is the average ring width index for the four years preceding the event and RWI_{post^e} is the average ring width index for the four years following the drought event. 'Recovery period' is the time needed, in years, to reach pre-disturbance growth levels again. 'Total

growth reduction' reflects the cumulative growth reduction in the year of disturbance as well as the associated years in the recovery period (ten years). To compute BAI for the final growth reduction comparison, we assumed stems to be circular, and used the equation:

$$BAI = \pi \times (R_t^2 - R_{t-1}^2)$$

where R_t^2 and R_{t-1}^2 are the squared radii of the tree for year t and the preceding one, respectively.

3.3.5 Statistical analyses

Pearson correlation analyses were run in R (Version 4.2.0) between the five frost variables and the detrended (spline) ring width indices of the corresponding and following year. Differences between provenances in the resilience indices were tested with a one-way ANOVA using provenance as a fixed factor, followed by a Tukey HSD post hoc test in the package DescTools (Version 0.99.45). Tests for homoscedasticity, linearity, and normal distribution were performed before analysis.

3.4 Results

We find low growth anomaly at all sites during the study period, but in varying frequencies, while the growth of all provenances was generally very similar across years and sites. In site 14a, there was an extreme growth decline observed in 1986 – 1987 (Figure 3-2). It takes a few years for the trees to recover, but then they experience a high growth period. The local provenance from the Acadia Research Forest has the highest recovery and the largest growth in 1990. Another decline in 1999 - 2000 has a more similar response between provenances. Another recovery follows this decline, and then the growth remains stable. Grimmer Parish, NB (site 14b), the coldest site, saw the fewest low growth anomalies. It has only one decline in 2006 and the most warm-adapted provenance from North Carolina is impacted the most. Site 14c in Fundy National Park experiences the most fluctuation in growth with only weak declines and no lagged effects. Growth declines in 1988, 1991, 1994, 1998, and 2009 impacted all trees similarly, and they recovered the following year. Site 95q (UNB Woodlot) experiences a strong decline and a prolonged lagged effect from 1994 - 1997. The trees from New Hampshire remain relatively stable while those from Maine and Quebec are impacted the most. This site also experienced a strong decline in 2009, where the recovery was immediate and similar across provenances.

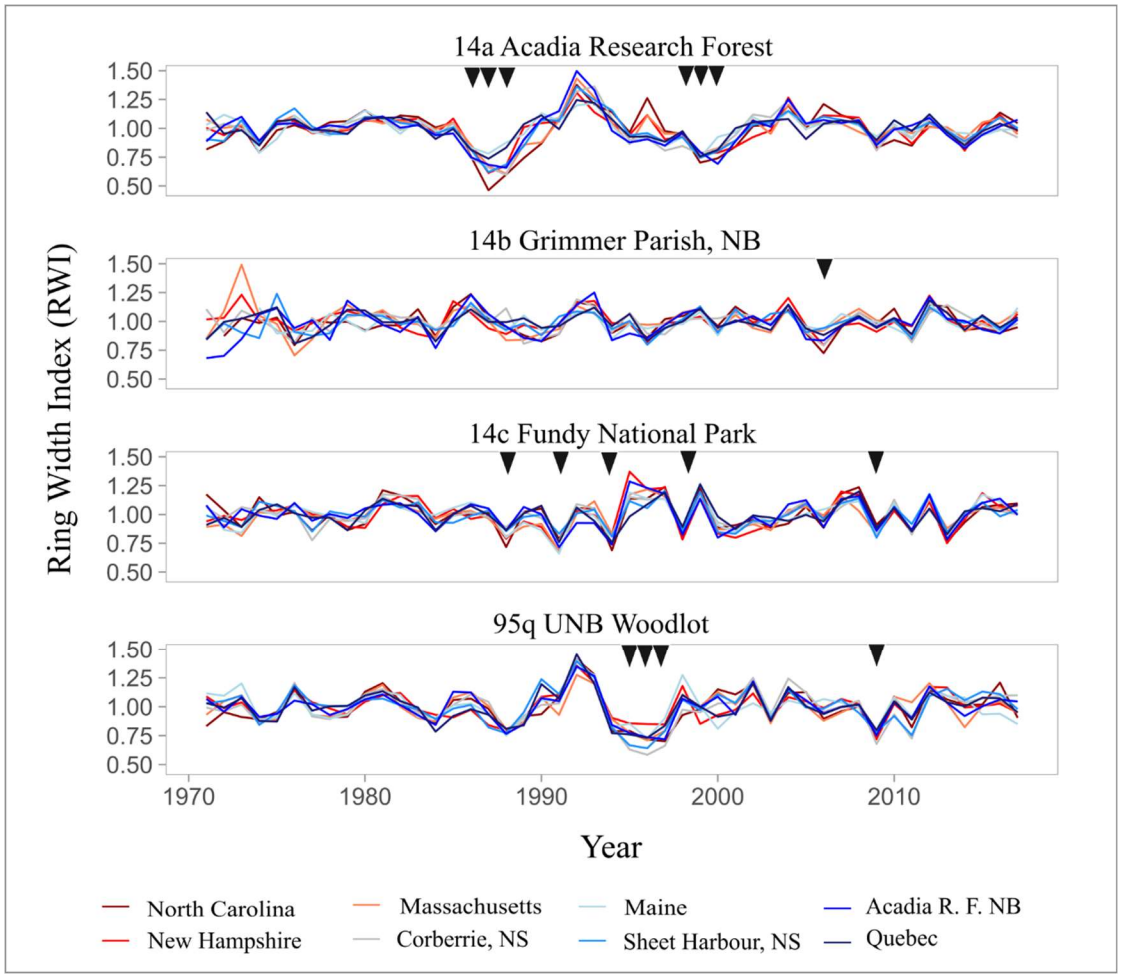

Figure 3-2 - Standardized ring width chronologies for each provenance growing at the four study sites. The color scheme indicates the mean annual temperature at the location of each seed source, from warmest (red) to coldest (blue). The arrows indicate pointer years.

3.4.1 Frost effect displays no consistent effect on interannual variation in growth

All provenances combined, the interannual growth variations at each site displayed weak and inconsistent correlations with frost variables, none of which were significant as the data was non-linear.

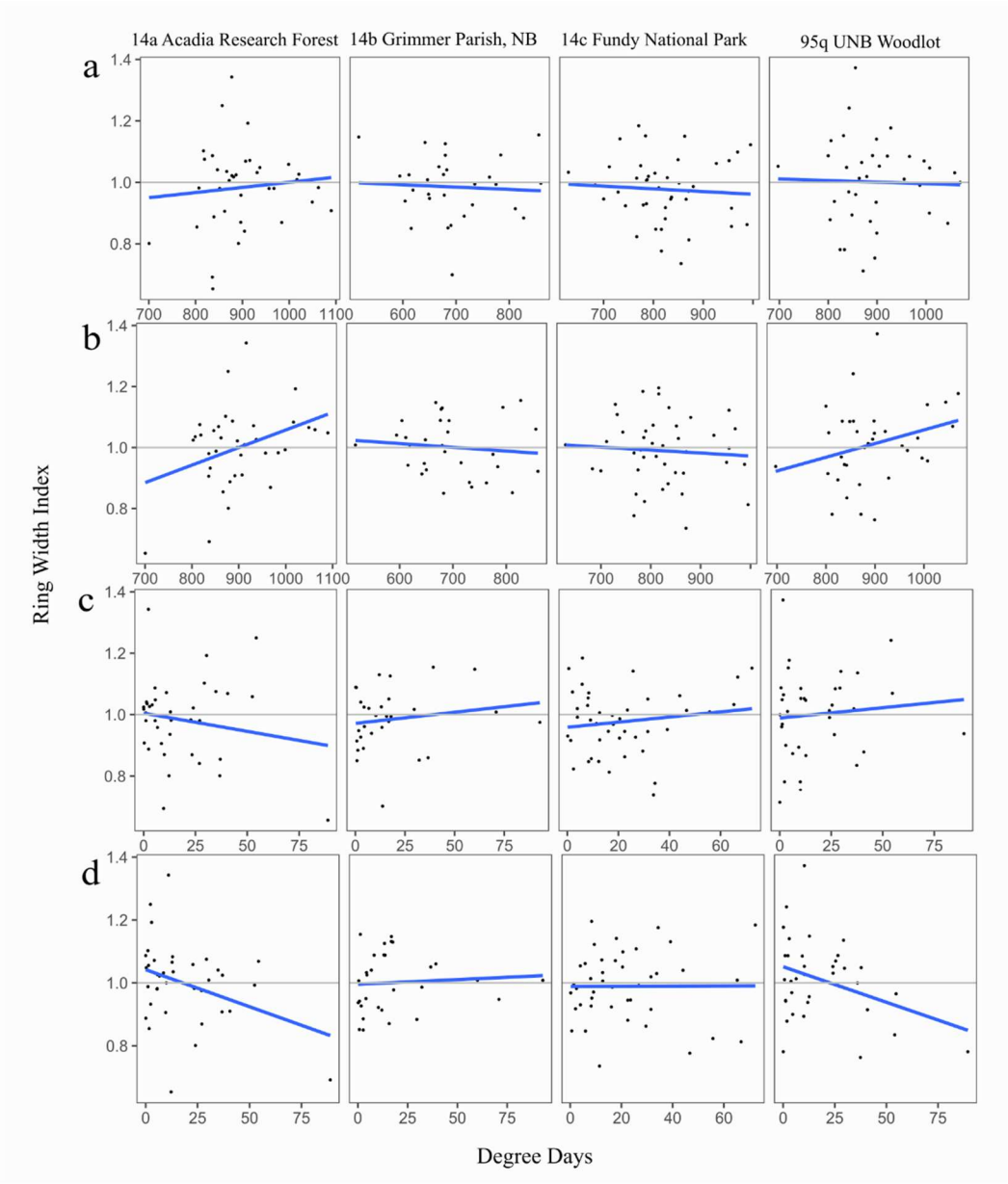

Figure 3-3 – Correlation between a) summer GDD before fall frost and current year RWI, b) summer GDD before fall frost and following year RWI, c) spring frost GDD and RWI of the current year, d) spring frost GDD of the following year's growth in each of the four sites. Spearman's rank correlation found no significant correlations.

3.4.2 No correlations between frost and low growth anomalies

To test our second hypothesis that only low growth anomalies are associated with frost anomalies, we assessed the relationship between spring and fall frost and low growth anomalies (negative pointer years) across sites. Contrary to our hypotheses, we find that the more damaging fall frosts are associated with lower growth declines (Figure 3-4), although none of the correlations are significant at $p < 0.05$. However, we do find that later, more damaging spring frosts are associated with lower growth anomalies. However, this negative effect of spring frost on growth is mostly due to a single event, the severe 1987 spring frost. Removing this value inverts the relationship (Figure 3-4). This 1987 low growth anomaly at site 14a (Acadia Research Forest) was synchronous with a – 4.1°C late spring frost occurring 89 degree-days (April 29) after the start to the growing season (March 29th), followed by a summer drought. This spring frost followed two milder spring frosts (-2.8 and -3.5°C) in the previous year (1986) after the start of the growing season, as well as an early fall frost with minimum temperatures dipping below zero (-1.1°C) in August, 1986.

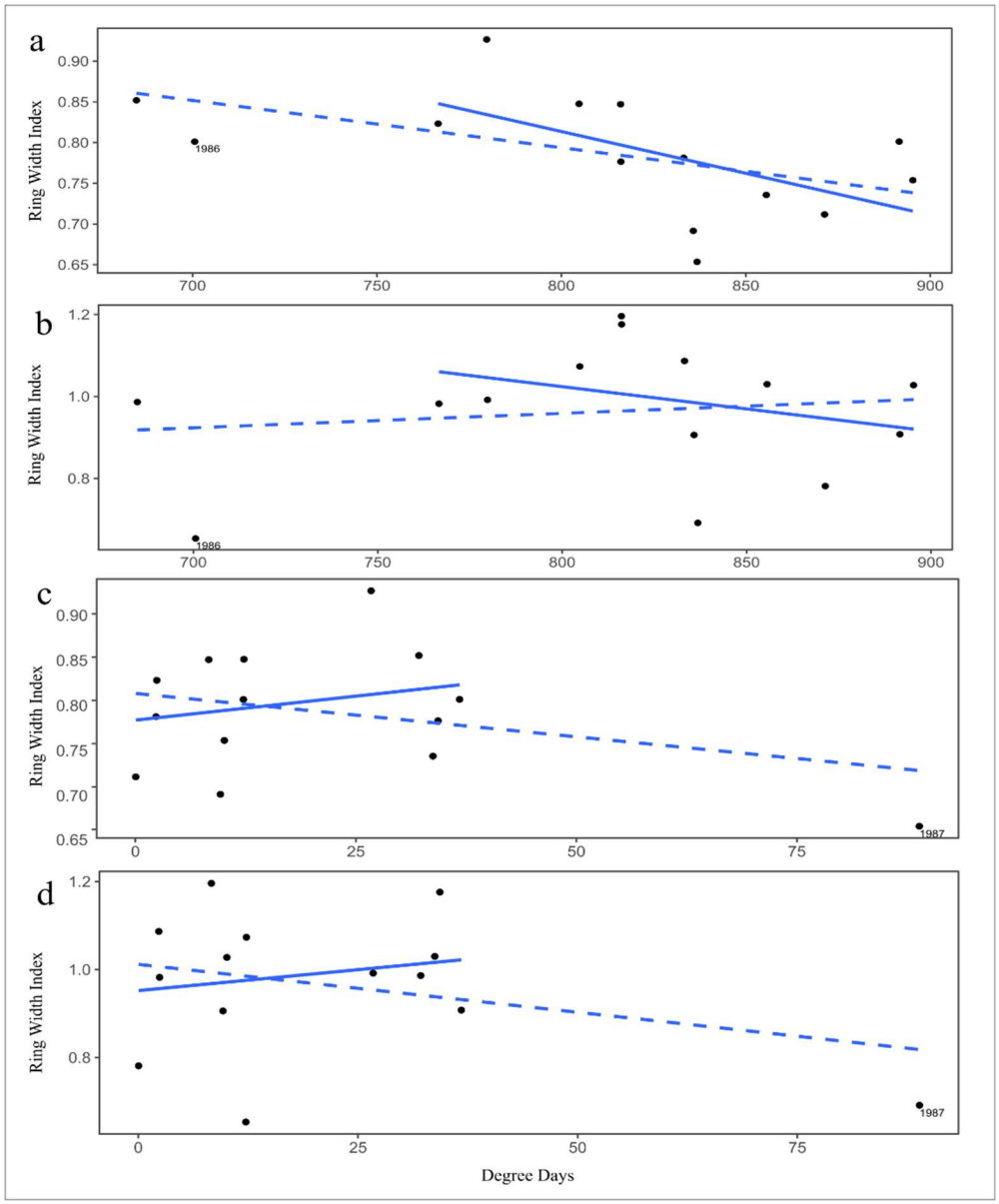

Figure 3-4 - Effects of spring and fall frost on red spruce low growth anomalies (RWI) across the four study sites. a) effect of fall frost on the same-year growth, b) effects of fall frost on the following-year growth, c) effect of spring frost on the same year growth, and d) effect of spring frost on the following-year growth. The solid lines are low growth years without the extreme events 1986 – 1987 and the dashed lines are including the extreme years, showing the change in trajectory.

3.4.3 Local seed sources display higher growth during the 1987 frost event

To test our third hypothesis of clinal differences between populations, we compared the growth response between provenances during low growth anomalies. We decided to center the analysis around the 1987 frost event at site 14a, given it displays the largest growth reduction combined with the most severe spring frost event following an early fall frost event in 1986. Indeed, the growth reduction in 1987 at site 14a was 35 ± 10%, compared to 29 ± 8% in site 95q, 25 ± 6% at 14a, while the growth reduction at 14b was marginal. Further, while other low growth anomalies were very similar among provenances, the differences between provenances were most pronounced during the 1987 event at site 14a, suggesting this event was sufficiently severe to highlight genetic differences between provenances and allowing us to test our hypothesis on provenances effects.

The comparison of resistance, resilience, and recovery indices between provenances for this event reveals a significant difference in resistance (initial capability to withstand stress) between the most warm-adapted provenance North Carolina and three of the cold-adapted provenances, Quebec, Maine, and New Brunswick (Figure 3-5). North Carolina displayed a low resistance score of 0.47, while New Brunswick, Maine and Quebec displayed significantly higher resistance indices of 0.75, 0.78, and 0.81, respectively (Figure 3-5). Such difference in resistance is due to significantly larger growth reductions during the 1987 frost event for North Carolina trees, which displayed 100, 94 and 70 % greater growth reduction than Maine, Quebec, and New Brunswick, respectively. When the RWI was converted to biomass, the North Carolina basal area

increment (BAI) that year was reduced by approximately 690 mm^2, while the Maine provenance was only reduced by 130 mm^2. We also report significant differences for resilience (the capability of trees to withstand stress, combined with their ability to recover) where Maine and Quebec seedlots displayed significantly higher resilience scores of 1.38 and 1.08, respectively, compared to a score of 0.66 for North Carolina trees. North Carolina took four and a half years to recover from this event, where Quebec recovered in just over a year and a half, and Maine recovered in just over a year.

During the 1987 event, the ranking of provenances mirrored the acclimation cold hardiness results from Acadia Research Forest (Chapter 2), with the most resistant and resilient provenances also experiencing the lowest rate of frost damage in the cold hardiness experiments (Figure 3-5). Vice versa, the highest rate of cold hardiness damage incurred was the warm-adapted provenance North Carolina, whose origin has a MAT that is 4 – 6 °C warmer than New Brunswick, was also found to have the highest total and average relative growth reduction and experienced the longest recovery period.

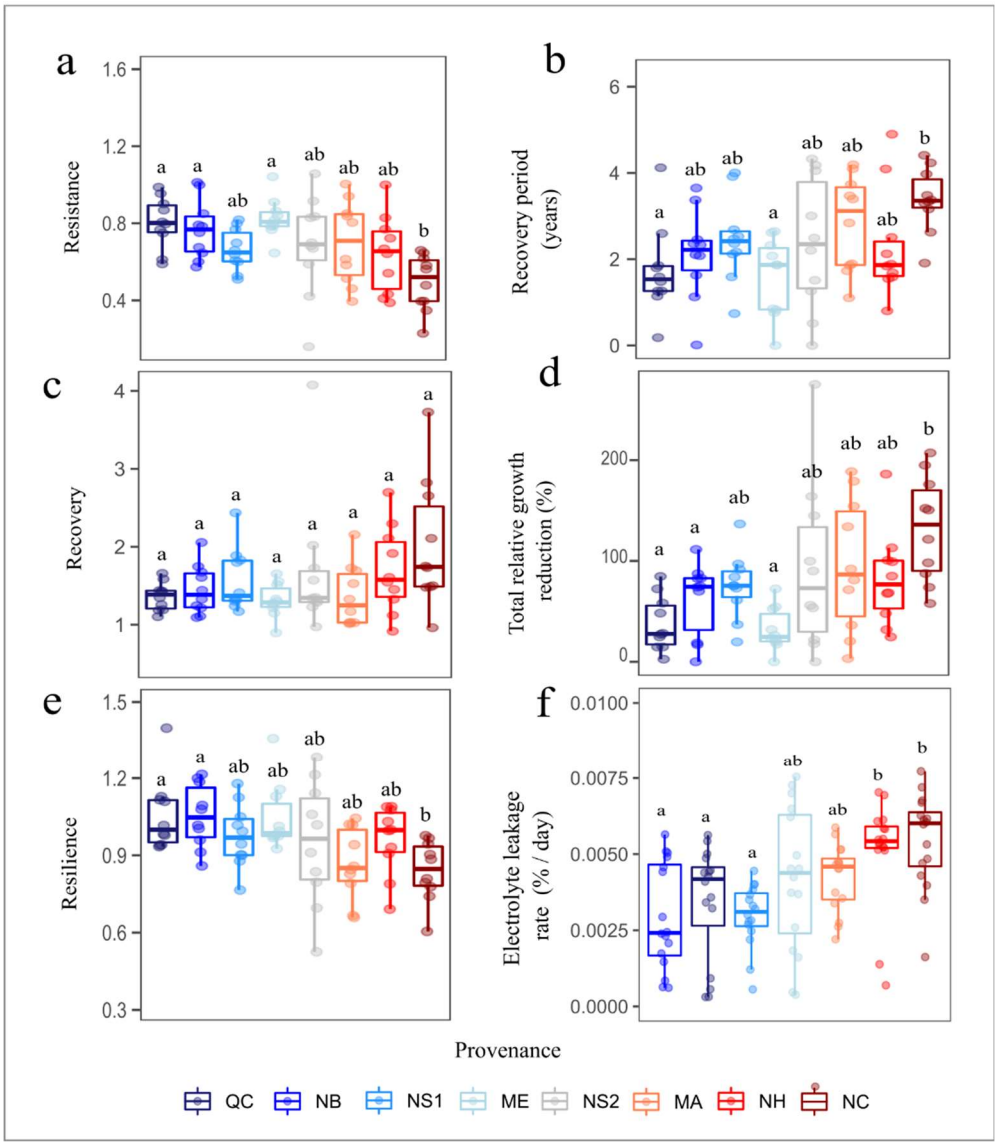

Figure 3-5 - Comparison between provenances for five resilience indices for the event in 1987 with the largest growth reduction. a) resistance, b) recovery period, c) recovery index, d) total relative growth reduction, e) resilience, f) acclimation frost hardiness (electrolyte leakage rates) results from Chapter 2. Letters indicate provenance groups with significant differences from one another ($p < 0.05$). Note: the NB provenance has the lowest MAT in the cold hardiness study as that provenance was from Rocky Brook, NB rather than the Acadia Research Forest.

3.5 Discussion

3.5.1 Little evidence of frost affecting red spruce growth

We evaluated the relationship between frost events and growth, and we tested whether frost events had a deleterious effect on red spruce growth overall (H1), or if the events covaried with low growth reductions (H2). We found limited evidence for a relationship between frost events and the growth of the red spruce over the last 50 years. The reduction in acid rain since the emendation of the Clean Air Act in 1990, which followed the severe decline of red spruce due to lower frost hardiness, has been associated with the recovery of red spruce trees, including increasing survival, health and growth (Kosiba et al. 2018, Mathias & Thomas, 2018). Kosiba et al. (2018) found that 75 % of trees in 90 % of plots they measured had experienced increased growth since 2001 and claim it was due to high temperatures outside the growing season. Yet, Mathias and Thomas (2018) found that since 1989 the most important drivers of red spruce growth were the reduction in pollution and increases in CO_2. Likely, it is a combination of many factors related to temperature and a reduction in acid rain. While warmer temperatures can lead to increased growth, especially in cold-limited forest ecosystems (D'Orangeville et al. 2016), they have also been associated with a premature deacclimation in woody plants (Vyse et al. 2019, Ladwig et al. 2019). While we agreed these factors would increase the growth of red spruce, we predicted it would also put the trees at risk of frost. Typical frost events occurring during or after deacclimation tend to damage the leaf primordia that compose the predeterminate growth found in red spruce buds, leading to the documented damages to current-year needles (DeHayes, 1992). The fact that we did

not record any frost events that were severe enough to negatively impact red spruce growth at the species level since 1987 is in line with earlier evidence of recovery for that species.

Frost events can only damage trees when they do not have sufficient cold hardiness in early fall or late spring, so it makes sense the overall relationship between frost events and growth would be insignificant. The relationships of RWI with each variable also changed dependent on the site, as conifer growth depends on soil texture and drainage, winter snowfall, and water availability among other factors (D'Orangeville et al. 2018). In a previous red spruce study using monthly averages, preliminary Pearson correlation tests between standardized BAI and GDD, seasonal CMI, temperature and precipitation revealed no significant correlation with temperature or GDD, but strong correlations with summer CMI and precipitation (Santos, 2022 MScF Thesis). The variety of frost events that occurred during the pointer years in this study suggest that not one, but many frost variables may lead to a growth reduction, especially when under previous stress from warmer temperatures (Kosiba et al., 2018; Ribbons, 2011; Verrico et al., 2019), excessive spring moisture (White et al., 2014), or low summer CMI (Moura dos Santos, 2020, MScF Thesis) which have all shown a negative relationship with red spruce growth in previous tree-ring studies. Although the severity of winter injury has been correlated with decreases in basal area increment and height growth in red spruce (Wilkinson 1990; Tobi et al. 1995), the lack of frost injury to our samples may explain in part why we found little evidence to support our first or second hypothesis.

3.5.2 Growth reduction follows multiple frost events in 1986 - 1987

While frost events as we defined them in this study displayed limited correlations with growth in New Brunswick, we aimed to quantify the effects of damaging frost anomalies, which are by definition rare and poorly replicated events. Such lack of replication makes it especially challenging to determine a clear causal link between frost and growth declines, and we cannot exclude that other undocumented factors may have played a role in the 1987 severe growth decline described here. Despite lack of evidence of frost injury, cold temperatures in late spring may still delay budbreak and reduce growth without causing frost injury. For example, a late spring frost at Acadia Research Forest on May 21 lead to no frost damage, instead increasing the cold hardiness of the trees relative to earlier measurements (Chapter 2), suggesting a sudden retraction in cold hardiness development during deacclimation, and perhaps a delay in bud development or growth. We know that cold acclimation is a dynamic developmental process that responds to environmental triggers (DeHayes, 1992). For example, winter injury response in red spruce has been reported to differ depending on the physiological state of the tree, and environmental conditions that caused injury one year may not be stressful to plants in a different state during another year (DeHayes, 1992). This may fully explain how the stress of multiple frost events over two seasons (1986 – 1987), coupled with the late spring frost and drought of 1987 create a confounding effect. The years 1986 – 1988 held historic climate records in Vermont, Virginia, and North Carolina as well (Mohnen, 1992). The northern areas had a one of the warmest Aprils on record, similar to New Brunswick that year, but frost was not accounted for in the studies. By mid-June this led to mild to moderate drought conditions. Contrastingly, in North Carolina, they

experienced a severe to extreme drought (Mohnen, 1992). This became a well-known year for studying red spruce response to atmospheric pollution, which contributed to the vast evidence of the impact of acid rain and pollution on forest health (Johnson et al. 1992, Johnson, 1992). If we consider that winter injury is more prevalent in high elevation red spruce sites and that high (and also low) elevation populations are under less stress due to reduced atmospheric pollution (Mathias and Thomas, 2018), then there is much evidence to support the recovery of red spruce. This is strong support to encourage management efforts of the species. One of these efforts recently suggested is assisted migration, where we move southern seed sources north in an effort to keep pace with a changing climate. This begs the question of which populations would be best adapted to conditions in New Brunswick.

3.5.3 Warm-adapted provenance likely experiencing maladaptation

Differences in cold hardiness between red spruce provenances have been detected in the past (DeHayes, 1990, DeHayes et al. 2001), as well as differences in rates of acclimation and deacclimation. We know that drought and cold hardiness response and phenology are important genetic components of red spruce adaptation to climate (Capblancq et al. 2022), so it makes sense that the difference in resilience indices coincides with our cold hardiness results from the previous chapter. Capblanq et al. (2022) found a strong negative correlation between seedling growth and climate transfer distance and estimated that around a third of the variation in red spruce height growth could be attributed to disruption of local adaptation caused by moving seedlings from their origin climate. We found that the most resistant and resilient provenances were also

the ones adapted to the local climate, and the same provenances also experienced the lowest rate of frost damage in our frost hardiness studies (Quebec, Maine, and New Brunswick). Vice versa, the highest rate of cold hardiness damage incurred was the warm-adapted provenance North Carolina, which was also found to have the highest total and average relative growth reduction and experienced the longest recovery period. North Carolina also experienced the greatest growth reduction from 1987 to 1988, providing strong confirmation that red spruce is genetically adapted to local climate conditions, and can be maladapted if seed is transferred beyond recommended seed transfer distances (Capblanc et al. 2022). The North Carolina provenance was also found to have the greatest variance in the resistance indices, leading to some heteroskedasticity in the results, but this may be in line with previous studies. This population may also have the highest within-provenance genetic diversity because of its unique population in the high-elevation Appalachians where they are secluded by a glacial refugia. This has been seen in other species like Butternut (*Juglans cinerea*) in trailing edge populations in New Brunswick (Schumacher, 2022). Li et al. (2020) also tested provenances transferred to colder climates and found a correlation of height and DBH with climate transfer distance, influenced by mean annual temperature, length of the frost-free period and number of growing degree days. This supports evidence that there is some variation in adaptation to cold or drought stress that should be considered in assisted migration strategies to avoid maladaptation (Capblancq et al. 2022). Sebastian-Azcona et al. (2019) found the same results in white spruce, where the southernmost and easternmost populations displayed the latest and most insufficient frost hardiness, while the climate of provenance origin explained a large proportion of the variance in height, mortality, and fall cold hardiness.

3.6 Conclusion

Tree-ring analysis played a large role in the discovery and documentation of the red spruce decline in the Appalachian Mountains, but there was much difficulty in disentangling observed growth patterns because many climatic factors and stand dynamics interact in affecting tree growth (Cook and Zedaker, 1992). Identifying the exact triggers of historical disturbance from tree rings or other types of ecological proxies can be difficult given that many factors drive forest dynamics (Pederson et al. 2014). Pointer years were crucial in this study, but the thresholds were different depending on the method used. Considering the young age of these trees (50 years) relative to their life expectancy (~300 years), there is a chance that the pointer years that were classified as strong or extreme growth reductions may not have been as strong if we were evaluating an old growth red spruce stand. Thus, the few growth reductions found in this study may have been part of a more natural growth cycle if the study length was increased. But, assisted migration should not be discarded as a valuable management strategy for red spruce, given the moderate levels of frost events recorded during this study and the risk for increasing divergences under increasing warming. The adaptability of red spruce to warming will mostly depend on the regional adaptability of southern populations (Capblancq et al. 2020).

We recommend using seed sources from no more than 1 – 2°C higher MAT for immediate assisted migration and we recommend further common garden trials be done to continue to assess the performance of high elevation red spruce populations (whether

growing in their local climates or moved north) in future climates where acid rain has been reduced, but temperatures continue to increase.

3.7 References

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Chapter 4 : Conclusion

4.1 Overview of thesis

The growth decline of red spruce is well-documented and has been associated with many variables. Acid rain and its negative impact on the cold hardiness of the species was a major contributor to the decline (Adams and Eagar, 2012; DeHayes et al. 1999; Johnson, 1983). The research put into the red spruce decline and the implementation of the Clean Air Act in the early 1990s highlighted how anthropogenic actions can alter forest health projections for the better. Climate change has been a more recent threat to red spruce, knowing the species' high sensitivity to temperature fluctuations (Strimbeck et al. 2015) and low genetic diversity (Capblanq et al. 2020). The history of red spruce showed the importance of exploring the more recent response of red spruce to cold events such as winter thaws, late-spring frosts and early fall frosts.

In Chapter 2, I use one of the most precise and objective approaches developed to assess cold hardiness in woody plants; electrolyte leakage. Protocols to measure electrolyte leakage are highly variable between studies and often do not account for the time-dependency of electrolyte leakage, where conductivity increases with time after freezing (incubation). Using one-year-old shoots of seven mature, range-wide provenances of red spruce (*Picea rubens* Sarg.), I measured the rate of electrolyte leakage over time at two target temperatures in fall 2021 during acclimation, twice while the trees were fully acclimated, and in spring 2022 during deacclimation. I then fit two different models to the time-varying electrolyte leakage rates to compare seasonal differences in cold hardiness between provenances. The first model derived from Murray et al. (1989),

uses a single parameter (κ), to determine the electrolyte leakage rate. The second, two-parameter asymptotic exponential model was tested which included a modifier for the asymptote value. I determined that model 1 is more appropriate than model 2 in the fall and spring overall, with the notable exception of late spring, when the second model is significantly better as the trees deacclimate and progress toward budbreak. Once budbreak occurs, the two-parameter asymptotic model (2) fits significantly better. I derived the rate of electrolyte leakage from model 1 to compare provenances, as it fit most data. My approach allowed me to detect significant differences between the most cold-adapted and warm-adapted seed sources during cold acclimation. This method can be applied to compare plant cold hardiness while controlling for the increasing electrolyte leakage over time since freezing.

In Chapter 3, I explored the clinal relationships of cold hardiness and growth during and after frost events. The analysis of annual growth from tree-rings provides a finer resolution of climate sensitivity, which allowed me to reveal impacts in red spruce from historic climatic extremes. In this study, I assessed whether frost events contributed to the radial growth of red spruce populations over the last 50 years. To do this, I evaluated the relationship between frost events and growth and found that frost events had limited effect on red spruce growth alone. I did find that at least some provenances' growth covaried with extreme climate anomalies. The most important result from this chapter was that regardless of the contribution of frost to the growth decline in 1987, warm-adapted populations were more sensitive to local conditions than cold-adapted populations. In other words, during times of stress, the warm-adapted provenances were often impacted more negatively than the local populations. Considering the warm-

adapted provenances have a lower frost tolerance during acclimation, I cannot rule out the impact this may have during future warming, where frost events are expected to increase.

4.2 Management implications

My results show that the local populations were the most adapted to local conditions, but temperature increases are imminent, and the "local is best" approach is no longer the best management practice (O'Neil and Gomez-Pineda, 2021). Assisted migration is becoming more widely used as an adaptation strategy to maintain the biodiversity, health, and productivity of Canada's forests under climate change. Motivations for studying local adaptation in trees include enhancing plantation productivity, selecting optimal source populations for increased productivity, and more recently, studies have considered local adaptation when evaluating a species capacity to respond to ongoing climate change (Capblanc et al. 2022, Sebastien-Azcona et al. 2019). Assessing the feasibility of assisted migration includes testing the variability of a species phenotypic response, as well as maladaptation to future climatic stressors.

Chapter 2 informs assisted migration because cold hardiness testing performed in the lab allows us to test for multiple temperatures and record the cellular breakdown of cells. This measures the rate of damage rather than just a single measurement at a time. This could allow for standardization of cold hardiness testing across seasons, species, and space. The methods in this chapter can provide a ranking of cold-adapted provenances in a short period of time to inform seed sourcing decisions. For example, a few weeks of testing mature trees in the autumn informed us that the warm-adapted red spruce

experience more measurable cold damage than the cold-adapted provenances. The methods in this chapter can be repeated on seedlings, saplings, or mature trees of any species that have been migrated to colder climates without having to travel to different seed sources. The conclusions from this chapter show that most red spruce provenances have sufficient cold hardiness until budbreak, and that in the spring, there is enough plasticity in temperature response in all of the provenances that there is no difference between them, but in the autumn during acclimation there is a significant difference between them.

Chapter 3 can help to inform species-specific recommendations for seed transfer and assisted migration in red spruce. As the climate changes, the use of local seed zones and guidelines may no longer apply (O'Neill & Gómez-Pineda, 2021). Yet, species-specific seed transfer distance research has been done for white and black spruce but is not readily available for red spruce (Pedlar et al. 2021). Because climate projections are uncertain, the current approach is to aim for the warmest climate that may be expected while ensuring that seedlots moved from warmer to colder climates are not moved so far that they risk cold damage (St.Clair et al. 2022). Thus, both Chapter 2 and Chapter 3 in this thesis are useful to inform species-specific seed transfer recommendations for red spruce. The New Hampshire population may be a good candidate for assisted migration as they have low cold hardiness damage in the spring, and low growth reductions during the high stress event in 1987, with a good recovery time. This provenance has a MAT 2°C warmer than Acadia Research Forest, and 4°C warmer than Rocky Brook, NB, which coincides with the current seed transfer distance recommendations. The issues surrounding tree seed supply in Canada is disquieting, and the many ongoing projects that

assess climate adaptability require ample seed supply. The seed we have must be allotted to the right growing conditions best for survival and optimal production. As many provinces look toward new management strategies such as the Triad approach (Lahey, 2018) or any form of intensive forestry, we must consider all aspects of clinal variation in our seed source to maximize the growing stock available for harvest.

4.3 Future study recommendations

Red spruce is known to acclimate and deacclimate in fall and spring, respectively, on par with other species, but the maximum depth of cold hardiness in the winter has been found to barely withstand mid-winter temperatures in some areas of its range (DeHayes 1992, DeHayes et al. 1999, Schaberg and DeHayes, 2000). Future studies on red spruce should focus on winter access and winter sampling of mature red spruce trees throughout its range to truly understand the current implications of climate change on midwinter stress of red spruce.

With a stronger record of events, a future study could project frost events into the future to assist future climate modelling and the impact it has on tree growth. Thus, I recommend future studies on red spruce or other species use older trees if assessing for radial growth. Yet other studies may continue to use seedlings to assess for both cold hardiness and height and diameter growth related to temperature adaptation. I also recommend a continuation of species-specific seed transfer distance research to inform seed banks and researchers such as the National Tree Seed Centre and Department of Natural Resources.

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Appendix A - Supplementary Material for Chapter 2

Table A 1 - Characteristics of red spruce provenance trials (sites) used in this chapter

	Maritimes	Rangewide
Trial ID	18-B2	14-A
Establish year	1962	1959
Location	Acadia Research Forest	
Tree spacing (m)	1.8 x 1.8	1.2 x 1.2
Altitude (m)	75	62
Year of thinning	2005	1973 & 1999
Mean annual temperature (°C)	5.51	5.55
Climate Moisture Index	-0.68	-0.64
Growing degree days (> 5°C)	1675	1679
Total nitrogen (%)	0.148	0.1575
Field capacity moisture (%)	13	10
Clay (%)	2.25	3.65
Silt (%)	25.3	27.75
Sand (%)	72.45	68.6

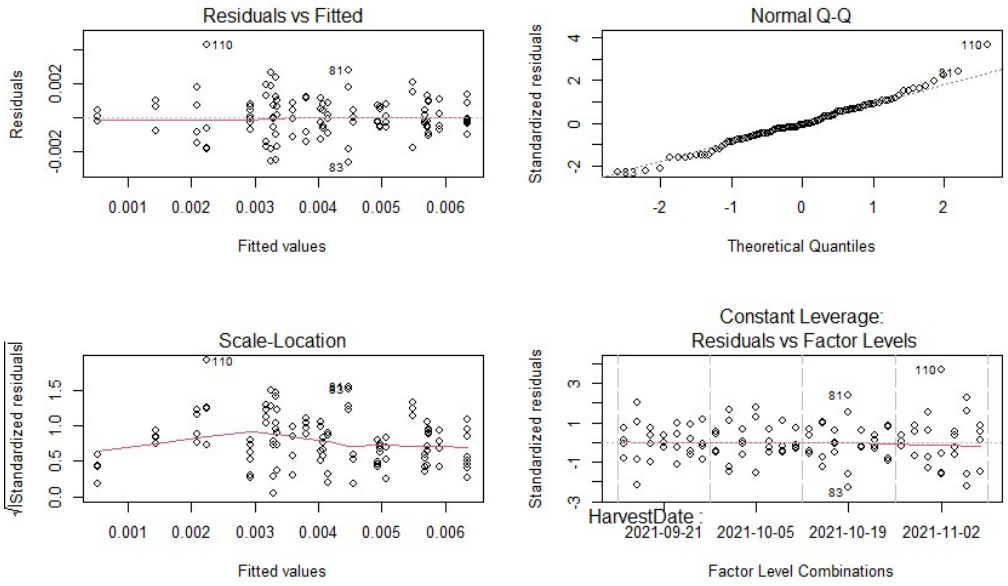

Figure A 1 - Tests for homoscedacity and normal distribution on the acclimation dataset.

Table A 2 - P values comparing seven red spruce provenances growing at Acadia research forest. Bold indicates significant differences of p value < 0.05.

	Acclimation				**Dormancy**		**Deacclimation**				
	Sep 21, 2021	Oct 5, 2021	Oct 19, 2021	Nov 2, 2021	Nov 16, 2021	Mar 28, 2022	Apr 11, 2022	Apr 25, 2022	May 9, 2022	May 23, 2022	Jun 6, 2022
NB	0.945	NA[1]	0.484	NA	0.406	0.512	0.880	0.149	0.115	0.720	**0.000**
NS	0.030	0.780	0.956	0.824	0.979	NA	0.576	0.257	0.157	0.416	**0.000**
QC	0.331	0.064	0.110	0.467	**0.001**	0.998	0.607	0.074	**0.011**	0.797	**0.000**
ME	0.018	NA	NA	NA	NA	NA	0.595	0.999	0.349	0.886	**0.019**
MA	0.928	0.136	**0.034**	NA	0.409	0.880	**0.011**	**0.042**	**0.031**	0.920	**0.000**
NH	0.090	0.401	0.086	0.938	0.985	NA	NA	0.085	**0.039**	0.796	0.215
NC	0.457	0.228	NA	NA	0.127	NA	0.861	0.329	0.370	**0.025**	**0.004**

[1] NA (does not fit) = one or both models do not fit the data and no comparison can be made.

Table A 3 - Post hoc TUKEY HSD results showing p-values for all provenances during acclimation. Provenances are in order of cold-adapted to warm-adapted. Bold is p-value < 0.01.

Provenance	NB	NS	QC	ME	MA	NH	NC
NB		0.129	0.946	1.000	0.099	**0.000**	**0.000**
NS			0.679	0.154	1.000	0.413	0.122
QC				0.963	0.604	**0.009**	**0.001**
ME					0.119	**0.000**	**0.000**
MA						0.486	0.157
NH							0.995
NC							

Table A 4 - Post hoc TUKEY HSD results showing p-values for all provenances during deacclimation and budbreak. Provenances are in order of cold-adapted to warm-adapted. Bold is p-value < 0.01.

Provenance	NB	NS	QC	ME	MA	NH	NC
NB		1.000	0.995	0.735	0.120	1.000	0.932
NS			0.996	0.719	0.127	1.000	0.924
QC				0.320	0.421	0.998	0.596
ME					**0.001**	0.652	1.000
MA						0.161	**0.006**
NH							0.887
NC							

Figure A 2 - Photos of swollen buds on May 23, 2022. Budbreak occurred between May 23 and June 6. The provenances are from top left: 2019, 2022, 2030, 2031, 2032, 2101, 2103, 15. The two local provenances from NB (bottom two) were furthest along.

Appendix B - Supplementary Material for Chapter 3

Table B 1 - characteristics of red spruce provenance sites. The sites we selected are the four rangewide sites

	Maritimes	Rangewide			
Trial ID	18-B2	14-A	14-B	14-C	95-Q
Establish year	1962	1959	1960	1960	1960
Location	Acadia Research Forest	Acadia Research Forest	Grimmer Parish	Fundy National Park	UNB Woodlot
Tree spacing (m)	1.8 x 1.8	1.2 x 1.2	1.2 x 1.2	1.2 x 1.2	1.5 x 1.8
Altitude (m)	75	62	239	273	71
Year of thinning	2005	1973 & 1999	No thinning	1979	No thinning
Mean annual temperature (°C)	5.51	5.55	2.61	4.92	5.7
Climate Moisture Index	-0.68	-0.64	4.68	3.36	-1
Growing degree days (> 5°C)	1675	1679	1290	1432	1710
Total nitrogen (%)	0.148	0.1575	0.282	0.474	0.229
Field capacity moisture (%)	13	10	11	13	20
Clay (%)	2.25	3.65	4.3	3.25	10.75
Silt (%)	25.3	27.75	34.85	42.9	50.1
Sand (%)	72.45	68.6	60.85	53.85	39.15

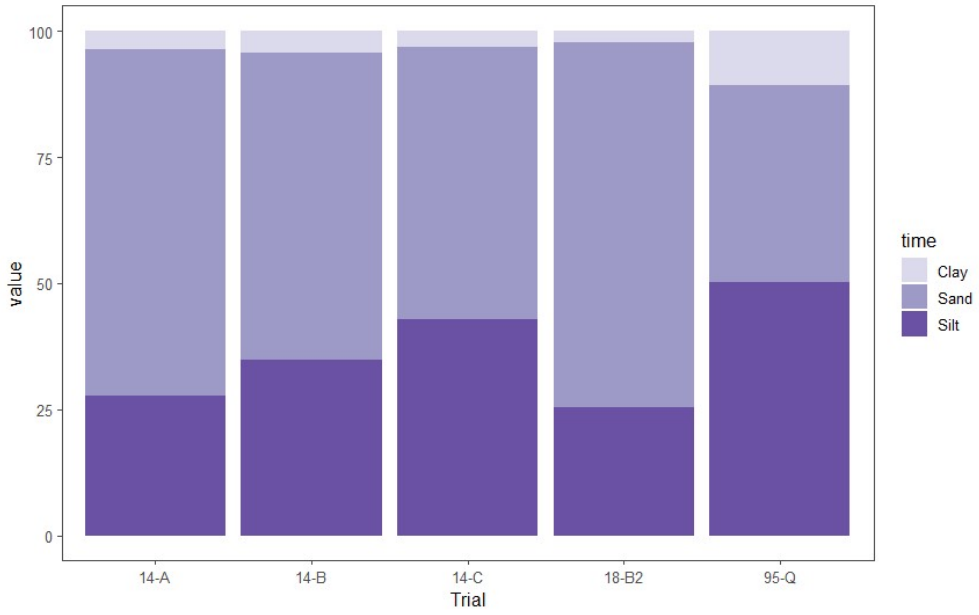

Figure B 1 - Difference in soil properties between the five sites.

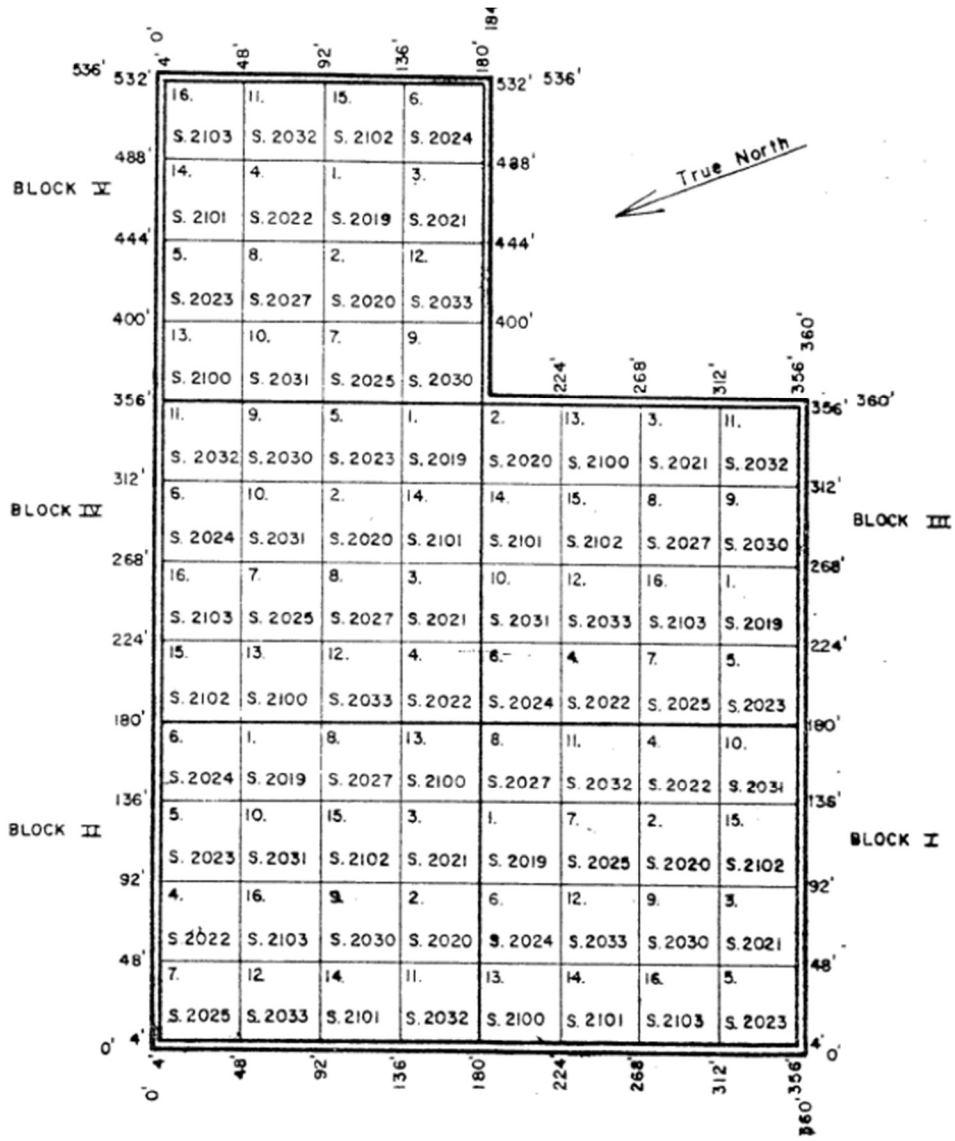

Figure B 2 - Range-wide (series 14-95) red spruce plantation layout example for one trial

Table B 2 – Spearman's rank correlation between six frost variables at each of the four trial sites. n = current year RWI, n+1 = following year RWI. Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

Frost event	Spring frost GDD		Winter thaw GDD		Fall frost GDD	
Year/ Site	n	n+1	n	n+1	n	n+1
14a	-0.07	-0.03	-0.06	-0.07	0.06	0.24
14b	0.17	0.26	0.36 .	0.16	-0.13	-0.14
14c	0.01	0.08	0.1	0.14	-0.11	-0.05
95q	0.13	-0.18	-0.19	-0.07	-0.03	0.29 .

Table B 3 -Spearman's rank correlation tests between spring frost GDD, fall frost GDD and growth during and following pointer years with and without the extreme event in 1987.

Data	Test	Cor	P value
Including early fall frosts	summer GDD before frost and RWI	-0.67	0.010
	summer GDD before frost and following year RWI	-0.04	0.891
Excluding early fall frosts	summer GDD before frost and RWI	-0.68	0.012
	summer GDD before frost and following year RWI	0.03	0.935
Including late spring frost	Spring frost GDD and RWI	-0.01	0.988
	Spring frost GDD and following year RWI	-0.07	0.808
Excluding late spring frost	Spring frost GDD and RWI	0.24	0.425
	Spring frost GDD and following year RWI	0.12	0.696

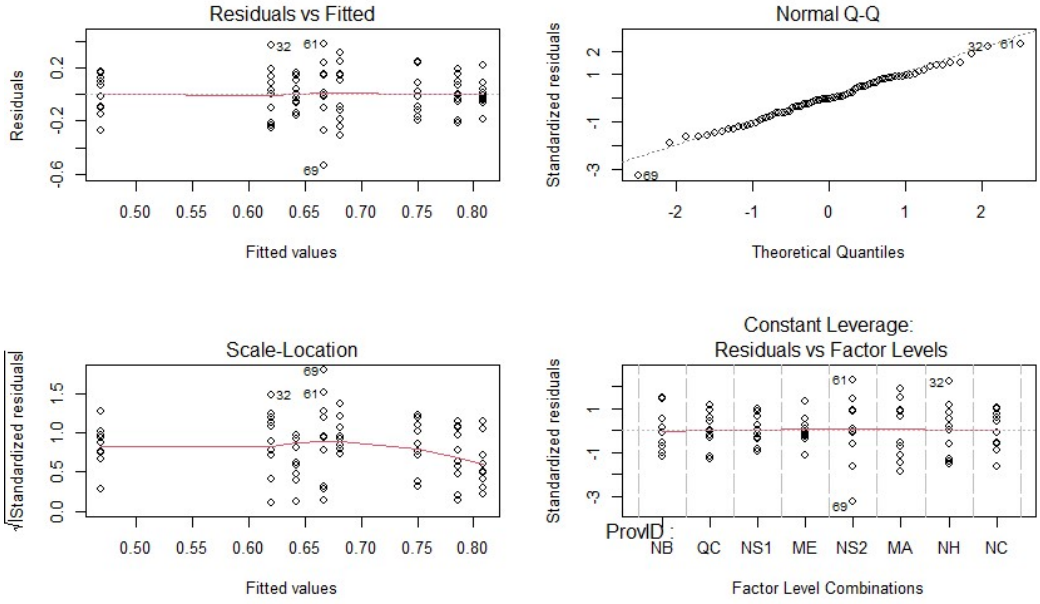

Figure B 3 - Example of results of tests for heteroscedasticity and normal distribution in the resistance comparison.

Table B 4 - P values for significant ANOVA results when comparing provenances across different measures of growth resilience indices during the 1987 event in site 14a. Dashes are not significant differences. All other provenance comparisons were not significantly different.

Provenances	resistance	resilience	recovery period (years)	total absolute growth reduction	relative growth reduction (%)	average absolute growth reduction	average relative growth reduction (%)
NC - QC	0.003	0.038	0.026	0.005	0.002	0.033	0.014
NC - ME	0.001	-	0.011	0.003	0.001	0.023	0.010
NC - NB	0.012	0.041	-	-	0.047	-	0.010

Table B 5 - An initial qualitative description of frost events that occurred during pointer years through analysis of daily weather data. WT = winter thaw, SF = spring frost. Bold indicates lag effects. Events based on date, not heat accumulation.

Site	Year	Event	Date start	Description
14a	1986	WT	1986-01-18	avg temperature > 0 for > 3 days, drops to -22 3 days later.
	1987	WT	1986-12-27	avg temp >0 for 1 day, drop to -17 2 days later
		WT	1987-03-09	avg temperature > 0 for > 1 days, drops to -21.5 2 days later.
	1988	WT	1988-01-31	avg temperature > 0 for > 3 days, drops to -19 2 days later.
		WT	1999-12-23	Tavg >2 for 2 days, drops to -17.5
	1999	SF	1999-05-13	Tmax > 20 from May 2 - 10th, followed by 4 days of Tmin <0
	2000	WT	2000-01-26	Tavg > 0 for > 1 day, drops to -18.5 2 days later
		SF	2000-04-14	Max temps > 4 for > 20 days, then 2 days of min temps < -4
14b	2006	SF	2006-04-30	Max temps > 4 for > 20 days, then 1 day of min temp < -4
14c	1988	WT	1988-01-25	Tavg >0 for >2 days, drops to -18 2 days later
		WT	1988-01-31	Tavg >0 for >3 days, drops to -19 1 day later. Biologically significant
		WT	1988-02-20	Tavg >0 for >1 day, drops to -18 2 days later. Third thaw this year.
		SF	1988-04-14	Max temps > 4 for > 10 days, then 2 days of min temps < -4
	1991	WT	1991-01-16	Tavg >0 for 1 day, drops to -16 2 days later
	1994	WT	1994-01-29	Tavg >0 for 1 day, drops to -25 2 days later
		WT	1994-02-20	Tavg > 6 for 2 days, drops to -17 2 days later. Biologically significant
		SF	1994-04-24	Max temps >4 for >20 days, then 3 days of min temps < -4
	1998	WT	1998-01-25	Tavg >0 for 1 day, drops to -19 2 days later
		WT	1998-02-12	Tavg >0 for 2 days, drops to -18 2 days later
		WT	1998-03-10	Tavg >0 for 2 days, drops to -16 2 days later, biologically significant
	2009	WT	2008-12-17	Tavg >3 for 2 days drops to -16
		WT	2008-12-26	Tavg >2 for 1 day drops to -16 1 day later
		SF	2009-04-20	Max temps >4 for >10 days, then 2 days of min temps < -4
95q	2009	WT	2008-12-13	Tavg >3 for 1 day, drops to -16 2 days later
		WT	2009-02-08	Tavg >0 for 1 day drops to -16 1 day later
		WT	2009-02-27	Tavg >3 for 1 day and drops to -17 2 days later
		WT	2009-03-12	Tavg >0 for 1 day drops to -17 2 days later
		SF	2009-04-21	Max temps >4 for >5 days, then 1 day of min temps < -4
	1995	WT	1995-03-09	Tavg >0 for 2 day drops to -18 3 days later
	1996	WT	1995-12-28	Tavg >0 for 1 day drops to -17 3 days later
		WT	1996-02-21	Tavg >0 for 7 days! drops to -15.5. Biologically significant
	1997	WT	1996-12-30	Tavg >0 for 1 day drops to -22 2 days later

Table B 6 - Pointer years with the frost variables from that year and the year prior for the provenances that were affected. Pointer years are in bold. Cropper value, derived from the NMW pointer year detection method, indicate the level of growth anomaly

Site		Year	Standard deviation from average growth	Spring frost GDD	Min temperature in spring	Winter thaw GDD	Number of winter thaws	Fall frost GDD	Number of fall events	RWI
14a	MA	1987	-	89	-4.1	13	3	837	5	0.67
		1988	**-1.4**	**10**	**-7.6**	**11**	**4**	**836**	**3**	**0.61**
	ME	1998	-	NA	NA	13	7	900	5	0.92
		1999	**-1.3**	**0**	**NA**	**18**	**6**	**983**	**4**	**0.74**
	NB2103	1987	-	89	-4.1	13	3	837	5	0.68
		1988	**-1.2**	**10**	**-7.6**	**11**	**4**	**836**	**3**	**0.66**
	NC	1986	-	12	-6.5	7	1	701	5	0.76
		1987	**-1.8**	**89**	**-4.1**	**13**	**3**	**837**	**5**	**0.46**
	NC	1998	-	NA	NA	13	7	900	5	0.95
		1999	**-1.5**	**0**	**NA**	**18**	**6**	**983**	**4**	**0.70**
	NC	1999	-	0	NA	18	6	983	4	0.70
		2000	**-1.3**	**37**	**-6.5**	**0**	**1**	**892**	**3**	**0.74**
	NH	1986	-	12	-6.5	7	1	701	5	0.82
		1987	**-1.6**	**89**	**-4.1**	**13**	**3**	**837**	**5**	**0.62**
	NH	1987	-	89	-4.1	13	3	837	5	0.62
		1988	**-1.3**	**10**	**-7.6**	**11**	**4**	**836**	**3**	**0.68**
	NS2100	1986	-	12	-6.5	7	1	701	5	0.82
		1987	**-1.6**	**89**	**-4.1**	**13**	**3**	**837**	**5**	**0.62**
	NS2100	1987	-	89	-4.1	13	3	837	5	0.62
		1988	**-1.2**	**10**	**-7.6**	**11**	**4**	**836**	**3**	**0.69**
	NS2101	1985	-	24	-5.6	13	5	878	4	1.04
		1986	**-1.0**	**12**	**-6.5**	**7**	**1**	**701**	**5**	**0.74**
	NS2101	1986	-	12	-6.5	7	1	701	5	0.74
		1987	**-1.3**	**89**	**-4.1**	**13**	**3**	**837**	**5**	**0.66**
	NS2101	1987	-	89	-4.1	13	3	837	5	0.66
		1988	**-1.3**	**10**	**-7.6**	**11**	**4**	**836**	**3**	**0.60**
		1986	-	12	-6.5	7	1	701	5	0.81

Site		Year	Standard deviation from average growth	Spring frost GDD	Min temperature in spring	Winter thaw GDD	Number of winter thaws	Fall frost GDD	Number of fall events	RWI
14b	NC	2005	-	1	-7.1	3	2	811	3	0.90
		2006	**-1.48**	**32**	**-6.0**	**1**	**1**	**685**	**4**	**0.72**
14c	MA	1990	-	11	-6.8	8	3	871	6	0.92
		1991	**-1.52**	**34**	**-9.5**	**2**	**2**	**856**	**2**	**0.68**
	ME	1990	-	11	-6.8	8	3	871	6	0.86
		1991	**-1.54**	**34**	**-9.5**	**2**	**2**	**856**	**2**	**0.66**
	NB2103	1993	-	47	-9.7	13	4	766	7	0.93
		1994	**-1.08**	**34**	**-12.6**	**14**	**3**	**816**	**2**	**0.74**
	NC	1987	-	56	-4.2	14	2	788	5	1.01
		1988	**-1.29**	**2**	**-9.6**	**10**	**3**	**767**	**4**	**0.72**
	NC	1990	-	11	-6.8	8	3	871	6	1.05
		1991	**-1.06**	**34**	**-9.5**	**2**	**2**	**856**	**2**	**0.79**
	NC	1993	-	47	-9.7	13	4	766	7	1.05
		1994	**-1.21**	**34**	**-12.6**	**14**	**3**	**816**	**2**	**0.69**
	NH	1990	-	11	-6.8	8	3	871	6	1.05
		1991	**-1.44**	**34**	**-9.5**	**2**	**2**	**856**	**2**	**0.68**
	NH	1997	-	6	-5.6	16	2	771	3	1.23
		1998	**-1.03**	**8**	**-5.8**	**13**	**7**	**816**	**4**	**0.78**
	NS2100	1976	-	8	-9.3	5	2	775	4	1.03
		1977	**-1.87**	**27**	**-5.9**	**2**	**1**	**780**	**3**	**0.85**
	NS2100	2008	-	1	-8.4	6	4	863	3	1.14
		2009	**-1.36**	**12**	**-8.5**	**9**	**4**	**805**	**4**	**0.80**
	NS2101	1976	-	8	-9.3	5	2	775	4	1.04
		1977	**-1.78**	**27**	**-5.9**	**2**	**1**	**780**	**3**	**0.78**
	NS2101	1990	-	11	-6.8	8	3	871	6	0.97
		1991	**-1.45**	**34**	**-9.5**	**2**	**2**	**856**	**2**	**0.77**
	NS2101	1993	-	47	-9.7	13	4	766	7	1.05
		1994	**-1.35**	**34**	**-12.6**	**14**	**3**	**816**	**2**	**0.75**
	QC	**1987**	**-1.4**	**89**	**-4.1**	**13**	**3**	**837**	**5**	**0.74**

Site		Year	Standard deviation from average growth	Spring frost GDD	Min temperature in spring	Winter thaw GDD	Number of winter thaws	Fall frost GDD	Number of fall events	RWI
	MA	1995	-	0	NA	8	4	913	4	0.78
		1996	**-1.3**	**0**	**-5.7**	**9**	**3**	**871**	**2**	**0.71**
	MA	2008	-	0	NA	3	3	925	3	1.02
		2009	**-1.3**	**10**	**-5.3**	**12**	**3**	**895**	**3**	**0.78**
	ME	2008	-	0	NA	3	3	925	3	1.00
		2009	**-1.4**	**10**	**-5.3**	**12**	**3**	**895**	**3**	**0.80**
	NB2103	2008	-	0	NA	3	3	925	3	1.02
		2009	-1.3	10	-5.3	12	3	895	3	0.75
	NC	1996	-	0	-5.7	9	3	871	2	0.72
		1997	**-1.0**	**2**	**-5.8**	**20**	**2**	**833**	**2**	**0.70**
	NC	2008	-	0	NA	3	3	925	3	1.00
		2009	**-1.4**	**10**	**-5.3**	**12**	**3**	**895**	**3**	**0.72**
	NS2100	1994	-	37	-12.5	8	3	899	4	0.82
		1995	**-1.3**	**0**	**NA**	**8**	**4**	**913**	**4**	**0.67**
	NS2100	1995	-	0	NA	8	4	913	4	0.67
		1996	**-1.4**	**0**	**-5.73**	**9**	**3**	**871**	**2**	**0.64**
	NS2101	1994	-	37	-12.5	8	3	899	4	0.84
		1995	**-1.2**	**0**	**NA**	**8**	**4**	**913**	**4**	**0.63**
	NS2101	1995	-	0	NA	8	4	913	4	0.63
		1996	**-1.4**	**0**	**-5.7**	**9**	**3**	**871**	**2**	**0.58**
	NS2101	2008	-	0	NA	3	3	925	3	1.05
		2009	**-1.5**	**10**	**-5.3**	**12**	**3**	**895**	**3**	**0.68**
	QC	1995	-	0	NA	8	4	913	4	0.76
		1996	**-1.2**	**0**	**-5.73**	**9**	**3**	**871**	**2**	**0.73**

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