Evaluation of phenotypic and physiologic characteristics of selected sources of white spruce, *Picea glauca* (Moench) Voss

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Abstract

White spruce is highly valued for its wood pulp in commercial forestry in Minnesota. Seed orchards have been developed using genotypes selected for increased volume production. I conducted three different experiments to study the variation of ecophysiologic traits among genotypes selected from the Minnesota Tree Improvement Cooperative's program to better characterize the phenotype of selected genotypes. In chapter 1, I analyzed wood specific gravity, tree volume, and leaf traits on 25-year old trees in a white spruce progeny test. Wood specific gravity was negatively correlated with tree volume. Needle traits, primarily specific leaf area (SLA), leaf area ratio (LAR) and leaf mass ratio (LMR), were positively correlated with wood volume. In chapter 2, I planted seedlings from four genotypes selected for superior volume growth and two wild sources in a common garden. I harvested ten trees from each genotype, each year for three years. I examined biomass allocation, tree allometry and assessed genetic correlations among allocation of biomass to major organs. The largest differences in biomass were found between the two wild sources that represented two different seed zones in Minnesota. Selected sources more closely resembled the southern, than the northern, wild source. The northern wild sources had slightly higher allocation to roots but otherwise no significant differences in allometry were found. In chapter 3, I set up an outdoor experiment by planting five selected- and two wild- seed sources into 1-gallon containers to test the effects of mid-winter warming on phenology and growth of white spruce. Bud-break time was delayed in plots that were warmed in February, and advanced in those warmed in March. Overall controls had the highest height growth and intermediate bud-break time. Climatic warming that takes place during winter months may delay or advance bud-break depending on the timing. Growth of white spruce is expected to decline with increased episodes of winter warming. Selected sources should be favored in reforestation across Minnesota because of the higher productivity and adaptability to local conditions.

Table of Contents

List of Tables

List of Figures

Introduction

White spruce, *Picea glauca* (Moench) Voss, is planted widely in commercial forest operations in Minnesota. Fast-growing genotypes have been selected and planted into orchards that supply seed, resulting in measurable gains in height and/or volume growth relative to other sources (Pike, Warren, & David, 2006; Weng, Tosh, & Fullarton, 2010). Seed collected from the Ottawa Valley of Canada has a history of producing trees with growth rates that exceed local sources in trials located in New York, Minnesota, northwestern Ontario and in the Petawawa region of southeastern Ontario (Dhir, 1976; Easley & Maynard, 1987; Mark R. Lesser & Parker, 2004; Stellrecht, Mohn, & Cromell, 1974). Selections from the Ottawa Valley region dominate tree improvement programs in the Midwestern US but the mechanism that underlies this growth anomaly is largely unknown.

Wind-pollinated conifers possess high levels of genetic diversity that exceed all other plants (Hamrick *et al.* 1992). White spruce is no exception, possessing polymorphisms at 61% of allozymes (Godt *et al.* 2001) along with little genetic structure, and modest clinal variation across latitudinal and longitudinal gradients (Furnier, Stine, Mohn, & Clyde, 1991; Jaramillo-Correa, Beaulieu, & Bousquet, 2001; Mark R. Lesser & Parker, 2004; Peng Li, Beaulieu, & Bousquet, 1997). Range-wide provenance trials indicate that extreme northern sources grow more slowly than all others in Minnesota, but few other geographic trends are evident within the state's boundaries (Radsliff *et al.* 1983). As a result, white spruce seed can generally be moved long distances before growth and survival is compromised (Li *et al.* 1997), but recommended transfer distances may be shorter in Minnesota than those used in Canada because of steep temperature and precipitation gradients along this southern range-edge.

In recent decades, tree breeders have integrated wood specific gravity into tree breeding programs that have an ultimate goal of increasing or sustaining wood yields for many different species (Cumbie, Isik, & Mckeand, 2012; Duchesne & Zhang, 2004; Tharakan, Volk, Nowak, &

Abrahamson, 2005; Zhang, Simpson, & Morgenstern, 1996). This has occurred because of concern that selection on growth will result in reduced dried wood volume as a consequence of genetic correlations between these traits (Corriveau, Beaulieu, & Mothe, 1987; Stellrecht et al., 1974; Zhang, 1995). Genetic correlations may persist in future generations depending on the strength of the correlation, the heritability of each trait, and plasticity across environments. White spruce is highly plastic and adaptable with respect to quantitative traits: phenotypes vary widely even for trees with a common familial genotype. In chapter 1, I investigated plasticity in white spruce genotypes by revisiting two replications of a progeny test planted in 1986. I measured genetic and phenotypic correlations between tree volume, wood specific gravity and leaf traits to understand potential shifts in phenotype that might occur with continued selection for volume growth.

Selection on wood volume alone may have other indirect consequences on allometric proportions of other plant organs. Trees allocate resources to build structures such as roots, stems, and reproductive organs according to their genotype and the environment in which the plant resides. In chapter 2, I explore biomass allocation in four selected and two wild sources to determine if selection has altered biomass allocation and whether genetic correlations forebear potential shifts in allometry in advanced-generation populations.

Climate change is expected to create new challenges to growing a boreal species on its southern range-edge like white spruce in Minnesota (Savolainen, Pyhäjärvi, Knürr, & Knurr, 2007), where predicted northward shifts could result in its local extirpation (Cherry & Parker 2003). Rising temperatures and more erratic precipitation patterns could lead to reduced snowpack and a greater incidence of winter thaws in Minnesota. White spruce is prone to early bud-break (Nienstaedt & King, 1970; Nienstaedt, 1972; Wilkinson, 1977) and may become more vulnerable to early spring frosts in a warmer climate (Colombo 1998). In chapter 3, I explored

the effects of artificial warming treatments applied in mid-winter on phenology (bud-break time) and growth of five selected and two wild sources of white spruce.

Chapter 1 Phenotypic and genetic correlations among tree volume, wood specific gravity and foliar traits in white spruce (*Picea glauca* (Moench) Voss) and implications for selection in Minnesota

Introduction

White spruce is highly valued by the wood products industry in North America and is widely planted across Minnesota. Seed orchards, comprised of genotypes selected for superior growth are a common seed source used for tree planting after timber harvesting on a commercial scale. Selection for fast growth is highly effective: differences between orchard-grown seedlings and local wild sources approach 30% in wood volume (Pike et al., 2006; Weng et al., 2010). However, negative correlations between growth rate and wood specific gravity (Chang & Kennedy, 1967; Corriveau et al., 1987; Duchesne & Zhang, 2004) may reduce wind-firmness of fast-growing genotypes even though net wood production after kiln-drying is not necessarily reduced (Stellrecht et al., 1974). Tradeoffs between growth rate and other wood traits such as fiber length (Beaulieu, 2003; Duchesne & Zhang, 2004) and veneer quality (Zhang, Yu, & Beaulieu, 2004) have also been found for white spruce. Quantifying tradeoffs that result from selection is a critical research need for orchard managers to optimize the genotypic composition of seed orchards to best meet their management objectives.

Growth rates, across plant taxa, are related to a myriad of traits that influence photosynthesis, light interception, and respiration (Reich et al., 2003). In global datasets, leaf traits that co-vary positively with growth rates across taxa include specific leaf area (SLA; $cm^2 \cdot g$), leaf area ratio (LAR; $cm^2 \cdot g^{-1}$) and leaf mass ratio (LMR; $g \cdot g^{-1}$) (Cornelissen et al., 2003). Subtle differences in leaf or needle morphology may lead to large differences in carbon-fixation across an entire tree (Reich, Tjoelker, Walters,

Vanderklein, & Buschena, 1998), but few studies have measured correlations between wood properties and leaf traits. Theoretically, traits that are positively genetically correlated with growth could be used to improve growth rates through indirect selection. By understanding mechanisms of growth and possible tradeoffs, the addition of foliar and/or wood traits to a tree improvement program could substantially increase wood production for future reforestation efforts.

The physical distance that a seed source may be transferred for reforestation work may be partially quantified through a study of genotype by environment interactions (herein referred to as g x e interactions). A significant g x e interaction signals that genotypic performances are inconsistent across sites, in which case, seed orchards with different genotype compositions are established for planting in defined seed zones. Alternatively, an insignificant 'g x e' interaction implies that genotypes are broadly adapted for planting across multiple sites without consequences for a trait of interest. In Minnesota, insignificant g x e interactions for height and volume growth have led to the state-wide deployment for selected genotypes in Minnesota (Klevorn, 1995). Field trials are costly, and generally fail to represent the full range of sites in which trees are deployed for commercial tree plantings. *A priori* knowledge of g x e interactions for wood density or foliar traits may improve deployment on sites that are untested or novel for the species to optimize the composition of seed sources for a targeted range of sites.

Nitrogen is an essential element in the photosynthetic apparatus of a plant, and therefore critical for plant growth. In plant tissue, the concentration of foliar nitrogen is influenced by environmental factors such as soils, latitude and mean annual temperature (Kang et al., 2011; Reich & Oleksyn, 2004). But foliar nitrogen also has genetic

underpinnings as revealed in common gardens (Oleksyn, Modrzynski, et al., 1998). Interior spruce (*Picea glauca* (Moench) Voss x *Picea engelmanii* Parry ex Engelm.) genotypes selected for fast-growth demonstrated higher efficiencies in nitrogen use (Hawkins, 2007; Miller & Hawkins, 2003, 2007). In loblolly pine, tall trees allocated proportionately more biomass to stem mass in high- vs low-nitrogen environments (B. Li, Allen, & McKeand, 1991). Traditional seed zones, based on abiotic variables (daylength, precipitation or temperature), may be enhanced with the addition of genotypic performance across a known soil/nitrogen gradient.

The first objective of this study was to compare genotypes planted at two distinct sites to study patterns of growth, foliar nitrogen, needle traits and wood specific gravity. The second objective was to test the hypothesis that growth rates are positively correlated to leaf traits, foliar nitrogen concentrations, and negatively correlated with wood specific gravity for genotypes selected for high wood production. This information may be used develop recommendations for seed orchard composition in the future.

Methods

Description of the study sites

In 1986 a progeny test, consisting of 292 open-pollinated half-sib families of white spruce, was established at five sites in Minnesota. Two sites are the focus of this study: one located near Finland, MN (47° 24' N, -91° 14' W, MAT=3.8°C, MAP=78 cm) hereafter referred to as "North" and one within the Nemadji State Forest in central Minnesota (46° 24' N, -92° 29' W, MAT=3.8°C, MAP=78 cm) (State Climatology Office, MN DNR Division of Ecological and Water Resources) hereafter referred to as "South." These sites were selected because tree survival was high in the 25th year,

exceeding 85% at both sites, and they represent distinct seed zones and floristic regions. Soils at each site were sampled and tested previously for macronutrients, texture, and organic matter. At North site, soil was a clay loam, pH 5.6, with 0.8% organic matter while South site had sandy loam soil with a pH of 5.5, also with 0.8% organic matter (Klevorn, 1995).

The progeny test was designed as a randomized complete block design with five replications and one 4-tree row plot per family in each replicate. All trees in the progeny test were planted in May 1986 at roughly 1.2 x 2.4 meter spacing. Tree heights were measured in 1990 and 1993, five and eight growing seasons, respectively, after planting (Klevorn, 1995). In 2000, both sites were thinned from four- to two-tree row plots, removing alternate trees in the row plots. Thinning increased spacing to 2.4 x 4.9 meters. Tree heights and diameters were measured again in 2005 and 2010 after 20 and 25 growing seasons, respectively. Tree volume was calculated for each tree (Equation 1) after (Ek, 1985), transformed to the cube-root to improve variance heterogeneity, and averaged by family using least-squared means to meet ANOVA assumptions of normality and stable variances (SAS Proc glm).

$$Volume\ (m^3) = \left((0.42 + 0.006 * 30 - Height) * Height * Basal\ Area\right) * \ 0.0283 \quad \text{(Equation 1)}$$

Criteria for genotypic selection

In 2008, a total of 284 trees were selected for intensive study, representing 30 families divided evenly among each of three selection tiers (10 genotypes per tier): top (ranks 1-91), middle (119-193), and low (202-283). Families from the top tier are most commonly represented in seed orchards for reforestation, while those from middle or lower tiers are generally excluded because of low performance. Ten trees were available

from each family at each site for this study. I selected one tree from each of the five two-tree row plots at each site, favoring co-dominant trees with no stem deformations for a total of five trees per site per family. When both trees in the row-plot were co-dominant I selected one tree at random. When neither tree was sufficient I skipped that replication but in most cases at least one suitable tree was available.

Protocol for collection of plant material

I collected one wood core and one branch sample per tree as described below for assessments of wood specific gravity and leaf traits, respectively. One increment core was collected at 1.3 meters above the ground, with a 12 mm-diameter standard increment borer (Haglof®). The increment core extended from bark to pith, and the bark plug was removed. The core was then placed into a precut PVC tube, and sealed with a cork for transportation to the lab. Within six hours of collection, the volume of each core was assessed using water displacement technique (Simpson, 1993; D. M. Smith, 1954). Each core was submerged in a flask of tap water with known volume, and the amount displaced was recorded to the nearest 1 ml. The cores were dried at 65° C for a minimum of five days and stored in a desiccant chamber until it reached room temperature. The core was removed and immediately weighed to the nearest 0.0001 grams. Wood specific gravity was calculated as the green volume divided by dried mass.

One branch, selected from the upper third of the south side of the tree crown was collected using pole pruners from each selected tree. Each branch was approximately one meter in length and contained multiple cohorts of needles. Branches were placed into individual plastic bags and refrigerated (1 °C) until processing. I excised approximately 40 needles from the previous year growth and scanned them using a back-lit scanner

(Hewlett Packard Scanjet 6100C). After scanning, needles were dried at 65°C for at least four days prior to weighing. Needle images were inspected for foreign particles that were erased, along with shadows, manually from each image. Using ImageJ software, the surface area of each composite needle sample was calculated (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2011). I calculated specific leaf area (SLA) as $cm^2 \cdot g^{-1}$.

I excised branch sections that contained one-year old needles from every full branch sample taken. The length of the reserved branch sections were measured to the nearest half centimeter and dried at 60°C for a minimum of five days. After drying, needles were removed and weighed to the nearest 0.001 gram. The dried woody material was also weighed to the nearest 0.001 gram. After weighing, a sample of needles was ground and assessed for leaf carbon and nitrogen on a COSTECH Analytical ECS 4010 (C. McFadden, University of Nebraska). Percent nitrogen (%N) was converted into nitrogen mass per unit leaf mass for the one-year old needles taken from the branch sample, $g \cdot cm^{-2}$ (N_{mass}). Nitrogen per leaf area (N_{area}) was calculated by multiplying SLA value by the needle mass for the one-year old needles. Branch leaf area ratio (LAR, $cm^2 \cdot g^{-1}$) was calculated using leaf area from SLA sample, but is scaled to the sum of the dried needle and woody stem of the branch. Branch leaf mass ratio, $g^1 \cdot g^{-1}$ (LMR), was calculated as the needle mass / needle+stem mass for the sample branch.

Statistical analysis

I assessed normality and variances for each trait visually, with SAS/STAT (Proc univariate, SAS Institute, Inc. 2011 version 9.3). I removed two outlier points that fell outside three standard deviations of the normal distribution, and applied a log

transformation to improve normality for all traits except tree diameters and volumes, which required no transformation.

I used analysis of variance in SAS/STAT (Proc glm) to compare the sites and to calculate genotype by environment interactions (g x e) with the model:

$$Y_{ijkl} = Site_i + Family_j\,(Selection\ Tier_k) + Site*Family(Selection\ tier)_{ijk} + e_{ijkl} \quad \text{Equation 2.}$$

where Y_{ijkl} is the observed value of the l^{th} tree in the i^{th} site and j^{th} family, nested in the k^{th} selection tier, with e_{ijkl} as the pooled error. I set all factors (site, family, and site*family interaction) as fixed variables to compare sites and to calculate the significance of site*family(selection tier) interaction.

I used a mixed models analysis to compare selection tiers for each trait (Proc mixed):

$$Y_{ijkl} = Selection\ tier_i + Site_j + Fam_k\,(Selection\ tier_j) + Site*Fam(Selection\ tier)_{ijk} + e_{ijkl} \quad \text{Equation 3}$$

Selection tier was set as a fixed effect (with 2 and 279 df), and all other factors were random effects with residuals assumed to be N~(0,1).

I used SAS/Stat (Proc mixed) to include a covariate value for tree volume to compare selection tiers with the model:

$$Y_{ijk} = Tree\ volume_i + Site_j + Selection\ Tier_k + e_{ijk} \quad \text{Equation 4.}$$

with site as a random variable, and selection tier as a fixed term. I used tree volume as the random covariate with leaf area ratio, leaf mass ratio and wood specific gravity as fixed variables for each of three analyses. Degrees of freedom were adjusted with Kenward-Roger correction (Kenward & Roger, 1997), and trait means were calculated at four different levels of the covariate. First I tested the null hypothesis that slopes for the selection tiers were equal to zero. Next I tested the null hypothesis that the slopes for the tiers were equivalent. If slopes were equivalent (i.e. I failed to reject Ho), the means for

selection tiers were compared with a model that assumed common slopes, using Tukey's adjusted test. When the slopes were not the same (i.e. I rejected Ho of common slopes), then I used SAS/STAT(Proc glm) to compare the slopes of the lines using orthogonal contrasts to compare the top and lowest tier, and the middle and lowest tiers.

I also calculated variance components for each trait/site combination using Restricted Maximum Likelihood in SAS/STAT (Proc varcomp) with a model containing family and error terms only. Narrow-sense heritability was calculated with the equation (Falconer & Mackay, 1996):

$$h^2 = \frac{4 * \sigma^2_{family}}{\sigma^2_{error} + \sigma^2_{family}} \quad \text{Equation 5.}$$

I calculated standard error for narrow-sense heritability with this formula:

$$SE = \frac{\frac{(1-h^2)}{4} * [1 + NR - 1) * \frac{h2}{4}]}{\sqrt{[\frac{NR}{2} * (NR-1) * (F-1)]}} \quad \text{Equation 6.}$$

Where N = sample size, R = number of replications and F = number of mother trees (Wright, 1976). I used SAS/STAT (Proc corr) to obtain Pearson correlations (phenotypic) using transformed variables for all trait combinations. I calculated genetic correlations from covariance components obtained in SAS/STAT (Proc mixed). The genetic correlation, r_g, was calculated for traits x and y:

$$r_g = \frac{\mathrm{cov}\, f(p)_{xy}}{(\sigma f(p)^2_x * \sigma f(p)^2_y)^{1/2}} \quad \text{Equation 7.}$$

where $cov\ f(p)_{xy}$ is the cross-product differences of traits x and y for each half-sib family; $\sigma f(p)^2_x$ is the variance between half sib families for trait x and $\sigma f(p)^2_y$ is the variance

between half sib families for trait *y*. The R matrix was estimated for site*family*tree, and a G-matrix was generated for each family.

Results

Trees at South site were significantly taller with larger volumes than the North site (Table 1-1, Figure 1-1). In addition, %N, N_{area}, N_{mass}, SLA, and branch LMR were significantly higher at South than North site. Wood specific gravity (WSG) and branch LAR did not differ significantly between sites (Table 1-1). Site by family (g x e) interactions were highly significant ($p<0.01$) for %N (Figure 1-2), and significant at $p<0.05$ for WSG and SLA (Figure 1-3).

Selection tiers differed significantly for tree height, diameter, volume, N_{area} (Figure 1-4), branch LMR, and branch LAR (Table 1-2). The top-tier trees were significantly larger than the low tier with greater LMR and LAR. However, N_{area} was lower in top compared to low tier trees (Table 1-2). Heritabilities for tree heights and diameters were generally lower at South site than at North site (Table 1-1). Heritability for %N was moderate at both sites. Heritability of branch LAR, and LMR varied between sites, generally being low at the North and moderate at the South site. SLA was moderately heritable at both sites.

In the analysis of covariance for branch LAR, the top tier differed from the low tier at $p<0.10$ only (Table 1-3). No differences among tiers were found for wood specific gravity or branch LMR in this analysis. Figures 1-5 through 1-7 portray the data as family means (least-squared means), with different symbols for top and low tier families. Middle tier families were intermediate between top and low-tiers and excluded from

Figures 1-5 through 1-7 for clarity. Top-tier families had higher values of branch LAR and LMR than low-tier families (Figure 1-5). Data is only shown for branch LAR; LMR was nearly identical. Low tier families generally had higher values of WSG than top-tier families (Figure 1-6). However, several families in the top-tier produced modest values of WSG. For N_{area}, I observed a negative trend with increasing volume by family, but otherwise lacked clear patterns (Figure 1-7). I also scaled N_{area} by average tree volume to infer stoichiometric relations for N in trees of different wood volumes, herein referred to as N_{volume} (Table 1-4). In spite of the higher %N on the South site, N_{volume} was approximately similar between sites, with consistently higher values of N_{volume} for low-tier genotypes than top-tier trees at both sites.

Tree volume was positively correlated, both phenotypically and genetically, with SLA, branch LAR and branch LMR (Figures 1-8 and 1-9, A-Table-1). Genetic correlations between tree volume and WSG, N_{area} and N_{mass} were significant and negative. Genetic correlations between WSG , %N and branch LMR were also significant and negative. Both N_{area} and N_{mass} were negatively correlated with branch LAR and branch LMR.

Discussion

My results demonstrate that differences of 0.011 m^3 in volume growth, approximately 33% of the site mean, are possible by selecting top- instead of low tier trees in this progeny test. The difference in volume between top- and low tiers is similar in magnitude to seed source trials comparing orchard-grown seed (consisting of genotypes selected for volume growth) to wild seed sources (Pike et al., 2006; Weng et al., 2010). Trees from the top tier were taller than the low tier at both North and South

sites, and had higher overall volumes, validating selection efforts. The sites differed only by 1° latitude, but represent two distinct forest types: the North site commonly supports spruce and fir, while oaks and other deciduous trees dominate the forests adjacent to the South site. Tree heights and diameters in my study were similar to another in the Lake States (Stellrecht et al., 1974): at year 15, our tree heights ranged from 1.0-4.9 meters, and diameters from 1.3 cm to 5.1 cm, compared to 4.6 to 6.9 meters, and diameters 6.3-10.2 cm.

Strong, significant genetic correlations between tree volume and foliar traits (branch LAR, LMR and SLA) suggest that top tier sources may invest more heavily into leaves and leaf area than lower tier families. Means for branch LAR and LMR were significantly different between top and low-tiers (Table 1-2), but taking an allometric approach (Table 1-3) suggests that low- and top tiers fall along the same allometric line. This result implies that top tier individuals are further along a shared developmental trajectory, and that allocations are not fundamentally different. Interspecific data sets of woody (Reich et al., 1998) and non-woody C3 plants (Poorter & Remkes, 1990) correlate growth positively with SLA, LMR and LAR since these foliar traits impact photosynthesis and light capture. Positive genetic correlations (Figure 1-8) suggest that breeding for increased growth rates might increase LAR, LMR and SLA in future generations, a result that may be verified through advanced-generation populations.

Tree volume and wood specific gravity (WSG) were negatively correlated mirroring other studies conducted in the Lake States (Beaulieu, 2003; Corriveau, Beaulieu, & Daoust, 1991; Merrill & Mohn, 1985). However, my data supports the contention that high growth rates are not necessarily a harbinger of reduced WSG

(Gaspar, Lousada, Rodrigues, Aguiar, & Almeida, 2009). Approximately half of the top-tier families produced an average WSG that was at or above the overall mean (Figure 1-6). Therefore, selection for genotypes that combine high volume and high WSG appears plausible for development of seed orchards.

Genotype by site interactions were not significant for tree volume in this dataset of 25-year old trees, nor were they significant for tree height after eight-or ten-years of growth for the same progeny test (Klevorn, 1995). Six of the ten families in the top-tier originated in the Ottawa Valley; four were selected from natural forests in Minnesota. My findings follow range-wide provenance trials that reveal exceptional growth of sources originating in the Ottawa Valley, Ontario (Radsliff, Mohn, & Cromell, 1983; Stellrecht et al., 1974). My results also support the continued state-wide deployment of top-tier genotypes to maintain high wood production.

Nitrogen content, in foliage of sample branches, was largely a function of stoichiometric limitations of the sites, rather than genotype because smaller, low-tier genotypes had higher amounts of N_{area} and N_{mass} than top-tier trees. This was confirmed by SLA, which was positively correlated with growth and negatively correlated with N_{area} and $N_{mass.}$ Fast-growth was correlated with increased nitrogen reserves in a controlled study of interior spruce (Miller & Hawkins, 2003). In loblolly pine, selected genotypes allocated biomass preferentially to stems in high N environments but this study did not assess nitrogen concentrations in the foliage of their study trees to capture stoichiometric differences (B. Li et al., 1991). Percent N was higher in the South site, suggesting that the South site was more N-rich, but this study lacks replication to determine whether

differences in growth between sites are due to foliar nitrogen, photoperiod, climatic differences or a combination of all.

Heritabilities were inconsistent for tree heights between the two sites, being high in the North and low in the South, and were generally more typical of the species at the South site for tree height and WSG (Corriveau et al., 1991; Polge, P.H.; Illy, 1967). A previous study of this progeny test reported a heritability (combined by sites) at year eight as 0.13 for tree height with standard error 0.02 (Klevorn, 1995), intermediate to my estimates that were separated by site. Narrow-sense heritabilities for tree height in other white spruce studies are also broad, ranging from 0.10 (Holst & Teich, 1969), 0.30 (Wilkinson, 1977), to 0.4-0.6 (Nienstaedt & Riemenschneider, 1985). Discrepancies in heritability estimates was also reported for clonal genotypes of white spruce at multiple test sites in Canada (Wahid et al., 2012). It is possible that error variation for WSG was increased at North site because of insufficient saturation of cores prior to volumetric sampling, but there is not sufficient data to support that the samples were more or less dry at one site over the other. Saturation is a prerequisite for the "maximum moisture content" method in assessing WSG (D. M. Smith, 1954), but I relied on latent moisture because each sample was removed from the ambient atmosphere by placing in a sealed cylinder and assessed volumetrically within a few hours of sampling. Instead, inconsistent heritabilities between sites may reflect varying levels of additive and non-additive gene expression, interactions with environments, and the relative small sample size of my selected population.

Management implications

Genotypes selected from the top tier were larger than the low tier at each of the two sites in my study: a typical northern boreal forest and a hardwood forest along the southern range-edge of white spruce. Genotype by environment interactions were not significant for tree volume, nitrogen per unit mass, nitrogen per unit leaf area, leaf area ratio and leaf mass ratio. Significant negative correlations with wood specific gravity indicate possible tradeoffs with advanced generation breeding for volume alone. However, roughly half of the top tier families combined high volumes and above average wood specific gravity, so that additional screening of top tier families may be warranted to favor trees that are favorable for both traits. Selection for fast growth may impact mechanical properties of wood more directly than wood specific gravity (Zhang, 1995), which merits further study depending on the final product desired for reforestation. Other traits that affect the value of the final end-product should also be considered to avoid negative tradeoffs that affect commercial value of forest products (Zhang & Morgenstern, 1995).

Branch LMR, LAR and SLA correlated strongly with wood volume, suggesting that carbon fixation may be more effective among top- than low tier genotypes. The modest heritabilities, combined with low g x e interactions, of SLA, branch LMR and LAR suggest that foliar traits may shift indirectly with subsequent generations of a controlled breeding program. Differences between sites and tiers for % foliar nitrogen and nitrogen per leaf area (N_{area}) were driven largely by stoichiometric limitations although I lacked evidence to support greater efficiencies in N-use by top tier genotypes. Tree improvement programs could apply this information rapidly by adjusting the genetic

composition of orchards to incorporate top tier genotypes for volume, along with modest wood specific gravity and high SLA/LAR to optimize wood production across sites.

Tables

Table 1-1. Least-squared means, standard errors, narrow sense heritabilities and standard errors for each trait at each site. Means that are significantly larger in a row are in boldface. Log-transformed data with outlier points removed is indicated with superscript t. N is approximately 150 at each site. WSG=Wood specific gravity. Nmass (grams of nitrogen per unit of branch mass), Narea (grams of nitrogen per unit of leaf area) branch Leaf Area Ratio (LAR) and branch Leaf Mass Ratio (LMR) were calculated for one sample branch per tree.

	North		South		p-value
Trait	**Mean (SE)**	**h^2 (se)**	**Mean (SE)**	**h^2 (se)**	**GxE**
Tree heights (m)	7.27 (0.08)	0.43 (0.03)	**8.19 (0.11)**	0.08 (0.01)	NS
Tree diameter (mm)	113.2 (1.76)	0.63 (0.04)	**122.5 (2.1)**	---	NS
Tree volume (m^3)	0.035 (0.001)	0.75 (0.04)	**0.045 (0.001)**	0.08 (0.01)	NS
WSG	0.365 (0.002)	---	0.359 (0.003)	0.57 (0.03)	< 0.05
Leaf % N	1.01 (0.01)	0.81 (0.04)	**1.08 (0.01)**	0.49 (0.03)	< 0.01
N_{mass} ($g\ g^{-1}$)	2.5 (0.08)	0.11 (0.01)	**2.9 (0.09)**	0.36 (0.02)	NS
N_{area} ($g\ cm^2$)	9.5 (0.33)	0.13 (0.01)	**11.3 (0.35)**	0.45 (0.03)	NS
SLA ($cm^2\ g^{-1}$)	3.98 (0.07)	0.57 (0.03)	**4.01 (0.09)**	0.21 (0.01)	< 0.05
LAR ($cm^2\ g^{-1}$)	1.85 (0.05)	0.03 (0.01)	1.97 (0.06)	0.45 (0.03)	NS
LMR ($g\ g^{-1}$)	0.47 (0.01)	0.04 (0.01)	**0.48 (0.01)**	0.12 (0.01)	NS

Table 1-2. Least-squared means and differences among selection tiers for tree height, diameter, leaf area ratio and leaf mass ratio. Letters below the standard errors depict differences among groups using Tukey's LSD adjusted for multiplicity.

		Selection Tier		
	Row	**Low**	**Middle**	**Top**
20-year heights, m	Mean	7.45	7.51	8.22
	SE	0.46	0.47	0.46
	Tukey's	b	b	a
20-year dbh, mm	Mean	111	115	128
	SE	4.9	5.0	5.0
	Tukey's	b	b	a
Volume, m^3	Mean	0.035	0.038	0.048
	SE	0.005	0.005	0.005
	Tukey's	b	b	a
Branch N_{area} ($g \cdot cm^2$)	Mean	2.322	2.303	2.160
	SE	0.109	0.109	0.110
	Tukey's	a	ab	b
Branch LAR, ($cm^2 g$-1)	Mean	0.057	0.058	0.072
	SE	0.006	0.006	0.006
	Tukey's	b	ab	a
Branch LMR, ($g \cdot g^{-1}$)	Mean	0.460	0.466	0.499
	SE	0.016	0.016	0.016
	Tukey's	b	ab	a

Table 1-3. Results from analysis of covariance with log-transformed tree volumes as a covariate (tVol). Trait variables included log-transformed(t) LAR, LMR, and WSG (wood specific gravity) between top- vs low selection tiers. Trees in the top selection tier had highest volumes in the progeny test.

		p-values				
Trait	**Covariate**	**Ho: slopes = 0**	**Ho: slopes are =**	**Ho: tiers are =**	**Top vs Low**	**Mid v Low**
t LAR	t Vol	$p < 0.05$	NS	NS	0.090	NS
t WSG	t Vol	$p < 0.01$	$p < 0.01$	NS	NS	NS
t LMR	t Vol	$p < 0.01$	$p < 0.01$	NS	NS	NS

Table 1-4. Nitrogen per mm^2 of leaf area (N_{area}), scaled to wood volume (m^3) at each site ($N/cm^2/m^3$). Values are least-squared means per site and selection tier.

	North		South	
	N_{area}	Volume	N_{area}	Volume
Top tier	8.47	0.0432	10.22	0.0531
Low tier	11.98	0.0313	11.989	0.032
	N_{volume}		N_{volume}	
Top tier	196.1		192.5	
Low tier	382.7		374.6	

Figures

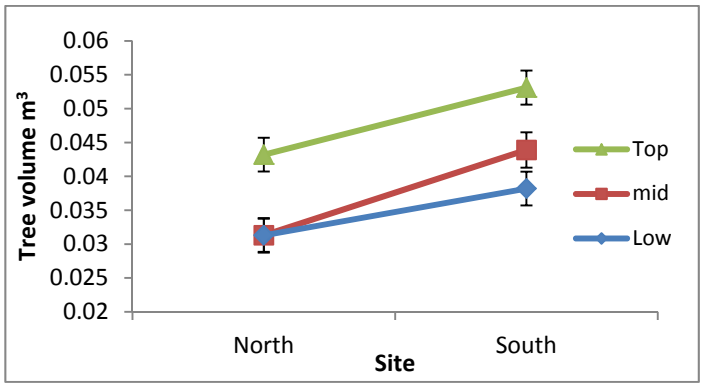


Figure 1-1. Tree volume for top, middle and low selection tiers at each of the two sites. The interaction was not significant at $p<0.05$.

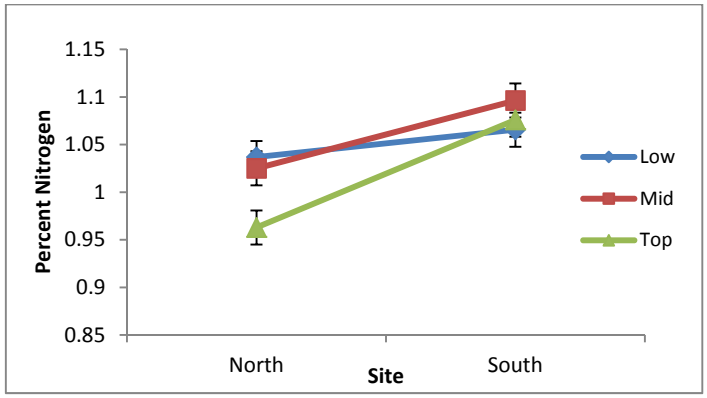


Figure 1-2. Percent nitrogen for three tiers at two sites. The g x e interaction was significant at $p<0.01$.

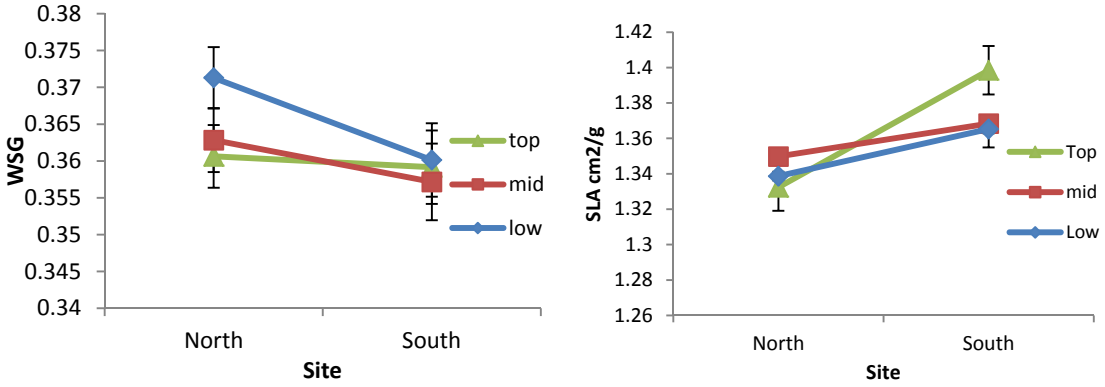


Figure 1-3. Wood specific gravity (WSG) and specific leaf area (SLA) by selection tier and site. Genotype by environment interactions were significant for both at $p<0.05$.

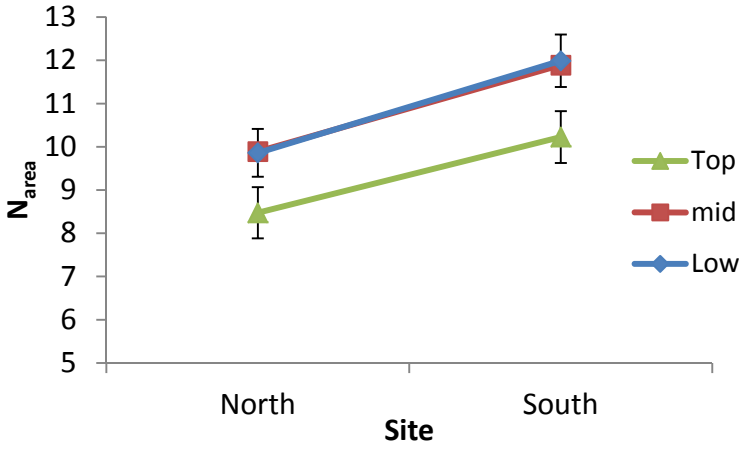


Figure 1-4. Nitrogen (grams) per cm^2 of leaf area (N_{area}) for top, mid and lowest tiers at north and south sites. The g x e interaction was not significant.

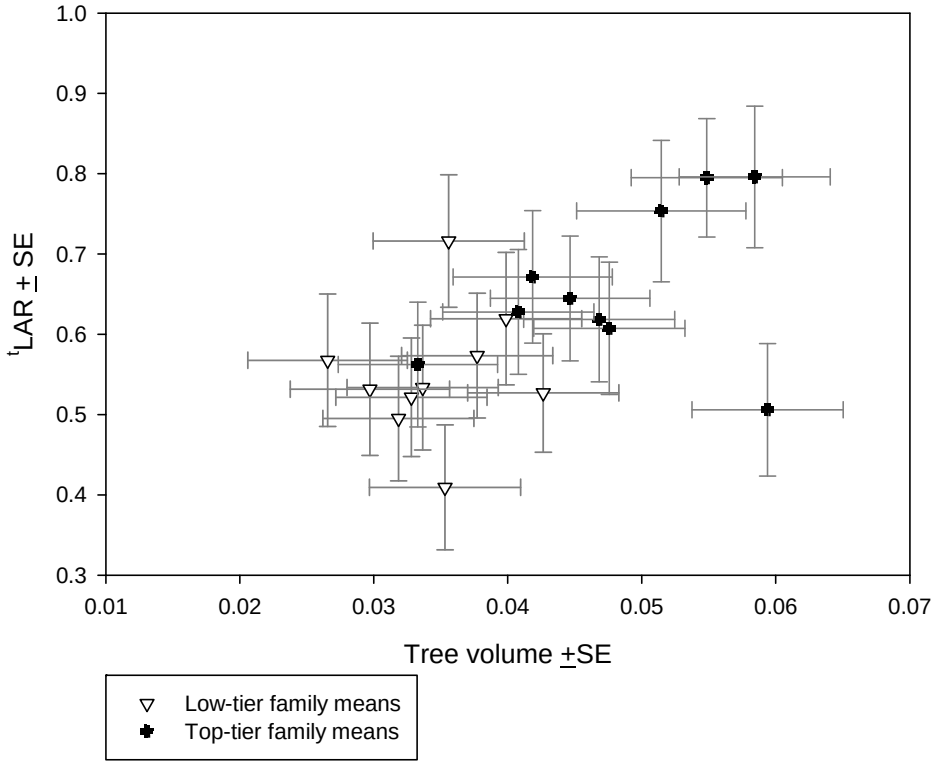


Figure 1-5. Leaf Area Ratio (LAR, log transformed) by tree volume (m^3) for top and low-tier families (least-squared means) Low- and top tiers had equal slopes, and the y-intercepts differed significantly at $p<0.05$.

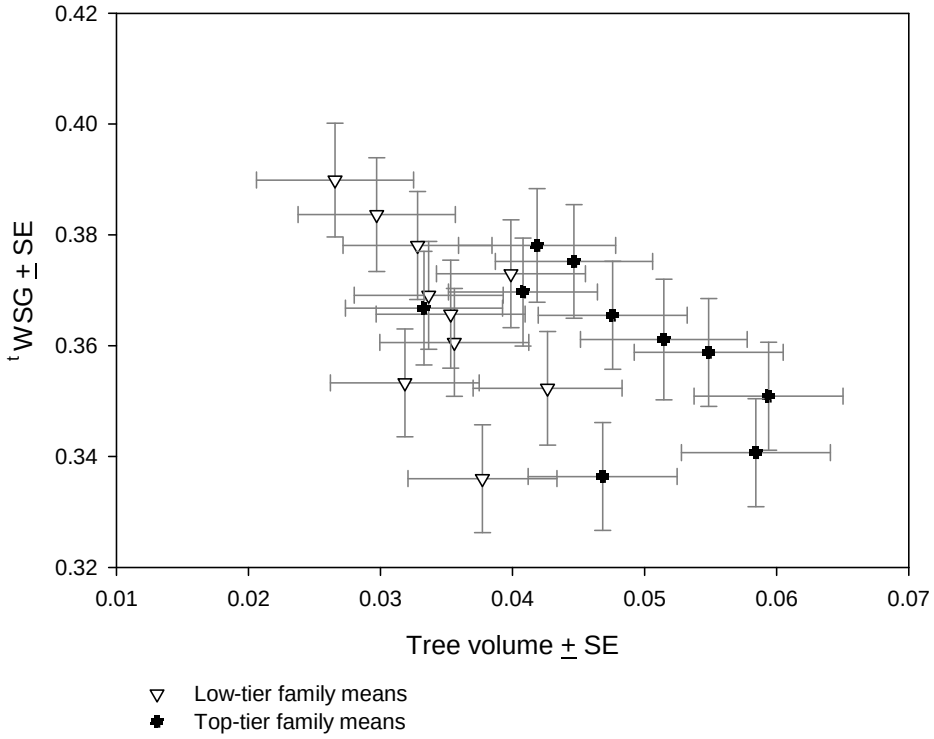


Figure 1-6. Wood specific gravity (WSG) vs tree volume (m^3) for families in top- and low selection tiers. Least-squared means are shown for volume and WSG with standard errors.

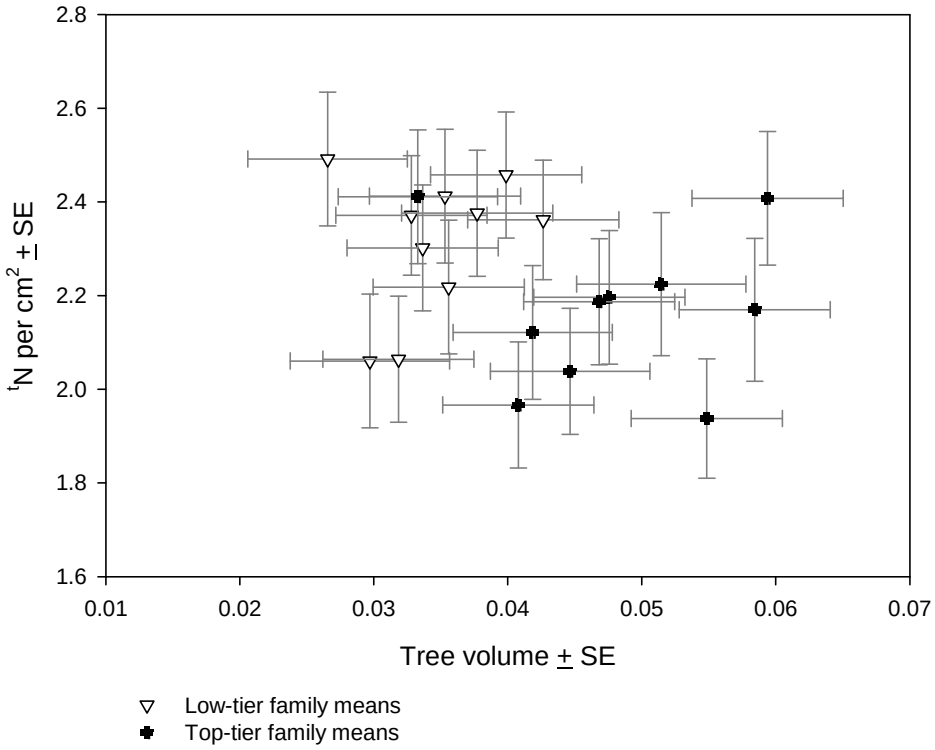


Figure 1-7. Nitrogen per leaf area (N_{area}) in grams / cm^2 by tree volume (m^3) for families in top and low selection tiers. Least-squared means are shown for both traits ± standard error.

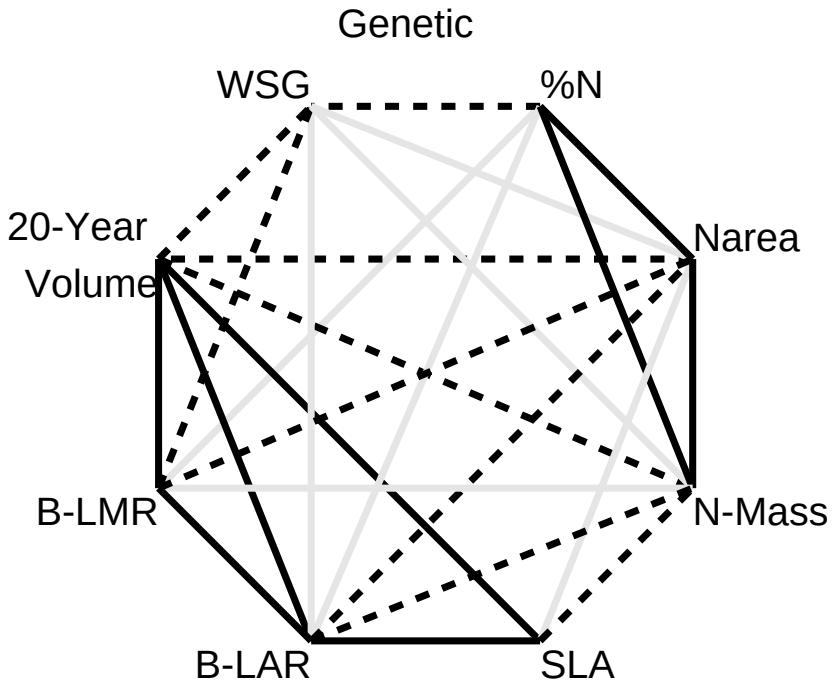


Figure 1-8. A pictorial correlogram depicting genotypic correlations. Dark, solid lines depict significant, positive genetic correlations ($p<0.05$). Dashed lines depict significant, negative correlations. Insignificant correlations are represented with solid grey lines. Abbreviations are described in Table 1. The calculations of genetic correlations are described in the Methods section.

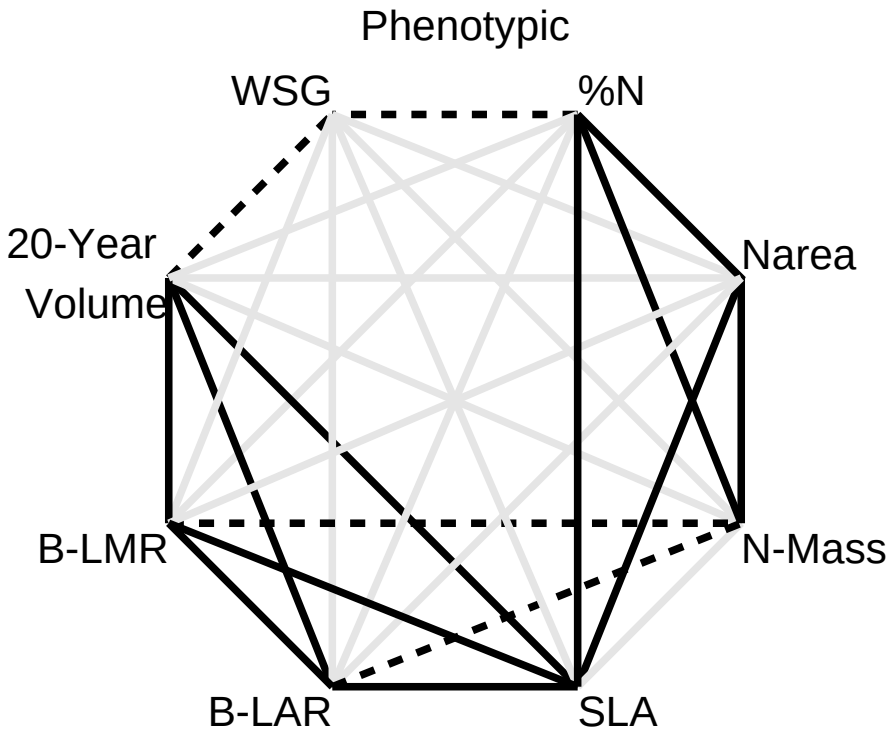


Figure 1-9 A pictorial correlogram depicting phenotypic correlations. Solid black lines depict significant, positive correlations (at $p<0.05$). Dashed black lines depict significant, negative correlations. Insignificant correlations are represented with solid grey lines. Abbreviations are described in Table 1.

Chapter 2 Biomass allocation between selected and wild sources of white spruce, *Picea glauca* (Moench) Voss

Introduction

Tree improvement programs have dramatically increased wood production through selection and breeding of commercially valuable forest tree species. In the southeastern US, wood volume of *Pinus taeda* (loblolly pine) has increased by 7-12% in first-generation orchards versus wild sources, and 17-30% with second-generation orchards (B. Li, Mckeand, & Weir, 1999). Increases in volume by planting selected *Picea glauca* sources range from 9-27% in Canada (Weng et al., 2010) and 15-30% in Minnesota (Pike, Warren, & David, 2002). White spruce seed collected from the Ottawa Valley of Ontario generally outcompetes locally adapted seed sources across the range of white spruce (Morgenstern, D'Eon, & Penner, 2006; Nitschke, 1986; Radsliff et al., 1983), and is exceptional even in northern Ontario and Minnesota (Mark R. Lesser & Parker, 2004; Stellrecht et al., 1974). The increases in wood production for white spruce are striking because they result from first-generation selection at latitudes that have considerably shorter growing seasons than the southeast US. When scaled to a landscape level, the additional wood production made possible through selection has significant economic value to the forest products industry.

High growth rates in select trees may be attributed to a variety of mechanisms. These include: longer growing-season length (Hannerz, Aitken, King, & Budge, 1999; Oleksyn, Reich, Tjoelker, & Chalupka, 2001), early bud-break (Wilkinson, 1977), and shifts in biomass allocation (Rweyongeza, Yeh, & Dhir, 2005). Seed size is also positively correlated with early growth in Scots pine (Castro, 1999; Reich, Oleksyn, &

Tjoelker, 1994). However, in white spruce, only 12% of two-year-old seedling growth could be attributed to seed size (Sylvie Carles et al., 2009) suggesting that seed size alone is not a strong predictor of growth. Shifts in biomass allocation among leaves, stems, and roots are also of particular interest since they could result in tradeoffs given the ecological values of photosynthesis, competition for light, and reproduction. In particular, changes in biomass allocation could result in inadequate seed production (Yousry & Barclay, 1992) or shallow root systems (Grossnickle, 2005) with implications for long-term tree survival. An imbalance in biomass among critical plant structures resulting from artificial selection could impact the fitness of planted trees or subsequent natural regeneration from planted stands.

Root systems are critical to plant growth because their architecture and relative size affect water and nutrient uptake. Root size and structure vary greatly among species (Kalliokoski, Nygren, & Sievanen, 2008), and are highly plastic, varying with light (Canham et al., 1996) and nutrient availability (Shipley & Meziane, 2002). Root mass allocations also vary with provenance in white spruce, being proportionately higher in northern than southern seed sources (S. Carles, Lamhamedi, Beaulieu, Stowe, & Margolis, 2011; Oleksyn et al., 2001). Changes in biomass allocations that reduce root mass could have particularly high impact on seedlings, because water acquisition is critical for the survival of small trees (Grossnickle, 2005). However, allocation to roots, often measured as root mass ratios (RMR; $g \cdot g^{-1}$), are not well studied. In one of the few studies to date, root length was more strongly correlated with relative growth rate among species than root mass (Reich et al., 1998) suggesting that not only biomass allocation but morphology may also be important.

Biomass allocations are governed by a combination of genetic and environmental factors. Breeding programs can separate sources of variation into their genetic and environmental components through pedigrees that result from breeder-imposed selection. Understanding whether traits are genetically correlated, or inherited together, is important because genetic correlations may constrain species responses if selection is antagonistic to the direction of the genetic correlation (Etterson & Shaw, 2001). Tree breeding programs that focus on increasing stem mass warrant *a priori* knowledge of genetic correlations to understand potential consequences of selection on other critical traits.

The primary objective of my study was to test the hypothesis that allometry, or relative proportions, of major organs will be conserved among seed sources selected for increased wood production. In other words, relative biomass for each organ (needles, roots, stems) for a variety of selected seed sources will fall along the same basic allometric line as wild, or slower growing, sources. The alternative, that seed sources selected for fast growth favor stem mass over roots or needles, would produce a different allometric line from wild sources. My study tests the common allometric hypothesis three different ways by comparing organ biomass between a) wild and selected sources of white spruce seedlings, b) the two wild sources and c) the two Ottawa Valley sources and d) select sources from Ottawa Valley and Minnesota. The last comparison (d) was performed to understand if allometry differs between two selected genotypes that originate from two different provenances.

Methods

Seed sources

Wild, open-pollinated seed was obtained from each of two seed zones by the Minnesota Department of Natural Resources State Nursery program (Appendix Figure 1): "W-MN-C" for wild sources from the Central zone near Hill City, Minnesota (Lat 46.9°N, Long 93.6°W), and "W-MN-N" for wild seed sources from the North East zone near Little Fork, Minnesota (Lat 48.4°N, Long 93.5°W). The wild sources differ primarily by latitude and not longitude. Open-pollinated seed was also collected from four genotypes at a clonal seed orchard owned by UPM-Blandin Paper Company in Grand Rapids, MN (Lat 47.2°N, Long 93.5°W). I chose four genotypes, herein referred to as "selected" sources or genotypes (designated as "S-MN-" or "S-ON-"), based on their half-sib progeny's performance (tree volumes) following the 20th growing season at a field trial of 292 families replicated at five sites in Minnesota (Klevorn, 1995). Two of the selected genotypes originated from natural stands in Minnesota (source names "S-MN-127" and "S-MN-132") and two originated from the Ottawa Valley in Southeastern Ontario, Canada near Beachburg (Lat 45.7°N, Long 76.8°W) (source names "S-ON-400" and "S-ON-419"). Genotypes "S-MN-127" and "S-MN-132" ranked 34 and 60, respectively, while Ottawa Valley sources "S-ON-400" and "S-ON-419" ranked 16 and 21, respectively, out of 292 genotypes at the progeny test. We were unable to acquire seed from the top 1 and 2 ranked families, so we chose the best seed sources that were available. This sample of four selected genotypes were ranked within the top-third for wood volume, and are currently used as a 'highly-improved' seed source for commercial reforestation in Minnesota.

Establishment of seedling study

Seed from the six sources (four selected sources, two wild sources) was germinated into 6A styroblocks (95 ml per cavity) at PRT nursery in Dryden Ontario in March, 2008. I used reserved seed from each seed-lot to calculate measures of seed areaby placing approximately 100 seeds on a back-lit scanner (Hewlett-Packard Scanjet 4c), and analyzing the image with ImageJ software (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, 1997-2012). To estimate seed mass, I weighed the subsample of seeds that had been scanned and calculated average mass per seed. No variances for seed mass could be estimated because each sample was weighed only once. The sample was large (330-700 seeds per sample). Our accuracy in the seed counts was facilitated by using ImageJ software to count the seeds that had been scanned. Seed size is a critical trait to include because large seeds tend to produce large seedlings, an effect that can be measurable for up to five years in Scots pine (Reich *et al.* 1994).

In spring 2009, 300 50-liter pots were filled with potting soil (50% peat moss, 15% perlite and 35% composted bark) at the Cloquet Forestry Center located in Cloquet, Minnesota (46°31' N Latitude and 92°30' W Longitude), at an elevation of 385 meters above sea level. The potting soil had been supplemented with approximately four liters of sandy-loam soil collected from a nearby soil pit. The pots were buried in pre-excavated trenches to within one decimeter of the pots' lip to insulate against temperature extremes. The trenches were underlain with landscape fabric to facilitate collection of roots that escaped the pots. A soaker hose was run along the pots to irrigate trees in the spring and summer months. In May 2009, 300 seedlings, 50 from each of the six seed

sources, were planted into the individual pots in a completely randomized design.

Sampling protocol

I reserved ten seedlings directly from the styroblocks in spring 2009 for each of the six sources to provide baseline data, herein referred to as "year 0" seedlings. In November 2009, 2010 and 2011, seedlings were destructively sampled, representing one (year 1), two (year 2), and three (year 3) growing seasons completed in the large pots. At each sampling date, ten seedlings from each of the six seed sources were carefully excavated. Trees were removed from the pots and sprayed with tap water to remove all potting material. Roots were cleaned on successive large and small width sieves so that all major roots and as many small roots as possible were recovered. Tree height, diameter at root collar, and length of the longest root were measured for each excavated seedling.

Trees were cut at the root collar; roots and stems+needles were placed into separate paper bags. All tissue samples were dried at 60°C for a minimum of 72 hours. After drying, needles were separated from stems and re-dried for an additional 24 hours. All primary variables are shown in Table 2-1. Dry weights (to the nearest 0.1 gram) were obtained separately for roots, stems and needles to calculate the whole-tree proportion of biomass for each major organ represented by root mass ratio (RMR), stem mass ratio (SMR), and leaf mass ratio, (LMR). Specific stem length (Stem length/ stem mass, SSL, $cm \cdot g^{-1}$) was calculated for each tree as well.

Intra-specific variation for critical leaf metrics are not well studied so I chose to include them as traits that may co-vary with wood growth. Approximately 40 needles were removed from current-year growth on the main leader, and scanned on a back-lit

scanner to obtain a measurement of specific leaf area (SLA, $cm^2 \cdot g^{-1}$). Needle samples for SLA were dried and weighed to the nearest 0.001 gram. SLA was subsequently used to calculate leaf area ratio (LAR, $cm^2 \cdot g^{-1}$), the amount of leaf area per unit plant mass.

Statistical analysis

Survival was 100 percent, permitting a balanced design, so that ten trees per seed source were sampled each year for all four years. All data was reviewed and I removed outlier points that fell outside the third standard deviation because transformations could not rectify the distributions. In total, one data-point was removed from 'year 0,' two were removed from 'year 1,' and three were removed from 'year 2' data. All subsequent analyses were conducted with various procedures in SAS/STAT® version 9.3 (SAS Institute, Cary NC). ANOVA assumptions of normality and variance stability were checked using Univariate procedure in SAS followed by a visual assessment of the residuals. In the year 1, roots were not weighed for one tree from each of the six sources because the roots were inadequately cleaned of dirt and debris prior to drying. Dried weights for roots, stems and needles from entire plants were log-transformed to improve variance stability and to obtain allometric coefficients.

For seed areas, I used ANOVA (SAS Proc glm), followed by a Tukey's HSD test to assess differences among the six sources when the p-value for source was significant. I assessed significance at a p-value of 0.05 for all results, except when noted otherwise. No analysis was performed on seed masses. For seedlings, ANOVA (SAS Proc glm) was used to measure significance for year, seed source, and year*seed source interactions (all variables fixed) for tree height, diameter, SLA, log needle mass, log stem mass, log root mass, and total biomass (roots+stems+needles). Because year was highly significant, I

performed ANOVA for each collection year on the same traits to compare the six seed sources. I used Tukey's HSD if the seed source factor was significant and orthogonal planned comparisons if not. Unlike Tukey's test, which requires a significant model p-value, orthogonal planned comparisons can be used whether the ANOVA is significant or not.

I used a multivariate analysis of variance to further investigate differences among seed sources for 'year 3' data using the following traits: log root mass, log stem mass, log needle mass, tree height, diameter at root collar, and SLA. My objective was to quantify the suite of traits that best classified seed sources with a discriminant analysis (SAS Proc discrim). I used pre-planned contrasts to make comparisons among sources (Table 2-2).

Ontogeny effects are strong in studies of biomass allocations (Coleman, McConnaughay, & Ackerly, 1994) and can be reasonably overcome by regressing the plant trait of interest against overall biomass and comparing slopes for different treatment effects (Espinoza, Magni, Martinez, & Ivkovic, 2013; Poorter & Nagel, 2000; Reich, 2002; Roa-Fuentes, Campo, & Parra-Tabla, 2012). This approach is useful to understand whether differences in growth among seed sources are associated with different allometric proportions, or if seed sources follow the same trajectory but grow at different rates. I used the general linear models procedure in SAS (Proc glm) on logarithmic-transformed weights of roots, stems, needles to compute allometric constants through regression. Log-transformed root mass, stem mass, and needle mass were each regressed on the log of combined weights (mass of root+stem+needles). In addition, I compared sources for specific stem length (tree height regressed over stem mass), root to stem ratio (root mass regressed over stem mass), and leaf area ratio (leaf area regressed

over needle biomass). I compared regression slopes with pre-planned contrasts (Table 2-2) as described earlier.

Lastly, I calculated correlations (genetic and phenotypic) for trait pairs across years. Genetic correlations differ from phenotypic correlations (Pearson correlations) in relating breeding values, or the relative quantity of a trait that is transmitted from parent to offspring. In contrast, phenotypic correlations measure the relationship between two traits but do not separate sources of environmental and genetic variation. The genetic correlation, r_g, was calculated for traits x and y in Equation 1:

$$r_g = \frac{\mathrm{cov}\, f(p)_{xy}}{(\sigma f(p)_x^2 * \sigma f(p)_y^2)^{1/2}} \quad \text{Equation 1}$$

where $cov\ f(p)_{xy}$ is the cross-product differences of traits x and y for each seed source; $\sigma f(p)^2_x$ is the variance between sources for trait x and $\sigma f(p)^2_y$ is the variance between sources for trait y. The R matrix was estimated for site*seed source*tree, and a G-matrix was generated for each seed source. I calculated Pearson correlations with SAS (Proc corr), and genetic correlations were calculated with SAS (Proc mixed).

Results

Seed traits

The analysis of variance was highly significant among sources for seed size ($p<0.001$), permitting the use of multiple comparisons and Tukey's test. Seed from S-ON-419 had the smallest seed area (Figure 2-1) compared to the other five sources. In contrast, seeds from S-ON-400 had the highest seed area compared to all other sources. In addition, seed areas were larger for the central wild source (W-MN-C) than the

northern source (W-MN-N). The regression between area and mass (Figure 2-2) resulted in a R^2 of 0.64 between average seed area vs seed mass.

Seedling traits

Seedlings were assessed first with full model ANOVA that included year, seed source, and the year*seed source interaction as factors for the following traits: tree height, diameter, SLA, longest root length, and masses of root, stem, needle and total tree mass. Year was highly significant for all traits (ANOVA p-value <0.001). A significant interaction (seed source by year) was found for SLA. In the full model, seed sources differed significantly in root mass, but no other traits differed significantly by seed source. Given the significant interaction, I analyzed traits separately by year (below).

Seed sources differed significantly only in year 0, at $p<0.10$ for tree diameter, and at $p<0.05$ for SLA, root mass, stem mass, needle mass, and total biomass (Table 2-3). Length of longest root did not differ among seed sources. Seed sources did not differ significantly for any trait in years 1-3.

Seedling traits: 'year 0'

In year 0, seedlings from a selected Ontario source (S-ON-400) were significantly taller than both wild sources (W-MN-N and W-MN-C), and S-MN-127 (Table 2-3). The other selected Ontario source, S-ON-419, had higher stem and needle mass than W-MN-C and S-MN-127, and higher stem mass than W-MN-C. Using orthogonal contrasts, I found significant differences between selected and wild sources for all traits except tree diameter and allocation to leaf biomass: selected sources were taller, supported more total biomass, and higher stem mass than the combined wild sources (Table 2-4). Also in year

0, trees from S-ON-400 were taller, with higher leaf area and SLA than S-ON-419 (Table 2-5).

Seedling traits: 'years 1' to 'year 3'

I also used pre-planned orthogonal contrasts to compare groups for years 1-3. First, I compared the wild seed sources to each other (W-MN-N and W-MN-C) and found no significant differences for any trait in years 1, 2 or 3 (data not shown). I also failed to detect differences between MN (S-MN-) and Ontario (S-ON-) selections at years 1, 2 or 3. I then compared the wild (W-) and selected sources (S-). Results varied among years. At year 1, selected sources had significantly more root, stem, needle, and total biomass than wild sources (Table 2-4). At year 2, no significant differences were found between selected and wild sources for any pre-planned comparison at $p<0.05$. However, by year 3, selected sources had higher leaf area and longer roots than wild sources (Table 2-4). Lastly, I observed differences between the two Ontario genotypes. I found no differences in year 1, but in year 2, S-ON-419 had higher SLA and longer roots than S-ON-400 (Table 2-5). By year 3, S-ON-419 trees were taller, with larger diameters, and greater stem mass than S-ON-400.

MANOVA

I used a multivariate analysis to test the hypothesis that sources differed significantly with all variables included in the model. MANOVA was highly significant in years 0-2 ($p<0.05$), and significant at $p<0.10$ in 'year 3.' I only report results from the oldest trees to reduce seed or cultural (nursery) effects in the dataset. By the end of the third growing season only the two selected sources from Ontario could be distinguished from one another (Table 2-6; $p<0.01$). A discriminant analysis revealed that 70% of the

variation could be attributed to the first canonical axis with a canonical correlation of 0.611 with an approximate standard error of 0.08 (Wilkes Lambda p-value=0.08). The second axis was not significant so discrimination is reported along the first axis (x-axis) only. Ontario source 419 (S-ON-419) populated the positive classes (high stem mass, heights), and S-ON-400 populated the smaller values of Can 1, (high root and needle masses) (Table 2-7). Stem and root mass posted the highest absolute values for canonical coefficients, and in opposing directions (Table 2-8). Wild northern sources (W-MN-N) were clustered in close proximity to S-ON-400, and wild central sources (W-MN-C) were in close proximity to S-ON-419. The two Minnesota selected genotypes were scattered across the middle of CAN1 (Figure 2-3).

Allometric regressions

To assess whether size differences were due to shifting allocation or just ontogenetic changes in allocation ratios I used an allometric approach. I first combined data across years to compare allometric coefficients between trait pairs. I compared slopes between the two wild sources, W-MN-North and W-MN-Central, which were statistically similar for all trait pairs except root mass vs total mass (Table 2-9, Figure 2-4). W-MN-Central trees tended to have larger root masses than the North seed source at years ‘1’and‘2,’ but followed the same allometric line. At ‘year 3’ the RMR was higher for North than Central sources affecting the slope. However, I question the biological significance of this difference as the slope differences appear to be very small (Figure 2-4) but this divergence may become more pronounced in future years. I also detected significant differences in allometric coefficients between the two Ontario sources with respect to stem mass ratios (stem mass vs total mass), and root to stem ratios (Table 2-9),

but again the differences in slopes are very small with sources following similar growth trajectories (Figures 2-5 and 2-6). Selected sources differed from wild sources for specific root length only (root length vs root mass). The slopes were significantly different but the assumptions of linearity are not met, so I only chose to interpret the data visually.

Genetic and phenotypic correlations

I used correlations to understand the genetic association between trait pairs. First I examined the genetic correlations between seedling height and other organ masses along with SLA. Seedling height was most strongly genetically correlated with needle mass, and negatively correlated with SLA although the latter was not significantly different from zero (Table 2-10). Next, I correlated total seedling mass with other organ masses and SLA. Total mass was most strongly genetically correlated with root mass, SLA, and to a lesser degree stem mass. Genetic correlations between needle mass and total mass were not significantly different from zero. Phenotypic correlations were all highly significant and strongly correlated (Tables 2-10 and 2-11).

I also correlated organ masses (roots, stems, and needles) to each other and to SLA. Genetic correlations between root and stem mass were significant (Table 2-11). SLA was significantly and positively correlated with root, stem and needle masses (Table 2-11). The phenotypic correlations were also highly significant and positive among roots, stems, and needles. Ironically, SLA was negatively correlated (Pearson r^2) with root, stem and needle masses.

Discussion

Breeding programs that favor tree volume have successfully increased woody biomass in artificially planted stands of white spruce, but the mechanisms that underpin the extra growth remain unclear. Within and among white spruce sources in my study, I found that ontogenetic trajectories of biomass allocation among root, stem, and needle masses were largely conserved. Each seed source was represented by fast- and slow-growing trees that fell along the same allometric line. White spruce fosters high genetic variation within its genomes so that resemblance among half-sibs is generally small (Furnier et al., 1991; Peng Li et al., 1997), a characteristic of most wind-pollinated conifers (Hamrick, Godt, & Sherman-Broyles, 1992). Differences in biomass among sources at any point in time were more closely related to ontogeny than to fundamental alteration of biomass allocation.

Differences among seed sizes, and early seedling growth

The six seed sources differentiated strongly with respect to seed size, but the effects were short-lived in seedlings. After one growing season in the new pots, seedling sizes did not emulate their seed sizes possibly as an effect of transplanting, or because of small effects of seed size on subsequent seedling size. However, biomass in 'year 0' seedlings, those removed from the styroblocks in which they germinated, also did not mirror patterns of seed size, as exemplified by S-ON-419. This family represented the fastest-growing genotype with larger heights and overall biomass than other sources, but possessed the smallest seed. Seed from S-ON-400 were larger than all other sources but produced only intermediate-sized seedlings in 'year 0.' This decline of seed-related effects occurred earlier than expected since seed mass effects can be detected for five or

more years in other species (Castro, 1999; Reich et al., 1994). Clearly, tree biomass was affected by factors other than seed size that may be related to genetics, or other factors such as phenology or resource-use efficiency.

The timing of phenological events, such as bud-break or bud-set, may affect growth patterns of white spruce to a greater degree than biomass alone. In Canada, northern sources of white spruce leaf out earlier and set buds later than southern sources (S. Carles et al., 2011; Mark R. Lesser & Parker, 2004). I did not include phenological traits in this study, but such traits are likely important: superior growth in selected genotypes of loblolly pine was associated with an extended late-season growth period (McKeand, Jett, & Jayawickrama, 1998). Early bud-burst was also associated with fast-growth in white spruce (Wilkinson, 1977), but families of white spruce selected for increased growth were also associated with delayed bud-set (Nienstaedt & King, 1970). Phenological traits are complicated by the influence of epigenetic proteins that attach to the DNA and alter translation (Besnard et al., 2008; Johnsen, Dæhlen, et al., 2005; Johnsen, Fossdal, et al., 2005). I attempted to minimize epigenetic effects by collecting seed from selected sources from one grafted orchard in a single year (2006), but these epigenetic effects can linger into subsequent generations (Galloway & Etterson, 2007). In spite of efforts to reduce confounding epigenetic effects, a trans-generational signal may linger impacting patterns of seasonal growth, as reflected in phenology. Further studies of the epigenome would be needed to determine the extent to which phenological traits are affected by a lingering epigenetic signal.

My results suggest that fast- and slow-growing genotypes that are identified through artificial selection may be distinguished, in part, by their needle morphology. In

natural populations across plant taxa, specific leaf area (SLA), leaf area and leaf mass ratios correlate strongly with relative growth (Lambers, Chapin, & Pons, 2008; Poorter & Remkes, 1990; Poorter et al., 2012; Reich & Oleksyn, 2004; Reich et al., 2003). My results underscore that shifts in needle traits are also possible through artificial selection imposed intraspecifically for white spruce. I also observed significant genetic correlations between stem mass and SLA that reinforce the possibility that extra growth in selected genotypes may result from increased carbon sequestration by improvements in light interception and a reduction of respiratory carbon losses (Reich et al., 2003). Selected genotypes that improve carbon sequestration through shifts in leaf morphology, instead of reductions in root mass, pose less of an ecological cost in terms of fitness for a tree breeding program. Tree breeders can incorporate leaf traits in selection criteria, potentially on young trees, provided that juvenile-adult correlations are demonstratively positive. The benefits to a breeding program may be realized by advancing the time and efficiency of selection efforts to construct seed orchards.

Comparisons between North and Central wild sources

Growth traits in forest trees, and the genetics that underpin them, vary predictably along clinal gradients. In my study, seeds from central provenances (W-MN-C) were larger than those from northern provenances (W-MN-N) supporting other published (Oleksyn, Modrzynski, et al., 1998) and unpublished reports of declining seed size with increased latitude (C. Vansickle, personal communication). In addition to possessing smaller seeds, seedlings from northern wild sources were shorter, and had smaller stem and root mass ratios than southern wild sources, a trend reported for Scots pine, Norway spruce, and white spruce (S. Carles et al., 2011; Oleksyn, Modrzynski, et al., 1998;

Oleksyn, Reich, Chalupka, & Tjoelker, 1999). Conversely, as MAT (mean annual temperature) for the provenance increases, the proportion of biomass allocated to roots decreases (S. Carles et al., 2011; Oleksyn, Tjoelker, & Reich, 1998). My results suggest the presence of a steep latitudinal cline for white spruce in Minnesota, across a relatively small geographic distance (1.5° latitude), although this finding is hindered by lack of replication. My results reinforce the management practice of conserving seed from southern range-edge populations for reforestation in Minnesota (Mark R. Lesser & Parker, 2004; Radsliff et al., 1983).

Comparisons between selected and wild sources

Selected sources were taller, with smaller root masses than wild, unselected sources, but generally tracked the same allometric line. My study supports a previous study in white spruce that found a low genetic basis for variation in biomass allocations (Rweyongeza et al., 2005). The root to stem ratio has a modest genetic underpinning in Scots pine, as revealed in range-wide provenance trials (Oleksyn, Tjoelker, et al., 1998), but is strongly influenced by environmental conditions in white spruce (Rweyongeza et al., 2005) and other plant species (McConnaughay & Coleman, 1999). Taller genotypes, such as S-ON-419, tended to have relatively smaller root masses compared with smaller genotypes such as S-ON-400. This finding contradicts another study in white spruce that failed to find differences in root masses even when heights were different among sources (S. Carles et al., 2011). My results indicate that the root to stem ratio is stable in white spruce, regardless of whether genotypes originated from wild stands or were selected for fast-growth.

Differences among selected genotypes

The four selected sources represent the broad range of phenotypes that can result after one-generation of selection. The differences between the two Ontario sources exceeded even the two wild sources, which was unexpected since the selected sources were collected from the same orchard and were both ranked among the type genotypes. In white spruce, seed originating from a northern seed orchard stops growth six days before a southern orchard, with an approximate difference of 2°Latitude (S. Carles et al., 2011). The larger of the two Ontario genotypes, (S-ON-419) had a high relative stem mass, height, and diameter and was similar to the central wild source, W-MN-C (Radsliff et al., 1983). In contrast, S-ON-400 had proportionately higher root and needle masses, bearing more similarity to the northern wild source (W-MN-N). It is possible that S-ON-400 exhibit slow early growth but older progeny test datasets fail to indicate this: seed source 400 was ranked 1 and 10 for tree volume, whereas family 419 was ranked 50 and 21, after 10 and 15 years respectively (unpublished data). This suggests that the slow growth of 400 may be due to a number of factors ranging from shortcomings in this specific seed-lot, seedling culture, poor handling of the styroblocks, or differences in site and soil conditions.

Management recommendations

To maximize growth potential in commercial reforestation efforts, central or southern sources of white spruce should be favored for planting programs instead of northern genotypes in Minnesota. However, in a changing climate, co-variation between traits that affect heat tolerance or drought stress may affect fast-growing sources in unexpected ways (Bigras, 2000, 2005) and require further study. Efforts to maintain growth rates at their current levels should focus on the use of selected sources from

orchards placed along the southern range edge of Minnesota to maximize epigenetic signals that may extend the growing season. Thus, care should be taken to avoid planting white spruce seed sources that originate from more than 2° latitude south in Minnesota, a distance of approximately 140 miles (222 kilometers). Seed zones developed by Rudolph (1956) for the lake states and in use by the MN DNR appear to maintain conservative latitudinal transfer distances.

Tables

Table 2-1. Traits used to assess biomass allocation among six different seed sources of white spruce. Traits followed with L were log-transformed prior to the analysis.

Trait	Description	Units
Tree height ^{L}	Measured from soil to tallest dominant branch, log –transformed	Centimeters
Tree diameter at root collar (diam)	Average of two measurements taken at 90 degree angles	Millimeters
Root mass ^{L}	Mass of roots after cleaning and drying, log-transformed	Grams
Stem mass ^{L}	Dried mass of stems – woody material only, log transformed	Grams
Needle mass ^{L}	Dried mass of needles, log-transformed.	Grams
Total mass	Dried mass of needles + roots + stems	Grams

Table 2-2. Pre-planned contrasts used to test differences in means and regression slopes of seedling traits. Seed collected from four different genotypes at a grafted orchard are prefaced with "S-." S-MN-127 and S-MN-132 are Minnesota, USA sources. S-ON-400 and S-ON-419 are Ottawa Valley, Ontario, Canada sources. Bulked wild sources, W-MN-C and W-MN-N refer to wild collections from Central and North East seed zones from Minnesota, respectively.

	Seed source					
Comparison	**S-MN-127**	**S-MN-132**	**S-ON-400**	**S-ON-419**	**W-MN-C**	**W-MN-N**
Selected (S-) vs North wild (W-MN-N)	1	1	1	1	0	-4
Selected (S-) vs Central wild (W-MN-C)	1	1	1	1	-4	0
W-MN-C vs W-MN-N	0	0	0	0	1	-1
S-MN vs S-ON	1	1	-1	-1	0	0
S-ON-400 v S-ON-419	0	0	1	-1	0	0

Table 2-3. Least-squared means for each seed source at year 0. All columns except diameter and SLA were back-transformed from logarithms. Letters indicate significant pairwise differences using Tukey's test adjusted for multiplicity. Largest values in each column are boldfaced.

Year 0	Height (cm)		Stem mass (g) ^{L}		Needle mass (g) ^{L}		SLA ($cm^2 \cdot g^{-1}$)		Longest root (mm)		Root mass (g) ^{L}		All mass (g) ^{L}	
S-MN-127	19.1	b	1.79	bc	2.08	b	**5.79**	**a**	13.3	a	1.74	a	5.61	a
S-MN-132	21.5	a	1.88	abc	2.24	ab	4.88	bc	13.0	a	1.69	a	5.81	a
S-ON-400	**22.7**	**a**	1.89	ab	2.24	ab	5.03	bc	12.9	a	1.65	a	5.78	a
S-ON-419	20.6	ab	**1.91**	**a**	**2.30**	**a**	4.50	c	13.3	a	1.73	a	5.94	a
W-MN-C	18.6	b	1.77	c	2.07	b	5.44	ab	12.5	a	1.64	a	5.57	a
W-MN-N	18.8	b	1.79	bc	2.14	ab	4.80	bc	12.2	a	1.67	a	5.60	a

Table 2-4. Significant contrasts between selected and wild sources. All traits were log-transformed prior to analysis; the back-transformed least-squared means are shown. Only contrasts with at least one significant comparison are shown. Boldfaced values represent the largest value for each contrast. Significance is indicated by *, **, and *** for $p<0.10$, $p<0.05$, and $p<0.01$, respectively.

Trait	Selected LSMean	p-value	Wild LSMean
Year 0			
Tree height ^{L}	**20.94**	***	18.68
All biomass ^{L}	**5.79**	*	5.58
Longest root ^{L}	**13.11**	**	12.32
Root mass ^{L}	**1.70**	**	1.66
Stem mass ^{L}	**1.87**	***	1.78
Needle mass ^{L}	**2.21**	***	2.11
Year 1			
All biomass ^{L}	**12.58**	**	10.67
Leaf area ^{L}	**14.92**	**	12.65
Root mass ^{L}	**4.86**	**	4.09
Stem mass ^{L}	**4.22**	**	3.62
Needle mass ^{L}	**3.62**	**	3.62
Year 2 - No differences			
Year 3			
Leaf area ^{L}	**189.8**	*	163.5
Longest root	**108.1**	**	97.3

Table 2-5. Planned contrasts between selected sources from Ottawa Valley Ontario, S-On-400 and S-On-419. Contrasts significant at $p<0.01$, $p<0.05$, and $p<0.10$ are indicated with ***, ** and *, respectively.

Trait	S-ON-400 vs S-ON-419		
	Year 0		
	On-400	On-419	p-value
Tree height [L]	**22.8**	20.7	***
Diameter	2.01	**2.23**	**
Root mass [L]	1.65	**1.74**	***
SLA	**5.03**	4.50	**
Year 1- No differences			
Year 2			
Longest root	89.0	**110.1**	**
SLA	3.57	**3.91**	**
Year 3			
Tree height [L]	73.4	**85.3**	*
Diameter	19.7	**22.4**	*
Stem mass [L]	56.0	**76.9**	*

Table 2-6. MANOVA p-values for overall seed source effects and pre-planned contrasts by year. Log tree height, root mass, stem mass, needle mass and SLA were used. The significance of the MANOVA is indicated at $p<0.01$, $p<0.05$, and $p<0.10$ with ***, ** and *, respectively.

	Year			
Effects	0	1	2	3
Overall seed source effect	***	**	**	*
Selected sources vs wild sources	***	**	NS	NS
W-HC vs W-LF	**	***	NS	NS
S-MN v S-ON	***	NS	NS	NS
S-ON-400 vs S-ON-419	***	NS	***	***

Table 2-7. Class means on canonical axis 1. Values are sorted from smallest (most negative) to largest to highlight that the largest discrepancy in sources occurred between S-ON-400 and S-ON-419.

Source	Can1
S-ON-400	-1.022
W-MN-N	-0.681
S-MN-127	-0.012
S-MN-132	0.033
W-MN-C	0.686
S-ON-419	1.106

Table 2-8. Pooled within-class standardized canonical coefficients. Values are sorted in order of largest absolute value (Stem mass) to smallest (Diameter).

Variable	Can1
Stem mass	2.368
Root mass	-1.31
Needle mass	-0.964
SLA	0.818
Tree height	0.359
Diameter	0.272

Table 2-9. For year 3 data, significant differences for the null hypothesis that slopes are equal between sources. Wild sources from a northern seed zone in Minnesota (W-MN-N) are compared with wild sources from central seed zone (W-MN-C) in the first column. Wild sources (W-) are pooled and compared with the average of selected sources (S-) (middle column). Comparisons between two sources from Ontario (S-ON-400 vs S-ON-419) are shown in the last column. Significance of contrasts is indicated by * for p-values <0.10, and ** for <0.05.

Trait	Trait pair	W-MN-N vs W-MN-C	W- vs S-	S-ON-400 vs S-ON-419
RMR	Root mass v All mass	**	NS	NS
SMR	Stem mass v All mass	NS	NS	**
LMR	Needle mass v All mass	NS	NS	NS
SSL	Height vs Stem mass	NS	NS	NS
LAR	Leaf area v All mass	NS	NS	NS
SRL	Root length v Root Mass	NS	**	NS
RS	Root Mass v Stem Mass	NS	NS	*

Table 2-10. Genetic and phenotypic correlations between tree height (log-transformed) and each major plant organ, and all biomass vs each organ. Correlations that are significantly different from zero are boldfaced. Standard errors are presented for genetic correlations, and p-values are shown for Pearson's correlations.

	Genetic				Phenotypic			
	Tree height^{L}		All mass^{L}		Tree height^{L}		All mass^{L}	
	r	SE	r	SE	r	P-val	r	P-val
Root mass^{L}	0.32	0.52	**0.68**	**0.33**	**0.95**	<.001	**0.99**	<.001
Stem mass^{L}	0.21	0.21	**0.39**	**0.17**	**0.95**	<.001	**0.99**	<.001
Needle mass^{L}	**1.04**	**0.23**	0.12	0.23	**0.92**	<.001	**0.99**	<.001
SLA	-0.11	0.66	**0.41**	**0.09**	**-0.82**	<.001	**-0.78**	<.001

Table 2-11. Genetic correlations (above diagonal) and phenotypic (Pearson's) correlations, below diagonal. Data for all four years was combined. All but SLA were log-transformed (L subscript) prior to analysis. P-values are provided for phenotypic correlations. Standard errors are shown for genetic correlations.

	Root mass^{L}		Stem mass^{L}		Needle mass^{L}		SLA	
	r	pval/SE	r	pval/SE	r	pval/SE	r	pval/SE
Root mass^{L}	---	---	**0.32**	0.14	0.12	0.39	**0.38**	0.07
Stem mass^{L}	**0.99**	<.001	---	---	0.03	0.07	**0.51**	0.07
Needle mass^{L}	**0.97**	<.001	**0.98**	<.001	---	---	**0.67**	0.09
SLA	**-0.79**	<.001	**-0.79**	<.001	**-0.76**	<.001	---	---

Figures

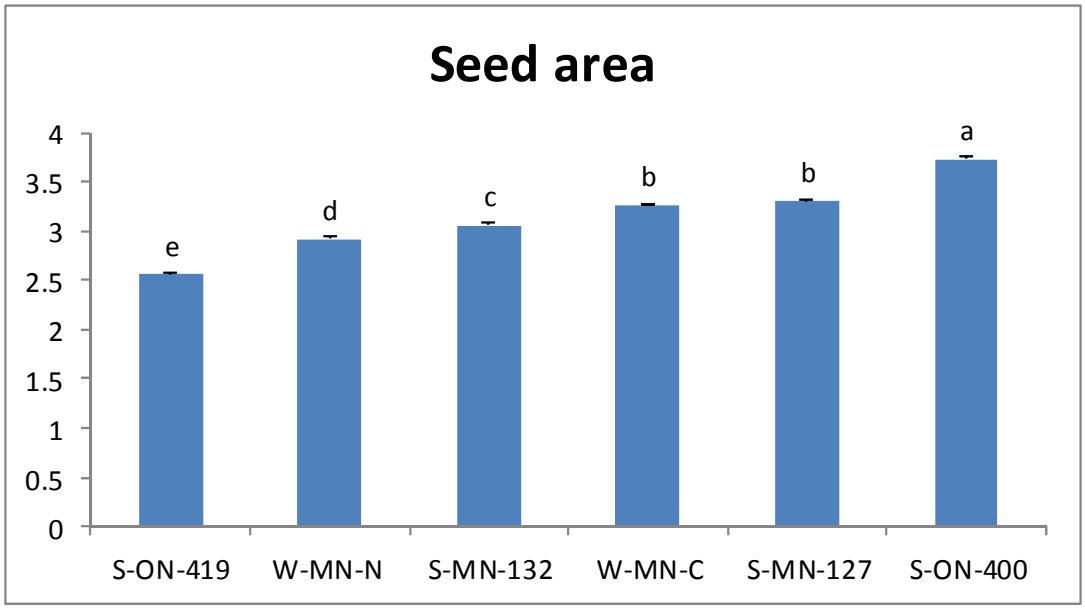


Figure 2-1. Average seed area (mm^2 + SE) for each source. Selected sources are prefixed with "S-" and wild sources with "W-." Differences at $p<0.05$ using Tukey's HSD test are indicated by different letters above the bars.

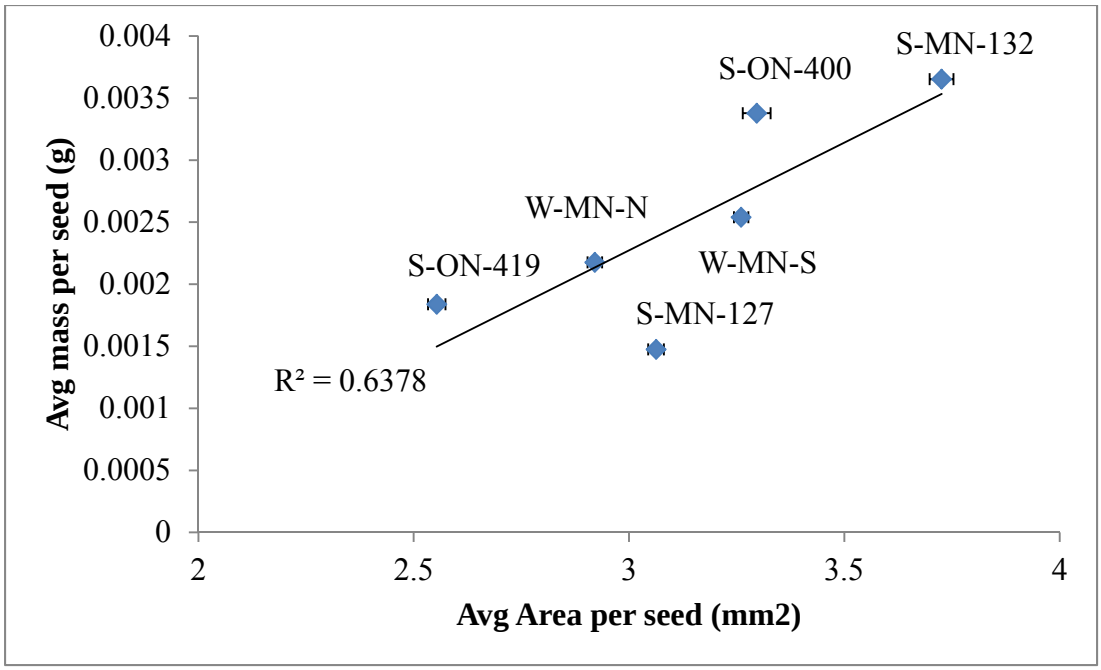


Figure 2-2. Seed mass vs seed area for sources. No variances were available for seed mass, but standard errors are shown for average seed area (x-axis).

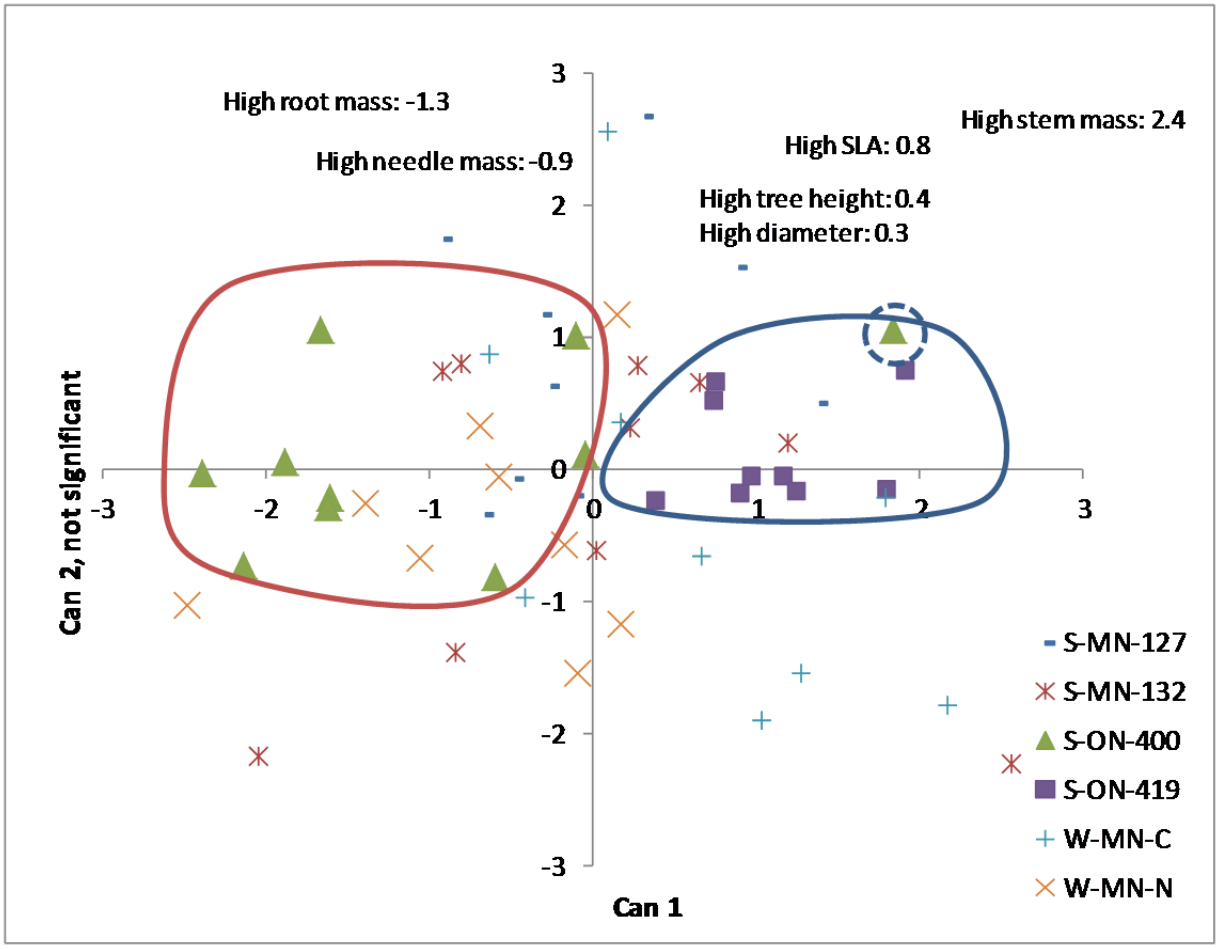


Figure 2-3. Canonical values for each tree sampled in year 3. The first canonical axis (Can1) was significant at $p<0.05$. Only the Ontario sources (400 and 419) were significantly different in multivariate space. One point for 400, that was not in sync with the others in the source, is circled with a dotted line. Standardized canonical coefficients for CAN1 are listed above the x axis.

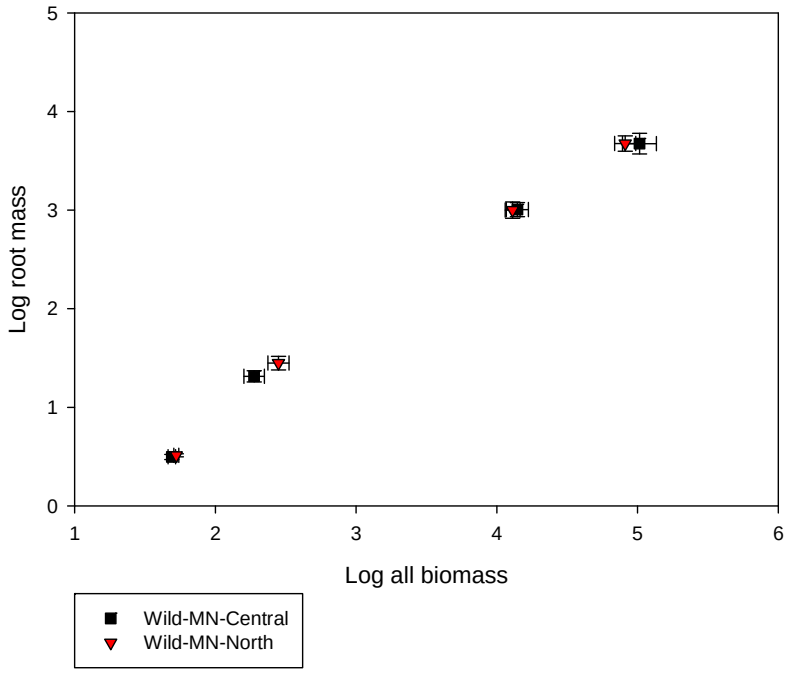


Figure 2-4. Root mass ratio for wild sources from Central Minnesota vs Northern Minnesota for years 0-3. Means ± standard errors for each year are shown.

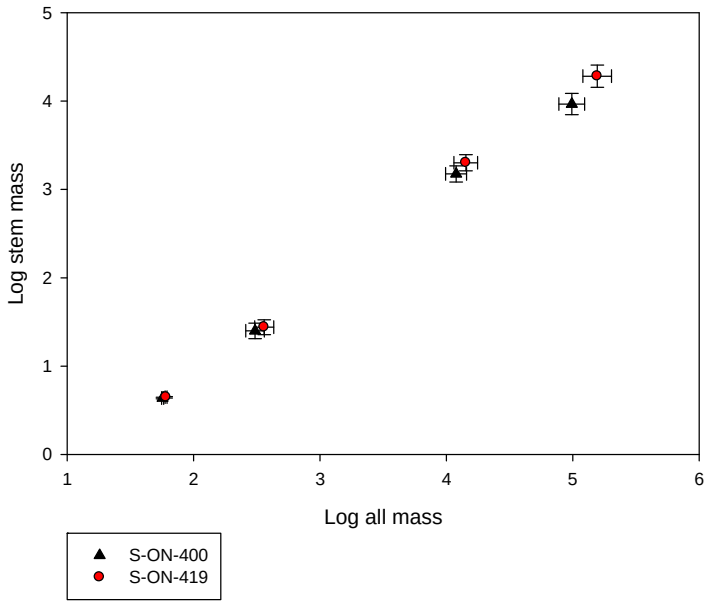


Figure 2-5. Stem mass ratio between selected Ontario sources 400 and 419. Means ± standard errors are shown for each year from 0-3.

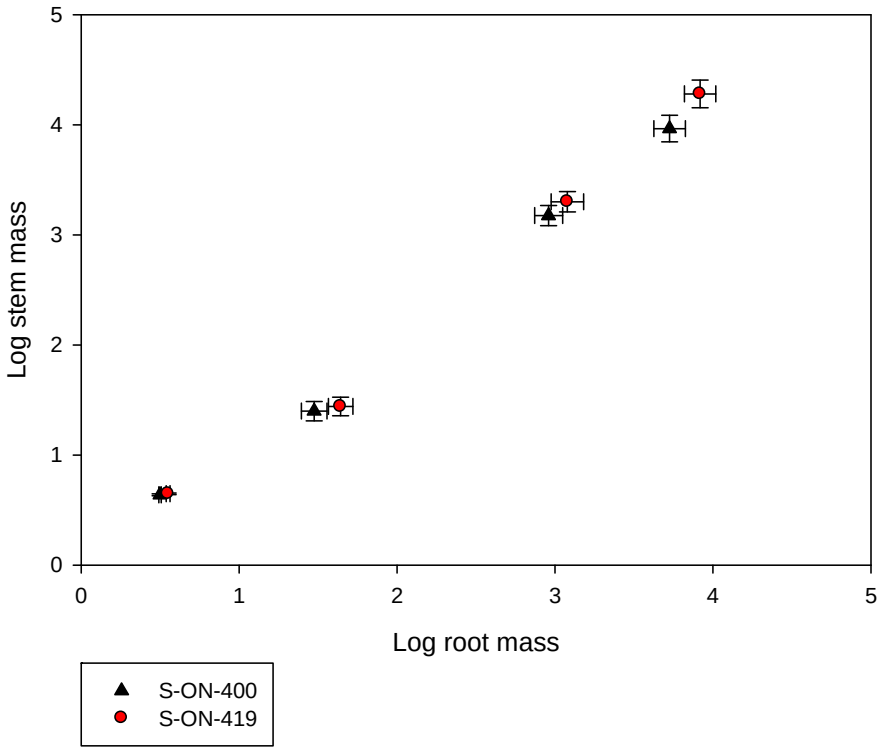


Figure 2-6. Root to stem mass ratio between selected Ontario sources 400 and 419. Means ± standard errors for each year 0-3 are shown.

Chapter 3 Effects of artificial warming during quiescence on bud-break and growth of white spruce, *Picea glauca* (Moench) Voss

Introduction

White spruce (*Picea glauca* Moench) is valued by the forest industry in the lake states for its wood-quality for paper-making and is commercially-planted across the state of Minnesota. Tree improvement programs have increased growth rates substantially, with volume increases of 30% for selected sources compared with wild, local, sources (Weng et al., 2010). However, white spruce's habit of early bud-burst makes it vulnerable to spring frost, leading to concerns that selection for increased wood production may aggravate this habit (Nienstaedt & King, 1970).

Phenological traits are indicators of synchrony between a plant and its environment, and include timing of bud-break, bud-set and growth rhythms. These traits are usually under strong genetic control, with their expression varying widely among provenances in common garden studies set in novel temperature, precipitation and/or photoperiodic conditions (Cannell & Willett, 1975; Hannerz et al., 1999; P. Li, Beaulieu, Corriveau, & Bousquet, 1993; Oleksyn et al., 2001; Pollard & Ying, 1979). Phenological traits are also linked to the tree's maternal environment through a combination of genetic and epigenetic mechanisms (Besnard et al., 2008; Kvaalen & Johnsen, 2008; Saxe, Cannell, Johnsen, Ryan, & Vourlitis, 2001). Studies of their expression may assist scientists in understanding a species' vulnerability to a rapidly changing climate.

Climate change is predicted to strongly influence boreal species like white spruce by forcing northward range shifts (Colombo, 1998; M.R. Lesser & Parker, 2006). Local

extirpation of white spruce is possible in Minnesota, where it resides along its southern range edge. The persistence of white spruce will be a function of the severity and timing of enhanced warming, and the presence of adaptive variation within stands located on range-edges (Savolainen et al., 2007).

Climatic changes in Minnesota are expected to be pronounced in winter months when trees are in a state of dormancy. The effects of changing winter temperatures on tree growth will depend on the severity and timing of warming relative to the state of dormancy. Three stages of dormancy are recognized for plants growing in seasonally cold environments: paradormancy, ectodormancy, and ecodormancy (Lang, Early, Martin, & Darnell, 1987). These stages are also known as first period of rest, second period of rest and quiescence, respectively (Hänninen & Pelkonen, 1989). Each stage is characterized by a complex of physiologic and cellular activity governed by the combined effects of day-length and temperature. The first-period of rest, or paradormancy, takes place from August through September after height growth ceases in white spruce (Nienstaedt, 1966; H. Smith & Kefford, 1964). Paradormancy is followed by ectodormancy, the deepest state of dormancy. In white spruce, completion of ectodormancy requires approximately six weeks of daily average temperatures below 0°C (Nienstaedt, 1966). Chilling is a key signal for the initiation and cessation of ectodormancy; in the absence of sufficient chilling, the cascade of events that lead to bud-break is broken, delaying bud-burst (Bailey & Harrington, 2006; Campbell & Sugano, 1979; Granhus, Fløistad, & Søgaard, 2009; Heide, 2003; Kriebel & Wang, 1962; Søgaard, Granhus, & Johnsen, 2009). Climatic warming events during ectodormancy can produce measurable effects on tree growth if the warming precludes the completion of

adequate chilling (Kriebel & Wang, 1962). The period after ectodormancy that precedes bud-break is known as ecodormancy, or more commonly "quiescence."

Quiescence begins sometime in January for white spruce in Minnesota, and spans the vernal equinox until bud-burst in April or May (Nienstaedt, 1966). Quiescence is understood as a period when symplastic pathways, that were closed during ectodormancy, are re-opened following sufficient chilling (P. L. Rinne, Kaikuranta, & van der Schoot, 2001; van der Schoot & Rinne, 2011). The environmental triggers that induce quiescence are not fully understood, but may be driven by the up-regulation of genes for gibberellin synthesis, which re-open pathways to the shoot apex. This in turn facilitates the expression of genes that are required for bud-burst to occur (P. Rinne et al., 2011). The transition between ectodormancy and quiescence is difficult to distinguish visually, requiring destructive sampling to assess levels of cellular activity.

The effects of warming during quiescence have been studied in several species with mixed results. In Norway spruce, the effects of quiescent-stage warming did not significantly alter the time to bud-burst (Hänninen & Pelkonen, 1989). In another study, Norway spruce was modestly resistant to premature de-hardening following mid-winter warming treatments (Westin, Sundblad, Strand, & Hällgren, 2000). In contrast, red spruce decline in New England was attributed to a rogue warm spell during early quiescence; balsam fir tolerated the warm spell without apparent physical consequences (Strimbeck, Schaberg, DeHayes, Shane, & Hawley, 1995). White spruce exhibits clinal variation in its tolerance to mid-winter low-temperatures, but is considerably more cold-tolerant than red spruce (Strimbeck, Kjellsen, Schaberg, & Murakami, 2007). However, white spruce is prone to early bud-break that may be exacerbated if climatic warming

during quiescence is accelerated (Hänninen, 2006). Selection for fast tree growth in tree improvement programs may further hasten early bud-break because of negative correlations between growth and time of bud-break (Nienstaedt & King, 1970; Nienstaedt, 1972) prompting researchers to question the potential risk of planting fast-growing genotypes in areas that commonly have late spring frosts.

The primary objective of this study is to observe the response of white spruce to episodic warming applied during the quiescent state. Phenological traits, such as bud development and season-end tree heights, are considered as response variables to test the hypothesis that bud-break time and growth are influenced by warming experienced during the quiescent stage in white spruce.

Methods

Study site, climate and warming treatment description

The experiment was located at the Cloquet Forestry Center located in Cloquet, Minnesota at 46°31' N Latitude and 92°30' W Longitude at an elevation of 385 meters above sea level. Climate data from an on-site NOAA weather station was used to determine historical maximum temperatures for February, March and April in the last 100 years. The approximate duration, and corresponding low temperatures of these warm spells was also ascertained from the weather data. Maximum temperatures served as a benchmark for this warming experiment: 13°C (1976) and 26°C (1946) for February and March respectively. Average low temperatures during these periods were -4°C to -1°C (February 1976) and 2°C to 4°C (March 1946). In 2010 and 2011, I applied warming treatments on white spruce seedlings for four days each in February and March. The objective of the experimental warming treatment was to approximately match or exceed

these historical maximum and minimum temperatures, and exceed the duration of warming by one day to test whether these enhanced warming periods affect bud-break time, tree height and growth during the subsequent growing season. I used a variety of known seed sources to represent the natural variation that occurs within the species.

Seed source and propagation

Open-pollinated (OP) seed was obtained from two main sources: bulked seed from wild collections and seed orchards established with genotypes selected for above-average height growth (A-Table 2). Orchard seed was collected from three different genotypes at one white spruce orchard owned by UPM Blandin in Grand Rapids, MN at Lat: 47°15' and Long: -93°29' MAT= 39.0°F (3.9°C), MAP= 24.7" (627.4 mm). Two additional genotypes was collected from the St Louis County Land Department (Minnesota) seed orchard located at 47°13' Latitude and -92°31' Long, MAT= 37.9°F (3.3°C), MAP= 27.7" (703.6 mm). Two wild sources were obtained from each of two MN DNR seed zones ("Baudette" represents the "North West" seed zone; "Hibbing" represents the "North Central" seed zone) from the MN DNR State Nursery in Badoura, MN (A-Figure 1). All seed was shipped to PRT greenhouse in Dryden, Ontario in March 2008. Seed was germinated in six-cubic inch styroblocks in custom screen peat moss and grown to a target height of 20 centimeters and a target diameter of 2.7 mm. In October 2008, seedlings were overwintered in their styroblocks in a 3°C cooler until planting in spring 2009 into nursery beds.

In spring 2009, 1008 seedlings were planted into individual pots, each pot with a total volume of 6.23 liters, 15 cm wide and 41 cm tall (TPOT2, Stuewe and Sons, Corvallis OR). The potting medium consisted of 50% peat moss, 15% perlite and 35%

composted bark (Berger soil mix #BM7, JR Johnson Supply, Roseville MN). This potting soil mix was chosen to best match growing requirements of white spruce trees. The pots were positioned into five nursery beds, constructed with 1.2 by 4.9 meter pressure-treated boards, lowered 30 cm into the ground, and overlain with cattle panels to support pots. Each bed contained 24 columns and seven rows, or 168 pots. Only the top 10 cm of the pots were above ground level. The beds were placed in an open area approximately 20 meters from forest edge, along the north side of a building. A wooden support rod, 5-meters in length, was suspended 1.4 meters above the tops of the pots. Trees were watered periodically as needed with a sprinkler. Treatments were applied after trees had experienced a full growing season outdoors in the pots. This was critical, since climatic conditions that occur the previous summer and fall impact the number of terminal buds placed (Chuine, Rehfeldt, & Aitken, 2006; Fraser, 1962).

Experimental design

One tree from each of the seven seed sources was randomly placed in each row of each nursery bed so that no seed source was represented disproportionately along the bed edges. Each of the five nursery beds was divided into three plots so that each plot (15 plots total) contained seven columns and eight rows (56 trees). Each treatment was replicated twice, once each in two plots. A total of seven treatments were randomly assigned, so that 14 plots were used for this study. Three trees from each seed source within each plot were designated for sub-sampling (21 trees per plot, 42 per treatment) and distinguished with colored cable-ties for repeated phenology measurements during the growing season. Edge trees were excluded from data collection.

Snow depth at the start of the February warming treatments was similar across years, 40 and 43 centimeters in 2010 and 2011, respectively. Ambient conditions for the duration of this study were similar to the 100 year averages. Approximately 30 cm of snow was carefully removed from treatment plots by hand, leaving a snow-depth of approximately 10 centimeters on each plot selected for treatment. At this depth, roughly 1/2 of each seedling remained under snow-cover in 2010 and roughly 1/3 of each seedling remained snow-covered in 2011. Snow depths in March were lower (than February) but snow removal for treatments was conducted similarly to the treatments in February.

Artificial warming was applied with one Kaglo® electric infrared lamp, hung from a support rod approximately 95 cm above the top each treated plot (Model MRM-2415, 240 Volts, 1500 watts, 6.5 amps, Kaglo Electronics Co., Inc. Bethlehem PA). A plastic tent, formed from greenhouse plastic (opaque to PAR) was placed over the entire plots and lamps to enhance warming and maintain uniform temperatures on the plots. Each lamp had a dial-up control to manually adjust the infrared output on the trees. Lamps were set to the lowest setting at night, and were adjusted during the day to ensure that the maximum temperature was achieved for 2-3 hours in the middle of each day.

Warming treatments were applied to two plots once each in February and again in March (Table 3-1). Snow was removed from treated plots as described previously, and two designated control plots measured effects of exposure due to snow removal alone. Two additional plots were warmed twice, once each in February and March (hereafter called “Feb+March warm” treatment). The experiment was repeated in 2011 without reassigning treatments to plots. After each warming treatment, all hardware was removed

(lamps and plastic tarps), and trees were left with no snow cover. No significant snow fell in February or March of either year, so plots that experienced snow removal in February and March remained exposed for the duration of the winter season. Following 2011 treatments, dead terminals were noted on 56 trees (15% of all sub-sampled trees) in the warmed plots, likely due to insufficient distance between lamps and tree tops. Trees with dead terminals were removed from further analysis.

Temperature sensors

Alcohol thermometers were placed in the center of the plots to monitor daytime temperatures. Thermometer readings were validated with periodic readings taken by an infrared gun aimed at foliage (Fluke® model 572), during one warming treatment in 2010 (Appendix-Figure 2). Data from the thermometers was recorded and a regression for the fit was calculated in control plot, with r^2=0.86 (A-Figure 2).

Temperature data recorders, placed in the center of each plot just above the snow, recorded temperature every 15 minutes (Hobo® Pendant Temperature loggers, Onset Computer Corporation) beginning in February 2010. Hoboes were left in place for the duration of the study. In the final analysis six treatments were compared: three warming treatments (Feb warm, March warm, Feb+March warm) and four controls (Feb control, March control, Feb+March control, unmanipulated control). In 2011, one thermocouple was placed approximately 10 cm beneath the soil surface into one pot located in the center of each plot to record soil temperature. Thermocouples were made by twisting type T 24 AWG thermocouple cable attached to a data logger (Campbell Scientific CR1000) with a multiplexer AMT 25 to multiply channels.

I used max and min daily temperatures from the Hoboes to calculate growing degree-days (GDD) using 1°C as a baseline temperature in each treatment (Man & Lu, 2010), since February 1 of each year. GDD are accumulated when the mean daily temperature (daily max temperature - daily min temperature) / 2; MDT) exceeded the base temperature of 1°C. If the MDT was less than the base temp, then GDD = 0.

I also calculated the number of chill days by counting the number of days from February 1 where the average daily temperature was lower than 1°C. Each day with MDT less than 1°C counted as one chill day, and the summation of chill days was tabulated. GDD and chill days are reported from February 1 to April 1 to cover the period just before, during and after treatments were applied.

Assessment of bud development and year-end tree size

In spring 2010 and 2011, bud-swelling and bud-burst was assessed on a sub-sample of individuals, 21 per plot (42 per treatment). Upon the first signs of swelling, terminal buds on sub-sampled trees in all plots were assessed every three to four days until fully extended. To assess bud development, terminal buds were visually inspected and assigned a developmental stage using a six-point scale based on Nienstaedt and King (1970). Bud-swelling was categorized as a "6" if in winter-condition, or a "5" with first observed swelling. Buds that were translucent, with evidence of green needles, were classified as "4." A broken bud-cap was classified as "3" the benchmark for bud-burst. The day to bud-burst since January 1 was determined from the first day that bud-burst was observed for each tree. Bud-categories 2 and 1 (Nienstaedt & King, 1970) were ignored since they occurred post bud- burst and were not analyzed further. Terminal buds later determined to be dead were removed from further analysis.

I measured terminal lengths (length of the terminal shoot from the base of the current year growth to the tip) approximately weekly when most trees had reached bud-break stage 2. Measurements were taken with a metal caliper for the first three weeks to the nearest 0.01 millimeter and to the nearest millimeter with a ruler for the last nine weeks. In October 2009 and again in October 2010 and 2011, I measured tree heights on all trees to the nearest 0.5 cm, and diameter of the lower stem, at the level of the pot, to the nearest 0.01 mm.

Statistical analyses

I used analysis of variance on the following traits: days to bud-burst and year-end tree height for 2010 and 2011 datasets. For bud-burst, I noted the first date when the bud-sheath was broken (stage 3), and converted the day to the number of days since January 1. I used a mixed-models procedure in SAS/Stat to compare treatments:

$$Y_{ijkl} = Trt_i + Plot_j + Seed\ source_k + Trt*Seed\ source*Plot_{ijk} + e_{ijk} \quad \text{(Equation 1.)}$$

Treatment (Trt) was set as a fixed effect; plot, seed source and the 3-way interaction were set as random effects with residuals assumed to be N~(0,1). A Kenward Roger adjustment was applied to correct the covariance structure of upward biases (Kackar & Harville, 1974). I compared treatments with pre-planned contrasts or Tukey's HSD test adjusted for multiplicity.

Terminal shoot lengths were measured on three trees of each seed source in each plot weekly (two plots per treatment). I calculated least-squared means of 42 trees for each treatment (three trees per seed source, seven seed sources per plot, two plots per treatment). I used repeated measures ANOVA (Proc mixed), with Kenward-Rogers adjustment, to compare differences among treatments with the shoot length at the first date as a covariate, treatment as a fixed variable, family as a random variable, and date as

the repeated measure. The R matrix was best fit with the autoregressive (ar(1)) covariance structure. I also used Tukey's test to compare the treatments at three different dates to observe where the divergence among treatments started occurring.

I fit shoot lengths from each growing season (from all sub-sampled trees) to a 4-parameter Richards function (Venus & Causton, 1979) with the equation:

$$\text{Terminal length (mm)} = a*(1-exp^{b-cT})^{1-d} \quad \text{(Equation 2.)}$$

Where *T*=day, a=upper asymptote of the curve and *b, c* and *d* are shape parameters. Overall R^2 for all trees together equaled 0.99. Average relative growth rate (AvgRGR) across the entire period was calculated with $c/(d+1)$ and weighted mean relative growth rate (wmRGR) was calculated as $a*c/(d+1)$(Hunt, 1982). I compared the parameters *a, b, c,* d, AvgRGR, and wmRGR with a mixed models ANOVA (SAS/Stat, proc mixed) with plot set as a random variable:

$$Y_{ijkl} = Trt_i + Plot_j + Trt*Plot_{ijk} + e_{ijkl} \quad \text{(Equation 3)}$$

All data was checked for normality and variance stability. Bud-burst was approximately normally distributed and skewed, but not improved with data transformations. Tree heights and diameters were approximately normally distributed with low heteroscaedasticity. For both years avgRGR and parameter '*a'* were approximately normal with stable variances. Parameters *b-d* were skewed to the right in 2010 and bimodal in 2011, neither of which were reconcilable with either logarithmic or exponential transformations. I compared all traits (days to bud-burst, parameter a, and year-end heights and diameters) using contrasts with the model:

$$Y_{ijkl} = \text{Seed source} + Plot_j + \text{Seed source}*Plot_{ijk} + e_{ijkl} \quad \text{(Equation 4.)}$$

Results

Weather conditions 2009-2011

Average ambient temperatures and precipitation recorded by the NOAA station for 2009-2011, the entire duration that the plants were situated in their pots, are shown in A-Tables 3 and A-Table-4. Average ambient temperatures in February were similar to the 100 year averages in both study years. In contrast, ambient temperatures in March and April 2010 were 7°C and 4°C higher, respectively, than the 100-year average. Precipitation during the first six months of 2010 and 2011 was lower than the 100-year average with the exception of January 2011 and June 2010 and 2011. Snowfall was below average for all months except January and April 2011 (A-Table 5). Ambient maximum and minimum temperatures during the months of the warming treatments are shown in A-Table 3. No aberrant warming spells during ectodormancy occurred, and temperatures during the fall of 2009 and 2010 were within 5° C of the 100-year average. The two years of this study differed primarily by spring temperatures, which were warmer than average in March and April 2010 than for the same period in 2011 (A-Table 6).

Warming degree-day and chilling sums, since January 1, for the different treatments applied in 2010-2011 are shown in Tables 3-2 and 3-3, respectively. We exceeded our target February max of 16°C in 2010 but were slightly lower (13°C) in 2011 (Tables 3-4 and 3-5). In March 2010, ambient temperatures were high, but our average max was approximately 20°C higher in warmed plots (Table 3-4). In March 2011 average max temperatures were just below the target of approximately 26°C. Warming degree-days were accumulated more slowly in 2011 than 2010, due to the

cooler ambient temperatures in the second year. In 2010, plots in the Feb+March warming treatment had accumulated twice as many growing degree-days (156 GDD) then the control plots (78 GDD). In 2011, the Feb+March warming plots had accumulated 53 GDD compared to 2.6 for the unmanipulated control plots.

In both 2010 and 2011, the unmanipulated control plots had the highest number of chill days by April 1, with 42 and 48, respectively (Tables 3-2 and 3-3). The Feb+March warming treatment had the least number of chill days in 2010 and 2011 with 32 and 52.5, respectively. In both years Feb warming had accumulated the second fewest chill days with 35.5 and 52 for 2010 and 2011, respectively (Tables 3-2 and 3-3).

Soils in the three warming treatments (February, March and Feb+March) began warming sooner than all other treatments (Table 3-6). By end of April 2011, Feb+March warming had accumulated twice as many degree days as the control plots. Both treatments applied in February 2011 (treatment and warming) were slower to thaw than control plots in 2011 likely because they were not insulated from extreme cold temperatures that occurred after snow removal.

Days to bud-burst

Bud-swelling was first observed on April 2 (Day of Year (DOY) = 92) and April 29 (DOY 119) in 2010 and 2011, respectively. Bud break was delayed in the Feb warm, relative to the Feb control, in both years, but differences were only significant in 2011 (Figure 3-1). Bud break was significantly earlier in the March warm treatment than the March control in both years (Figure 3-1). In 2010, the Feb+March treatment was similar to the Feb warm and the untouched control was intermediate to all treatments (Figure 3-2). In 2011, Feb warming was significantly later than either the March warm or the

unmanipulated control. The Feb+Mar warming treatment was similar to the unmanipulated control and the Feb warm treatments.

Weekly terminal lengths

Terminal shoots were first measured on May 23 (DOY 123), ending July 12 (DOY 193) in 2010, and beginning May 10 (DOY 130), ending July 22 (DOY 203) in 2011. Treatments were similar until DOY 161 in 2010 and 176 in 2011 (Figure 3-3, 3-4) when they diverged. Treatment ranks remained largely stable after divergence in both years (Tables 3-7, 3-8). By DOY 193 in 2010, terminals lengths of trees in the March warm and untouched controls were significantly longer than Feb warm plots (Table 3-7). By DOY 203 in 2011, terminal lengths of March warm and Feb+March control were significantly longer than February warm, Feb control , Feb+Mar warm and March control plots (Table 3-8). The repeated measures results were similar to the Tukey's results from the last day of collection (DOY 193 and 203 in 2010 and 2011, respectively), so they are not reported further.

Parameters derived from the Richards function were significant for parameter '*a*' only, the asymptote of terminal lengths. For this parameter, March warm and March control treatments differed significantly (Figure 3-5). In both years, parameter '*a'* was greater for the March warm than the control but did not differ between Feb warm and Feb control. No differences in parameter 'a' could be detected from the unmanipulated control (Figure 3-6). Curvature parameters (parameters *b-d*) were largely unaffected by treatments or too skewed to produce differences that were detectable by ANOVA. I was not able to detect significant differences for parameters b, c, or d or AvgRGR or weighted mean relative growth rate (wmRGR) among treatments for both years.

Year-end heights and diameters

Survival was 100% and no visible necrosis or chlorosis of needles was consistently associated with any treatment in either year. Tree heights were significantly taller in the February control plots than the February warming plots and similar between March warm and March control treatments in both years (Figure 3-7). Trees were taller in the untouched controls compared to the Feb warm, March warm and Feb+March warm (Figure 3-8), with significant differences in 2010 but not 2011.

Genetic and phenotypic correlations

Genetic correlations were significant and negative between bud-break time and height in 2011 (Table 3-9). Genetic correlations in 2010 were negative, but the standard error was high (1.07), precluding significance. Genetic correlations between diameter and bud-break time were significant and negative for both years (Table 3-9). Phenotypic correlations between bud-break time and height were negative but not significant for either year.

Discussion

Artificial warming treatments applied in February significantly delayed budburst time and reduced overall tree height relative to unmanipulated and snow-removal controls. This contrasts with studies that experimentally advanced bud-break time by subjecting trees to consistently elevated temperatures during quiescence (Hänninen, Slaney, & Linder, 2007). Bud break time may be delayed if elevated temperatures during ectodormancy, the stage preceding quiescence, preclude chilling requirements from being met (Heide, 2003; Søgaard et al., 2009). A phenological delay, attributed to incomplete chilling during ectodormancy, was reported for entire plant populations in Tibet (Yu,

Luedeling, & Xu, 2010). Quiescent-stage warming also delayed bud-break in Douglas fir, likely due to insufficient chilling units (Bailey & Harrington, 2006). Trees in my study experienced normal ambient temperatures during ectodormancy (the preceding fall and early winter in 2009 and 2010), fulfilling the chilling requirements each of the two years of the study (Nienstaedt, 1966). My data supports the idea that chilling during quiescence also affects bud-break time, even after ectodormancy chilling requirements are met (Partanen, Hänninen, & Häkkinen, 2005). Intermittent cold temperatures that reverse dehardening processes may further increase the number of degree-days required to break-buds (Leinonen, Repo, & Hänninen, 1997; Repo, 1991). One effect of global warming in northern Minnesota could be a delay in bud-burst of white spruce if periodic warming occurs during quiescence, even if chilling requirements for ectodormancy are met.

Trees warmed in March broke bud significantly earlier than trees warmed in February. This acceleration in bud-break time resulting from winter warming has been reported in hardwood trees (Fu, Campioli, Deckmyn, & Janssens, 2012). In addition, bud-break was advanced in Norway spruce following a controlled 14-day warming spell (Granhus et al., 2009). The discrepancy in bud-break time between Feb and March warming treatments in my study may be attributed to several factors. For one, a deeper dormancy state in February may have reduced receptivity to warming preventing the accumulation of growing degree-days (Wisniewski, Sauter, Fuchigami, & Stepien, 1997). In March, trees were either more receptive to warming, were not affected by the loss of chill days, or some combination of both. The response of tree seedlings to high

temperatures during quiescence is not consistent, suggesting that quiescence is best understood as two separate stages instead of one (Partanen et al., 2005).

The cumulative warming treatment, Feb+March warm, did not differ from the unmanipulated controls with respect to bud-break time in either year of this study in spite of experiencing the highest number of growing degree-days (GDD) and fewest chill days of all treatments. This further supports the idea that a loss of chill days in February was not further compounded by a loss of chill days in March, in which case bud-break in the cumulative treatment would have experienced a heightened delay. The cumulative warming treatment had acquired almost twice as many GDD as the control in 2010 and several-fold more in 2011, suggesting that warming days accrued in February and March were also not additive. In continental boreal climates like Minnesota, white spruce are well-adapted to natural fluctuations in quiescent-state temperatures. This adaptation may be buffered by additional chilling requirements during quiescence to prevent premature bud-break. Warming in February produced delays in bud-break likely due to insufficient chilling while warming in March were receptive to warming degree days, hastening their bud-break. In the cumulative treatment, the effects of chilling losses that resulted from warming in February was canceled out by the accumulation of warming days that occurred following warming in March.

Tree heights in all treatments were reduced, relative to unmanipulated controls, whether warmed or experienced snow-removal only. My study is unique because treatments were warmed and subjected to intermittent periods of cold, subfreezing temperatures. Ambient temperatures that followed the February and March treatments differed starkly: following the February treatment, trees were exposed to consistently low

temperatures, dipping to lows of 7° to -13°C, while March temperatures rarely dipped below 0°C. The effect of extreme cold temperatures after extreme warming may have re-hardened tissues, triggering metabolic events increasing carbon losses (Repo, 1991). The combination of exposure, through snow removal, and warming in the winter months may have induced embolisms that reduced overall growth (Mayr & Charra-Vaskou, 2007). Alternatively, warming may have stimulated the diversion of stored carbon to respiration (Schaberg et al., 1996) or photo-protection via the xanthophyll cycle (Ottander, Campbell, & Oquist, 1995). White spruce is susceptible to needle damage in the winter because its foliage lacks anthocyanins, but in spite of the extreme temperatures I observed no needle necrosis, indicating the likelihood of active photo-protection. In contrast, a natural January winter thaw produced widespread dieback and decline in red spruce in New England (Strimbeck et al., 1995). The deep dormancy achieved by white spruce may insure stronger protection from mid-winter thaws than a species with a more southerly range, like red spruce (Strimbeck et al., 2007). Likewise, mid-winter thaws were not associated with de-hardening in Norway spruce (Westin et al., 2000). In spite of the reduction in tree height, white spruce exhibited resiliency to the effects of mid-winter warming as exemplified by their survival, and a lack of tissue chlorosis and necrosis.

The early bud-break time that resulted from the March warming treatment did not enhance tree heights. This supports the idea that the additional gains in early photosynthesis by leafing-out early were offset by carbon losses from other physiological processes. This finding seemingly contradicts negative correlations between bud-break time and tree height in my data, but perhaps the artificial warming failed to override inherent bud-break time. A previous study of white spruce reported genetic correlations

between bud-break date and height elongation of -0.66 and -0.93 (Wilkinson, 1977), similar to my findings. The difference in average bud-break time among sources was approximately four days compared to differences in treatment means of approximately two days. These differences are small compared to differences of 21 days among trees in wild stands (Nienstaedt & Teich, 1972). The risk for damage from spring frost reduces with age since bud-break time is strongly ontogenetic, and is delayed as the tree matures (Nienstaedt & King, 1970; Partanen et al., 2005). In most years the two-day difference in bud-break time would not pose a great risk for early frost damage, but the risk for a spring frost remains in young seedlings.

Ambient temperatures clearly have an impact on time to bud-burst: the timing of bud-break was almost a full month earlier in 2010 than 2011, supporting the contention that the accumulation of heat sums is the main driver of bud-break in the spring (Blum, 1988). My results support the possibility that photoperiod may help trigger the transition from latent quiescence to the onset of degree-days accumulation. This idea is supported by Owens & Molder (1977) who postulated that bud-break is best modeled when mitotic events restart in the buds, an event that occurs the same time each year regardless of temperature. Recent studies have reported that photoperiod and temperature stimulate ATP-ase activity in the plasma membrane that precedes bud-break (van der Schoot & Rinne, 2011). A second rest period, culminating at the vernal equinox, was more recently proposed as a stage of dormancy Norway spruce (Partanen et al., 2005). My March warming treatments occurred just prior to the vernal equinox in both years so that differences in bud-break time that I observed between February and March warming

treatments may be also supported by the completion of a secondary rest phase in late February to early March triggered by changes in photoperiod.

Management implications

White spruce responded to quiescent-stage warming with reductions in height growth, and delays or advances in bud-break depending on the timing of applied heating. I observed no mortality or obvious necrosis on the needles indicating that the trees maintained functionality in spite of the stress imposed by the treatments. My results suggest that quiescence may be best modeled in two stages, early and late quiescence, corresponding to Feb and March, respectively. Tree heights were reduced following quiescent-stage warming that could portend a decline of growth in a future climate with less snowpack and more extreme mid-winter temperatures.

The delays in bud-break associated with warming in February may offset the risk of damage from early spring frosts. Bud-break was negatively genetically correlated with growth, but bud-burst time is strongly ontogenetic and delayed with maturity so the risk for spring frost damage will diminish with age. Tree-tree variation in bud-break time was high, so that trees with early bud-break were observed in all seed sources. Because of this, white spruce seedlings should be planted under an overstory or away from topographically low-areas to safeguard young seedlings from the damaging effects of spring frosts from radiational cooling.

Tables

Table 3-1. Treatments used for experimental warming. Each treatment was represented by two plots of 56 trees each. Treatments were randomly assigned to plots.

Feb warm	Snow removed + warmed 4 days in February
Feb control	Snow removed
March warm	Snow removed + warmed 4 days in March
March control	Snow removed in March
Feb+March warm	Snow removed + warmed Feb and March
Feb+March control	Snow removed twice, once each in Feb and March
Control	Unmanipulated

Table 3-2. Cumulative growing degree-days (base temperature of 1°C) and number of chilling days (mean daily temperature less than 1°C) by April 1, 1°C for 2010 treatment and control beds. Values were averaged across two plots, using temperature hoboes.

	Warming days			Chilling days		
Treatment in 2010	Feb	March	Sum	Feb	March	Sum
Feb warm	18.7	95.9	114.6	24.5	11.0	35.5
Feb control	0.0	96.7	96.7	28.0	10.5	38.5
March warm	0.0	102.5	102.5	28.0	10.5	38.5
March control	0.0	102.2	102.2	28.0	11.0	39.0
Feb+March warm	22.2	133.5	155.8	23.5	8.5	32.0
Feb+March control	0.0	108.0	108.0	28.0	10.0	38.0
Control	0.0	77.6	77.6	28.0	14.0	42.0

Table 3-3. Cumulative growing degree-days (base temperature of 1°C) and number of chilling days (mean daily temperature less than 1°C) by April 1, 1°C for 2011 treatment and control beds. Values were averaged across two plots, using thermocouples.

T-couples	Growing Degree Days			No. Chilling Days		
Treatment in 2011	Feb	March	Sum	Feb	March	Sum
Feb warm	19.0	7.0	26.0	24.0	28.0	52.0
Feb control	2.6	7.8	10.5	26.5	27.5	54.0
March warm	0.0	28.7	28.7	28.0	22.5	50.5
March control	0.0	5.8	5.8	28.0	28.0	56.0
Feb+March warm	15.1	37.5	52.7	25.0	27.5	52.5
Feb+March control	3.4	9.1	12.5	26.0	26.0	52.0
Control	0.7	1.9	2.6	27.5	21.0	48.5

Table 3-4. Daily maximum temperatures (°C) for warmed and control plots during four days of treatment application in Feb and March, 2010.

	Feb max temps				
Day -->	1	2	3	4	Avg max
Feb warm	10.5	25.4	14.9	15.2	16.5
Feb control	-3.1	-1.8	0.2	-4.9	-2.4
Control	-5.7	-6.9	-7.9	-8.0	-7.1
	March max temps				
Day -->	1	2	3	4	Avg max
March warm	47.3	76.1	62.7	67.8	63.5
March control	36.6	51.2	40.4	36.8	41.3
Control	34.3	40.0	36.7	35.1	36.5

Table 3-5. Daily maximum temperatures (°C) for warmed and control plots during four days of treatment application in Feb and March, 2011.

2011	Feb max temps				
Day -->	1	2	3	4	Avg max
Feb warm	8.3	18.2	23.3	15.0	16.2
Feb control	3.5	6.4	10.8	5.8	6.6
Control	1.2	9.3	4.2	-0.6	3.5
	March max temps				
Day -->	1	2	3	4	Avg max
March warm	25.0	14.5	26.5	31.1	24.3
March control	10.4	0.2	0.9	3.6	3.8
Control	5.6	0.1	1.5	1.9	2.3

Table 3-6. Growing degree-days for soils, one decimeter beneath the soil surface, calculated as the differences between the average daily temperature and the base temp of 1°C, for 2011. Values were averaged across two plots, using thermocouples. Warming days = 0 when the average daily temperature (between highest and lowest temperature for any day) is lower than 1°C.

Treatment in 2011	Soil Growing Degree-Days			
	Feb	March	April	Sum
Feb warm	0.00	0.00	22.15	22.15
Feb control	0.00	0.00	22.64	22.64
March warm	0.00	0.00	27.95	27.95
March control	0.00	0.00	29.14	29.14
Feb+March warm	0.68	2.44	58.00	61.12
Feb+March control	0.00	0.00	29.75	29.75
Control	0.00	0.00	30.40	30.40

Table 3-7. Terminal lengths (mm) in spring 2010 (least-squared means) for treatments at three points in time. Differences significant by Tukey's LSD test at $p<0.05$ are indicated by different letters within each column. Day is the day since January 1. Control is the unmanipulated plots. Feb control, March control had snow removed but were not heated. Feb warm, March warm were warmed for one week in Feb and March respectively. No significant differences were found in Day 144.

2010	144	SE	Rank	LSD	165	SE	Rank	LSD	193	SE	Rank	LSD
Control	50.05	2.192	4	a	123.1	2.192	1	b	131	2.192	1	b
March warm	46.38	3.113	7	a	118.7	3.113	2	ab	127.1	3.113	2	b
FM warm	53.31	3.136	1	a	116.8	3.136	3	ab	123.1	3.136	3	ab
March control	52.59	3.091	2	a	114.6	3.091	4	ab	120.7	3.091	4	ab
Feb. control	49.28	3.089	5	a	113.8	3.089	6	ab	120.5	3.089	5	ab
FM control	48.61	3.093	6	a	114.5	3.093	5	ab	120.5	3.093	6	ab
Feb. warm	50.50	3.09	3	a	108.9	3.09	7	a	114.2	3.09	7	a

Table 3-8. White spruce seedling terminal lengths (mm) and standard errors (SE) after heating treatments in spring 2011 at three points in time. Differences significant by Tukey's LSD test at $p<0.05$ are indicated by different letters within each column.

2011	Day				Day				Day			
Treatment	**130**	**SE**	**Rank**	**LSD**	**176**	**SE**	**Rank**	**LSD**	**203**	**SE**	**Rank**	**LSD**
Feb+Mar control	8.13	3.64	4	a	70.37	3.64	1	c	89.80	3.64	1	c
March warm	8.43	3.63	3	a	68.60	3.63	2	bc	87.48	3.63	2	c
Control	7.74	3.63	6	a	57.14	3.63	3	abc	76.90	3.63	3	bc
Feb+March warm	7.99	3.67	5	a	58.97	3.67	4	abc	72.26	3.67	4	b
Feb. control	9.95	3.65	1	a	55.14	3.65	5	ab	70.88	3.65	5	b
Feb. warm	9.78	3.66	2	a	54.07	3.66	6	ab	67.92	3.66	6	ab
March control	4.55	3.66	7	a	44.31	3.66	7	a	54.90	3.66	7	a

Table 3-9. Genetic (above the diagonal) and phenotypic (below the diagonal) correlations for date to bud-burst in 2010 (BB-2010, top box), and 2011 (BB-2011, lower box). Genetic correlations are shown with standard errors, and Pearson correlations are shown with p-values, where NS means not significantly different at $p < 0.05$.

	BB 2010	Height 2010	Diameter 2010
BB-2010	x	-0.77 (1.07)	-0.65 (0.32)
Height 2010	-0.09 (NS)	x	0.82 (0.38)
Diameter 2010	-0.05 (NS)	0.58 (p<0.001)	x
	BB 2011	Height 2011	Diameter 2011
BB-2011	x	-0.56 (0.40)	-0.91 (0.28)
Height 2011	-0.10 (NS)	x	0.54 (0.57)
Diameter 2011	-0.13 (p<0.05)	0.67 (p<0.001)	x

Figures

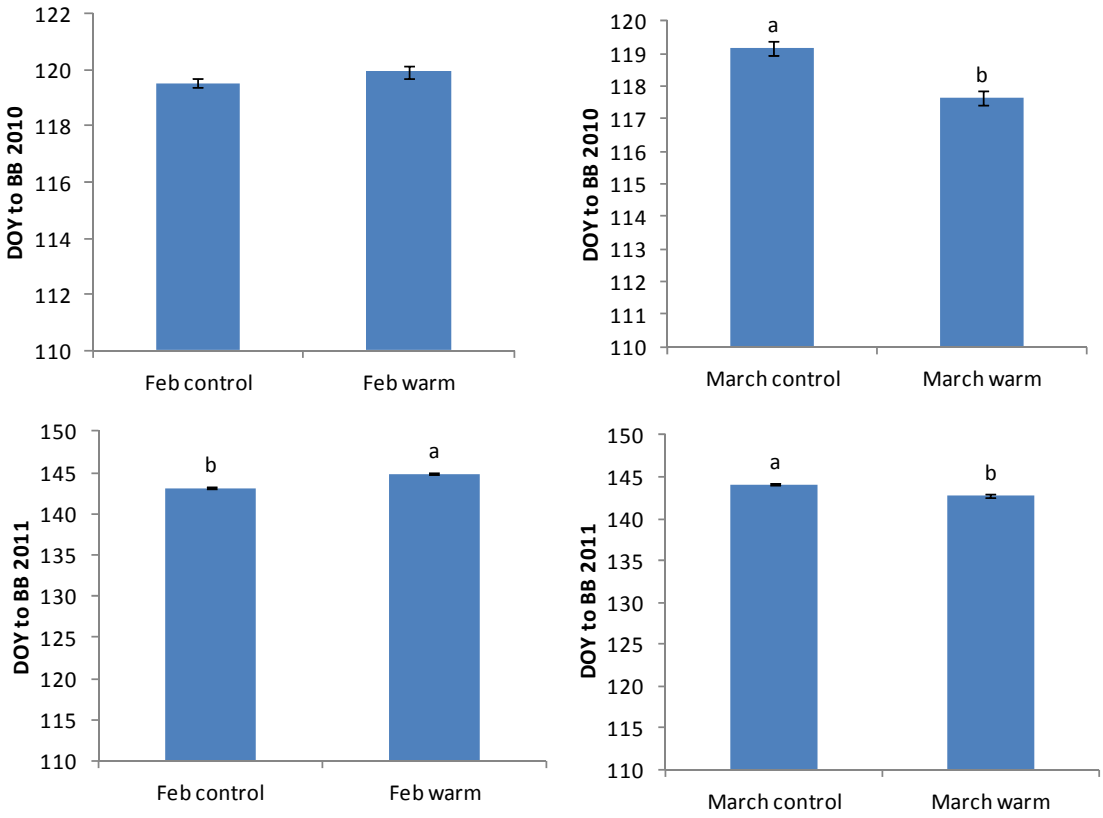


Figure 3-1. Average (least-squared means) day of year (DOY) to bud-burst, stage "3," for treatments applied in 2010 (top) and 2011 (lower). Treatments are described in Table 2-2. Significant differences at $p<0.05$ using contrasts are indicated by letters above the bars. No differences were found in 2011 between February warm and control.

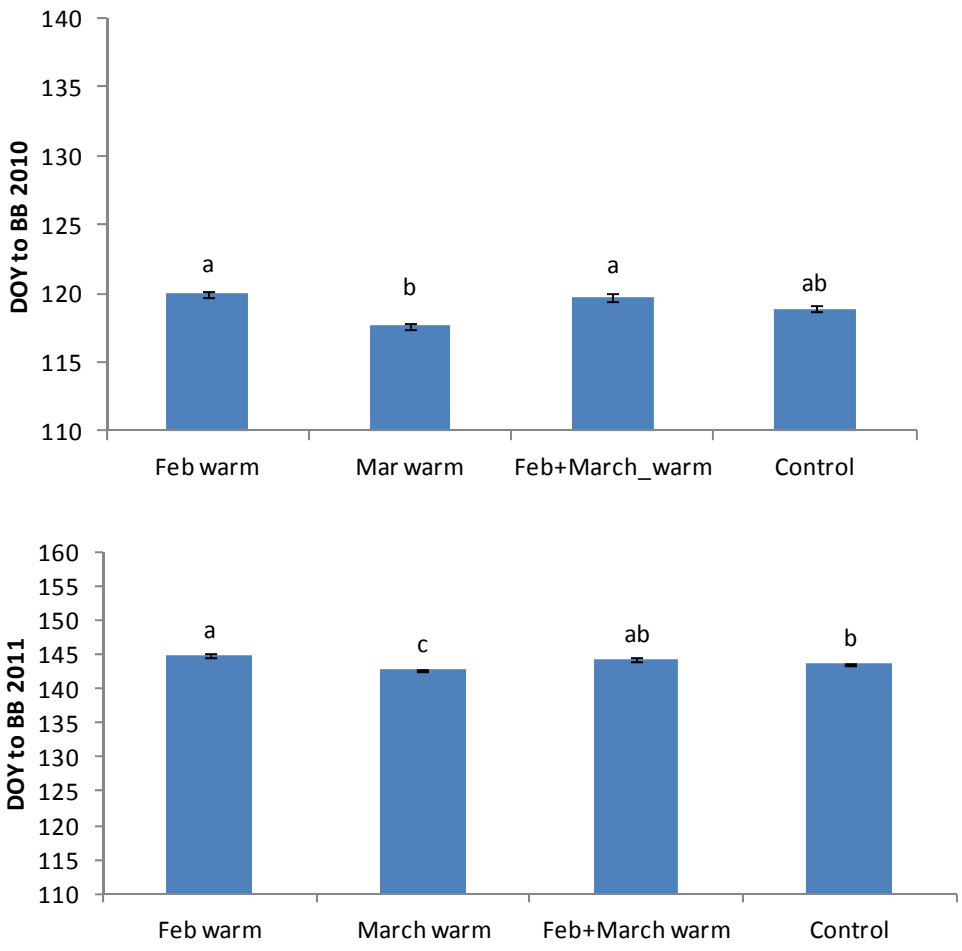


Figure 3-2. Average (least-squared mean) day of year (DOY) to bud-burst, stage "3," for treatments applied in February and March 2010 (top) and 2011 (bottom). The experimental control is depicted as well (no snow removed, no warming). Different letters above the bar indicate significant differences with Tukey's adjusted least-squared means at $p < 0.05$.

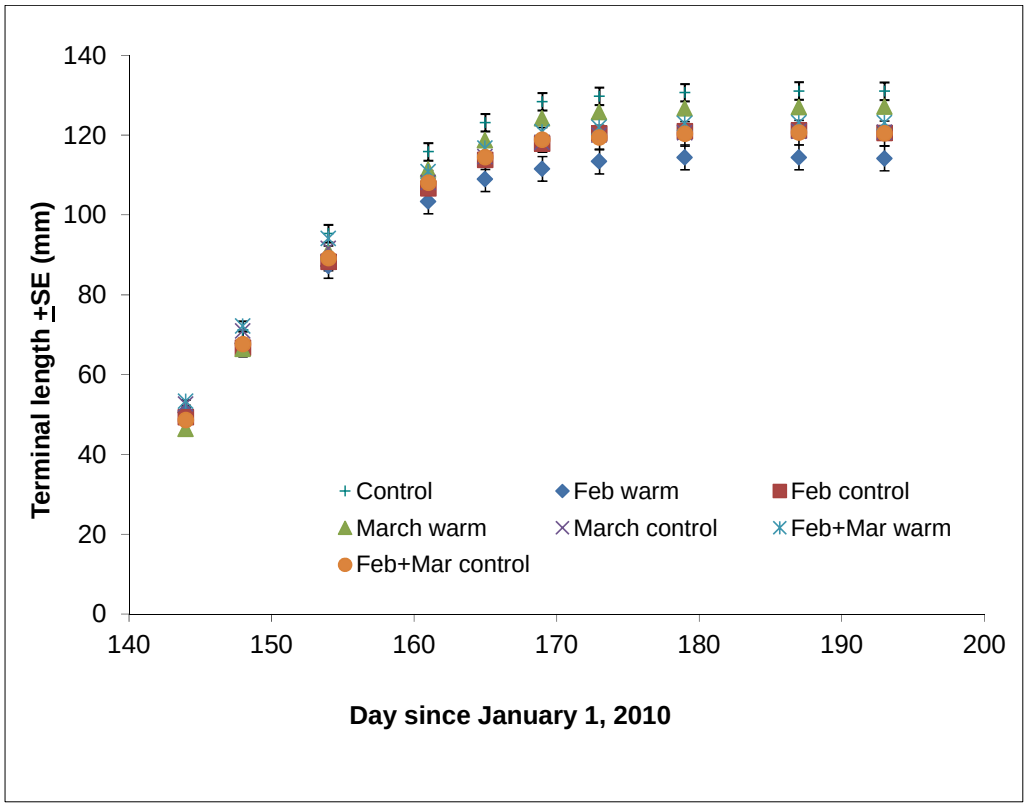


Figure 3-3 Average periodic terminal bud lengths (mm) for treatments in 2010.

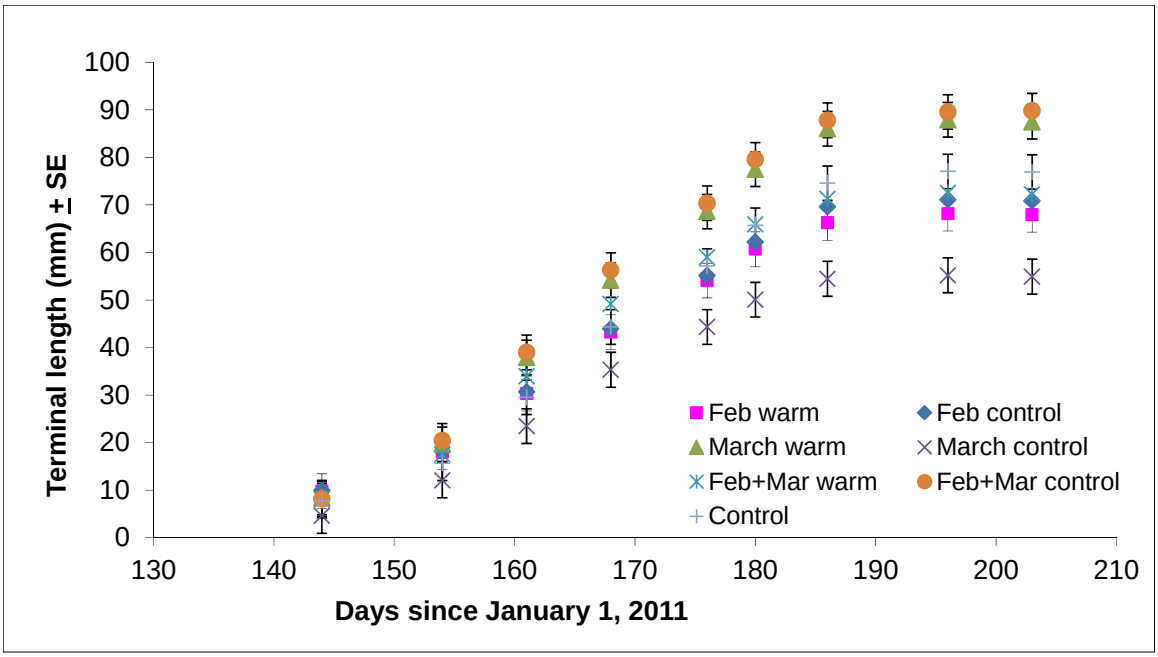


Figure 3-4. Average periodic terminal bud lengths (mm) for treatments in 2011.

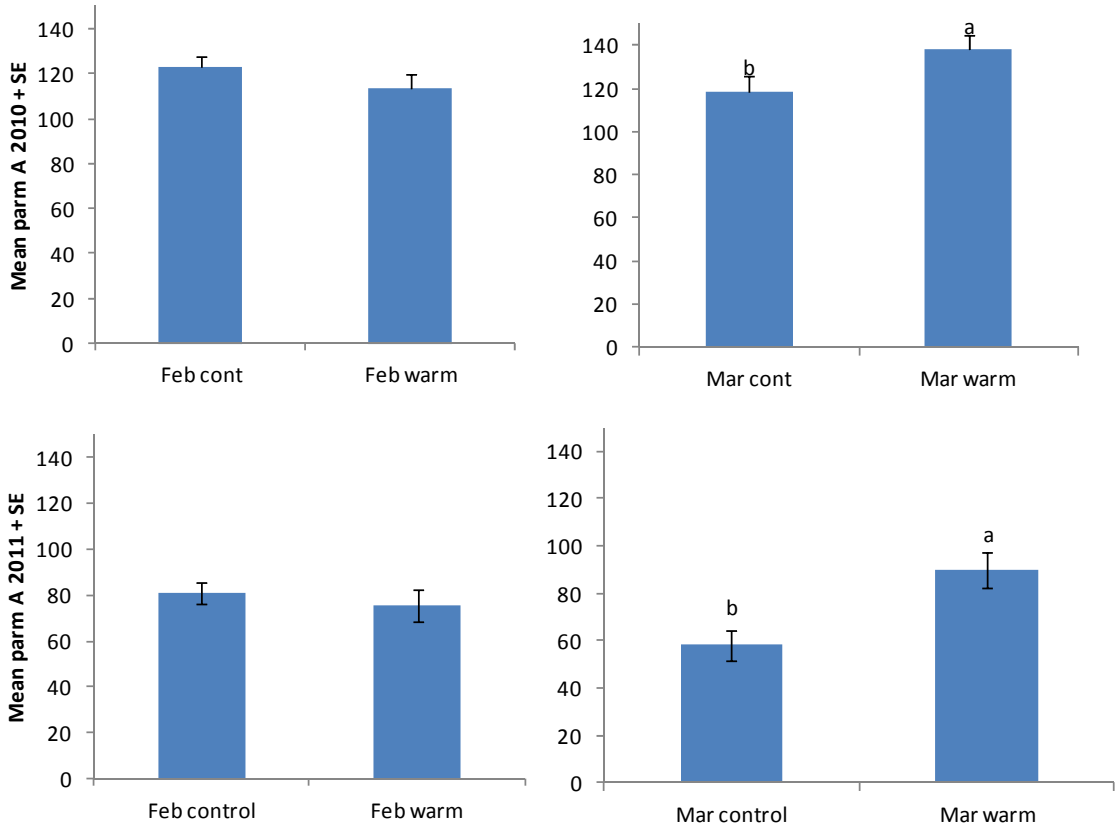


Figure 3-5. Parameter *a* (Parm A) among treatments in 2010 (top) and 2011 (lower). Parm A represents the upper asymptote of growth. Different letters above the bar indicate significant differences with contrasts at $p < 0.05$.

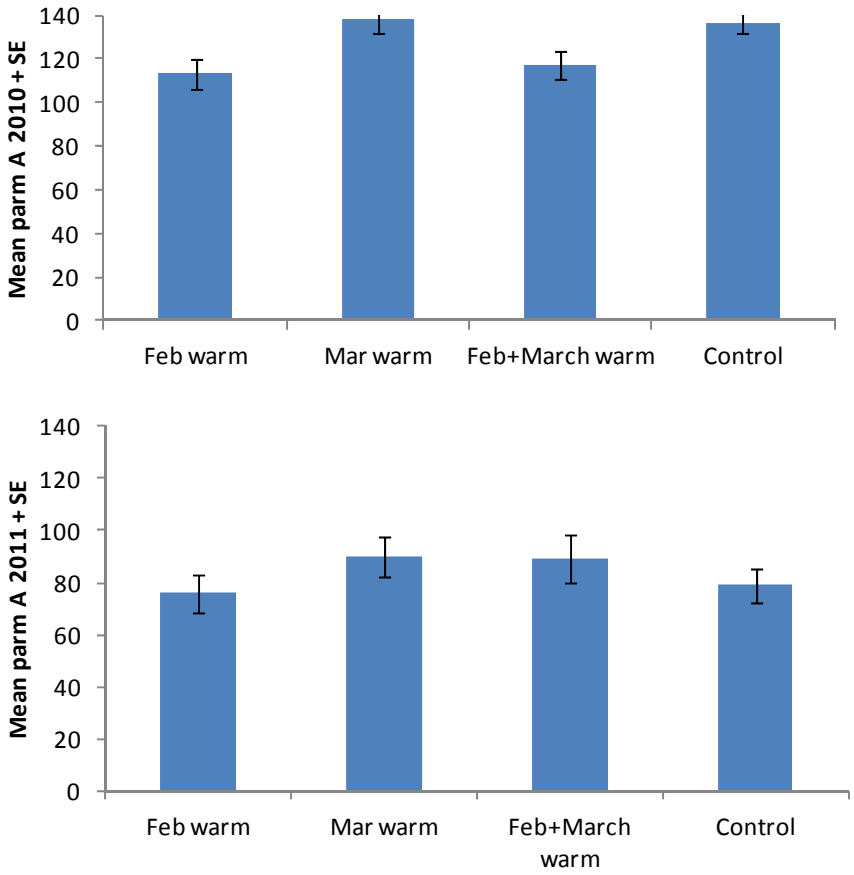


Figure 3-6. Parameter *a* (Parm A) among treatments in 2010 (top) and 2011 (lower) between treatments: February warm, March warm and overall (untouched) control. Parm A represents the upper asymptote of growth. No pairwise comparisons were significant using Tukey's HSD at $p < 0.05$.

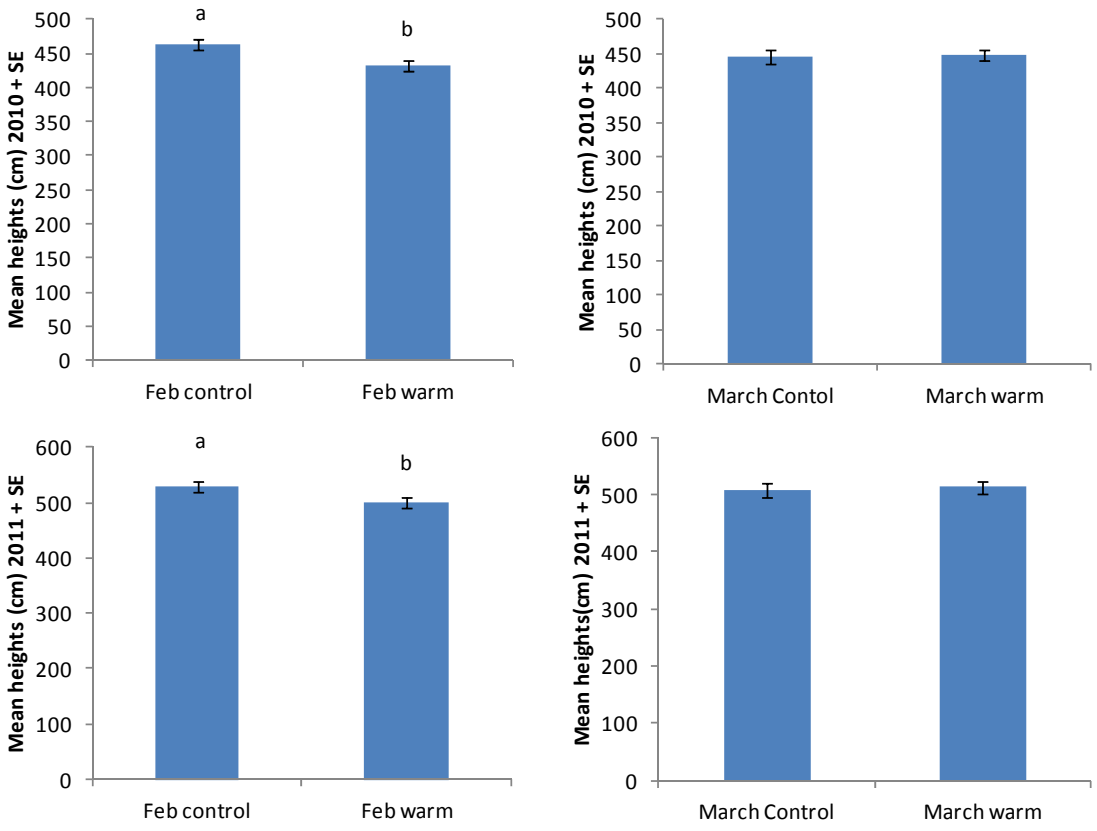


Figure 3-7. Least-squared mean tree heights (cm) by treatment in fall 2010 (top) and fall 2011 (lower). Treatments that differ significantly using contrasts at $p<0.05$ are indicated with different letters. N=42 trees for each bar.

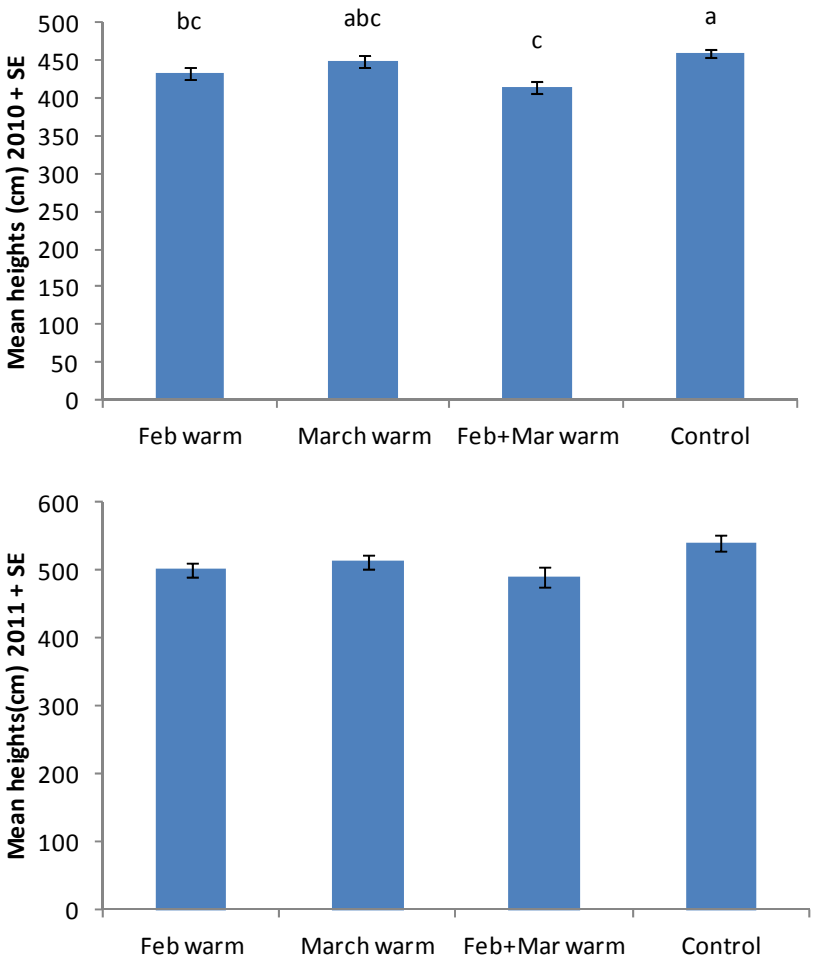


Figure 3-8. Least-squared mean tree heights (cm) in fall 2010 (top) and 2011 (lower) between treatments warmed in February, March, and the untouched control. Significant differences using Tukey's HSD are indicated with different letters at $p<0.05$. No differences occurred in 2011. N=42 trees for each bar.

Conclusions

- Selected sources of white spruce showed no significant g x e interaction for volume after 25 years of growth: trees in the top-selection tier were significantly larger than low- and mid- tier selected genotypes across sites, resulting in over 30% more wood volume per tree.
- Leaf traits (LMR, LAR) were positively genetically correlated with volume growth and were moderately heritable. Advanced-generation selection for increased volume growth may shift the phenotypic of these traits. Foliar nitrogen was dominated by stoichiometric limitations of the sites, and was not genotype-specific.
- Wood specific gravity was negatively correlated with volume but exhibited significant gxe interactions. In addition, the correlation was not consistent across tree size classes: in the highest volume class, top-tier selections had significantly higher values of WSG than low-tier selections.
- Allometric proportions of root, stem and needle mass were conserved between selected and wild sources. Large and small genotypes allocated biomass along approximately similar allometric lines.
- Biomass allocations were only significantly different between northern and southern wild sources for Root Mass Ratio. Southern sources had a lower RMR than northern wild sources.
- Artificial warming conducted in early February delayed bud-break time in white spruce while warming in March advanced it. Untouched controls were intermediate. In control plots, selected sources had slightly earlier bud-break than wild sources, by approximately two days.
- The effects of warming during in Feb and March were canceled out. Delays in bud-break that occurred after warming in February were negated by warming again in March.
- White spruce growth is expected to decline if the frequency of mid-winter warming spells increase in the future.

Cited References

Bailey, J. D., & Harrington, C. A. (2006). Temperature regulation of bud-burst phenology within and among years in a young Douglas-fir (Pseudotsuga menziesii) plantation in western Washington, USA. *Tree Physiology*, *26*(4), 421–30.

Beaulieu, J. (2003). Genetic variation in tracheid length and relationships with growth and wood traits in eastern white spruce (Picea glauca). *Wood and Fiber Science*, *35*(4), 609–616.

Besnard, G., Achere, V., Jeandroz, S., Johnsen, Ø., Faivre Rampart, P., Baumann, R., … Favre, J.-M. (2008). Does maternal environmental condition during reproductive development induce genotypic selection in *Picea abies*? *Annals of Forest Science*, *65*(109), 1–6. doi:10.1051/forest

Bigras, F. J. (2000). Selection of white spruce families in the context of climate change: heat tolerance. *Tree Physiology*, *20*(18), 1227–1234.

Bigras, F. J. (2005). Photosynthetic response of white spruce families to drought stress. *New Forests*, *29*(2), 135–148. doi:10.1007/s11056-005-0245-9

Blum, B. M. (1988). Variation in the phenology of bud flushing in white and red spruce. *Canadian Journal of Forest Research*, *18*, 315–319.

Campbell, R. K., & Sugano, A. I. (1979). Genecology of bud-burst phenology in Douglas-fir: response to flushing temperature and chilling. *Botanical Gazette*, *140*(2), 223–231.

Canham, C. D., Berkowitz, A. R., Kelly, V. R., Lovett, G. M., Ollinger, S. V., & Schnurr, J. (1996). Biomass allocation and multiple resource limitation in tree seedlings. *Canadian Journal of Forest Research*, *26*(9), 1521–1530.

Cannell, M. G. R., & Willett, S. C. (1975). Rates and times at which needles are initiated in buds on differing provenances of *Pinus contorta* and *Picea sitchensis* in Scotland. *Canadian Journal of Forest Research*, *5*, 367–80.

Carles, S., Lamhamedi, M. S., Beaulieu, J., Stowe, D. C., & Margolis, H. A. (2011). Differences in growth and mineral nutrition of seedlings produced from ten white spruce seed orchards. *New Forests*. doi:10.1007/s11056-011-9247-y

Carles, Sylvie, Lamhamedi, M. S., Beaulieu, J., Stowe, D. C., Colas, F., & Margolis, H. A. (2009). Genetic variation in seed size and germination patterns and their effect on white spruce seedling characteristics. *Silvae Genetica*, *58*(4), 152–161.

Castro, J. (1999). Seed mass versus seedling performance in Scots pine: a maternally dependent trait. *New Phytologist*, *144*(1), 153–161. doi:10.1046/j.1469-8137.1999.00495.x

Chang, C. I., & Kennedy, R. W. (1967). Influence of specific gravity and growth rate on dry wood production in plantation-grown white spruce. *The Forestry Chronicle*, *43*(2), 165–173.

Chuine, I., Rehfeldt, G. E., & Aitken, S. N. (2006). Height growth determinants and adaptation to temperature in pines: a case study of *Pinus contorta* and *Pinus monticola*. *Canadian Journal of Forest Research*, *36*(5), 1059–1066. doi:10.1139/X06-005

Coleman, J. S., McConnaughay, K. D. M., & Ackerly, D. D. (1994). Interpreting phenotypic variation in plants. *Trends in Ecology and Evolution*, *9*(5), 187–191.

Colombo, S. J. (1998). Climatic warming and its effect on bud burst and risk of frost damage to white spruce in Canada. *The Forestry Chronicle*, *74*(4), 567–577.

Cornelissen, J. H. C., Lavorel, S., Garnier, E., Díaz, S., Buchmann, N., Gurvich, D. E., … Poorter, H. (2003). A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Australian Journal of Botany*, *51*(4), 335. doi:10.1071/BT02124

Corriveau, A., Beaulieu, J., & Daoust, G. (1991). Heritability and genetic correlations of wood characters of Upper Ottawa Valley white spruce populations grown in Quebec. *The Forestry Chronicle*, *67*(6), 698–705.

Corriveau, A., Beaulieu, J., & Mothe, F. (1987). Wood density of natural white spruce populations in Quebec. *Canadian Journal of Forest Research*, *17*(7), 675–682.

Cumbie, W. P., Isik, F., & Mckeand, S. E. (2012). Genetic Improvement of Sawtimber Potential in Loblolly Pine. *Forest Science*, *58*(2), 168–177.

Dhir, N. K. (1976). Stand, family, and site effects in upper Ottawa valley white spruce. In *Proceedings of the twelfth Lake States Forest Tree Improvement Conference* (pp. p. 88–97). St. Paul, MN: USDA Forest Serv. Gen. Tech. Rept. NC-26.

Duchesne, I., & Zhang, S. Y. (2004). Variation in tree growth, wood density, and pulp fiber properties of 35 white spruce (Picea glauca (Moench) Voss) families grown in Quebec. *Wood and Fiber Science*, *36*(4), 467–475.

Easley, D., & Maynard, C. A. (1987). Nineteen-year results of white spruce provenance tests in New York State. In *Proceedings of the North Central Tree Improvement Conference*. East Lansing, MI.

Ek, A. R. (1985). A formula for the total cubic foot stem volume of small trees in the Lake States. *Northern Journal of Applied Forestry*, *2*(1), 3.

Espinoza, S. E., Magni, C. R., Martinez, V. A., & Ivkovic, M. (2013). The effect of water availability on plastic responses and biomass allocation in early growth traits of *Pinus radiata* D . Don, *22*(1), 3–14.

Etterson, J. R., & Shaw, R. G. (2001). Constraint to adaptive evolution in response to global warming. *Science*, *294*(5540), 151–154.

Falconer, D. S., & Mackay, T. F. C. (1996). *Quantitative Genetics* (Fourth Edi., p. 459). Essex, England: Pearson Education Ltd.

Fraser, D. A. (1962). Apical and radial growth of white spruce (*Picea glauca* (Moench) Voss) at Chalk River, Ontario, Canada. *Canadian Journal of Botany*, *40*, 659–668.

Fu, Y. H., Campioli, M., Deckmyn, G., & Janssens, I. A. (2012). The impact of winter and spring temperatures on temperate tree budburst dates: results from an experimental climate manipulation. *PloS One*, *7*(10), e47324. doi:10.1371/journal.pone.0047324

Furnier, G., Stine, M., Mohn, C. A., & Clyde, M. A. (1991). Geographic patterns of variation in allozymes and height growth in white spruce. *Canadian Journal of Forest Research*, *21*(5), 707–712.

Galloway, L. F., & Etterson, J. R. (2007). Transgenerational Plasticity Is Adaptive in the Wild. *Science*, *318*(November), 2006–2008.

Gaspar, M. J., Lousada, J. L., Rodrigues, J. C., Aguiar, A., & Almeida, M. H. (2009). Does selecting for improved growth affect wood quality of *Pinus pinaster* in Portugal? *Forest Ecology and Management*, *258*(2), 115–121. doi:10.1016/j.foreco.2009.03.046

Granhus, A., Fløistad, I. S., & Søgaard, G. (2009). Bud burst timing in Picea abies seedlings as affected by temperature during dormancy induction and mild spells during chilling. *Tree Physiology*, *29*(4), 497. doi:10.1093/treephys/tpn039

Grossnickle, S. C. (2005). Importance of root growth in overcoming planting stress. *New Forests*, *30*(2-3), 273–294. doi:10.1007/s11056-004-8303-2

Hamrick, J. L., Godt, M. J. W., & Sherman-Broyles, S. L. (1992). Factors influencing levels of genetic diversity in woody plant species. *New Forests*, *6*(1), 95–124.

Hannerz, M., Aitken, S. N., King, J., & Budge, S. (1999). Effects of genetic selection for growth on frost hardiness in western hemlock. *Canadian Journal of Forest Research*, *29*, 509–516.

Hänninen, H. (2006). Climate warming and the risk of frost damage to boreal forest trees: identification of critical ecophysiological traits. *Tree physiology*, *26*(7), 889.

Hänninen, H., & Pelkonen, P. (1989). Dormancy release in *Pinus sylvestris* L . and *Picea abies* (L .) Karst. seedlings : effects of intermittent warm periods during chilling. *Trees Structure and Function*, *3*(3), 179–184.

Hänninen, H., Slaney, M., & Linder, S. (2007). Dormancy release of Norway spruce under climatic warming: testing ecophysiological models of bud burst with a whole-tree chamber experiment. *Tree Physiology*, *27*(2), 291.

Hawkins, B. J. (2007). Family variation in nutritional and growth traits in Douglas-fir seedlings. *Tree Physiology*, *27*, 911–919.

Heide, O. M. (2003). High autumn temperature delays spring bud burst in boreal trees, counterbalancing the effect of climatic warming. *Tree Physiology*, *23*(13), 931–6.

Holst, M. J., & Teich, A. H. (1969). Heritability estimates in Ontario white spruce. *Silvae Genetica*, *18*(1-2), 23–27.

Hunt, R. S. (1982). *Plant growth curves: the functional approach to plant growth analysis*. London: Edward Arnold.

Jaramillo-Correa, J. P., Beaulieu, J., & Bousquet, J. (2001). Contrasting evolutionary forces driving population structure at expressed sequence tag polymorphisms, allozymes and quantitative traits in white spruce. *Molecular Ecology*, *10*(11), 2729–2740.

Johnsen, Ø., Dæhlen, O. G., Østreng, G., Skrøppa, T., Johnsen, O., Daehlen, O. G., & Ostreng, G. (2005). Daylength and temperature during seed production interactively affect adaptive performance of Picea abies progenies. *New Phytologist*, *168*(3), 589–596. doi:10.1111/j.1469-8137.2005.01538.x

Johnsen, Ø., Fossdal, C. G., Nagy, N., MØlmann, J., Dæhlen, O., & Skrøppa, T. (2005). Climatic adaptation in *Picea abies* progenies is affected by the temperature during zygotic embryogenesis and seed maturation. *Plant, Cell & Environment*, *28*(9), 1090–1102.

Kackar, R. N., & Harville, D. A. (1974). Approximations for standard errors estimators of fixed and random effects in mixed linear models. *Journal of the American Statistical Association*, *79*, 773–795.

Kalliokoski, T., Nygren, P., & Sievanen, R. (2008). Coarse root architecture of three boreal tree species growing in mixed stands. *Silva Fennica*, *42*(2), 189–210.

Kang, H., Zhuang, H., Wu, L., Liu, Q., Shen, G., Berg, B., … Liu, C. (2011). Variation in leaf nitrogen and phosphorus stoichiometry in *Picea abies* across Europe: An analysis based on local observations. *Forest Ecology and Management*, *261*(2), 195–202. doi:10.1016/j.foreco.2010.10.004

Kenward, M. G., & Roger, J. H. (1997). Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics*, *53*, 983–997.

Klevorn, R. E. (1995). *Genotype-environment interaction for height growth in white spruce*. MS Thesis, University of Minnesota.

Kriebel, H. B., & Wang, C. W. (1962). The interaction between provenance and degree of chilling in bud-break of sugar maple. *Silvae Genetica*, *11*(5/6), 125–130.

Kvaalen, H., & Johnsen, O. (2008). Timing of bud set in *Picea abies* is regulated by a memory of temperature during zygotic and somatic embryogenesis. *New Phytologist*, *177*(1), 49–59. doi:10.1111/j.1469-8137.2007.02222.x

Lambers, H., Chapin, F., & Pons, T. L. (2008). *Plant Physiological Ecology, Second edition* (p. 604). New York, NY: Springer Science + Business media. doi:10,1007/978-0-387-78341-3_7

Lang, G. A., Early, J. D., Martin, G. C., & Darnell, R. L. (1987). Endo-, para-, and ecodormancy: Physiological terminology and classification for dormancy research. *Hort Science*, *22*, 371–377.

Leinonen, I., Repo, T., & Hänninen, H. (1997). Changing environmental effects on frost hardiness of Scots pine during dehardening. *Annals of Botany*, *79*(2), 133.

Lesser, M.R., & Parker, W. H. (2006). Comparison of canonical correlation and regression based focal point seed zones of white spruce. *Canadian Journal of Forest Research*, *36*(6), 1572–1586. doi:10.1139/X06-047

Lesser, Mark R., & Parker, W. H. (2004). Genetic variation in *Picea glauca* for growth and phenological traits from provenance tests in Ontario. *Silvae Genetica*, *53*(4-5), 141–148.

Li, B., Allen, H. L., & McKeand, S. E. (1991). Nitrogen and family effects on biomass allocation of loblolly pine seedlings. *Forest Science*, *37*(1), 271–283.

Li, B., Mckeand, S., & Weir, R. (1999). Impact of forest genetics on sustainable forestry — results from two cycles of loblolly pine breeding in the US. *Journal of Sustainable Forestry*, *10*(1-2), 79–85.

Li, P., Beaulieu, J., Corriveau, A., & Bousquet, J. (1993). Genetic variation in juvenile growth and phenology in a white spruce provenance-progeny test. *Silvae genetica*, *42*(1), 52–52.

Li, Peng, Beaulieu, J., & Bousquet, J. (1997). Genetic structure and patterns of genetic variation among populations in eastern white spruce (Picea glauca). *Canadian Journal of Forest Research*, *27*(2), 189–198.

Man, R., & Lu, P. (2010). Effects of thermal model and base temperature on estimates of thermal time to bud break in white spruce seedlings. *Canadian Journal of Forest Research*, *40*(9), 1815–1820. doi:10.1139/X10-129

Mayr, S., & Charra-Vaskou, K. (2007). Winter at the alpine timberline causes complex within-tree patterns of water potential and embolism in *Picea abies*. *Physiologia Plantarum*, *131*(1), 131–139. doi:10.1111/j.1399-3054.2007.00942.x

McConnaughay, K., & Coleman, J. (1999). Biomass allocation in plants: ontogeny or optimality? A test along three resource gradients. *Ecology*, *80*(8), 2581–2593.

McKeand, S. E., Jett, J. B., & Jayawickrama, K. J. S. (1998). Phenological variation in height and diameter growth in provenances and families of loblolly pine. *New Forests*, *16*(1), 11–25.

Merrill, R. E., & Mohn, C. A. (1985). Heritability and genetic correlations for stem diameter and branch characteristics in white spruce. *Canadian Journal of Forest Research*, *15*, 494–497.

Miller, B. D., & Hawkins, B. J. (2003). Nitrogen uptake and utilization by slow-and fast-growing families of interior spruce under contrasting fertility regimes. *Canadian Journal of Forest Research*, *33*(6), 959–966. doi:10.1139/X03-017

Miller, B. D., & Hawkins, B. J. (2007). Ammonium and nitrate uptake, nitrogen productivity and biomass allocation in interior spruce families with contrasting growth rates and mineral nutrient preconditioning. *Tree Physiology*, *27*(6), 901–9.

Morgenstern, K., D'Eon, S., & Penner, M. (2006). White spruce growth to age 44 in a provenance test at the Petawawa Research Forest. *The Forestry Chronicle*, *82*(4), 572–578.

Nienstaedt, H. (1966). Dormancy and dormancy release in white spruce. *Forest Science*, *12*(3), 374–384.

Nienstaedt, H. (1972). Degree day requirements for bud flushing in white spruce - variation and inheritance. In *Proceedings, Eighth Central States Forest Tree Improvement Conference, October* (pp. 28–32).

Nienstaedt, H., & King, J. P. (1970). Breeding for delayed budbreak in *Picea glauca* (Moench) Voss-potential frost avoidance and growth gains. In *Second World Consultation on Forest Tree Breeding* (pp. 61–80). Rome, Italy, FAO.

Nienstaedt, H., & Riemenschneider, D. (1985). Changes in heritability estimates with age and site in white spruce, *Picea glauca* (Moench) Voss. *Silvae Genetica*, *34*, 34–41.

Nienstaedt, H., & Teich, A. H. (1972). Genetics of white spruce. *USDA Forest Service*, *Res. Pap.*(WO-15), 24 pp.

Nitschke, K. J. (1986). A seedbed comparison of two seed sources of white spruce (*Picea glauca* (Moench.) Voss) at the Maine State Forest Nursery. *Tree Planters Notes*, 27–28.

Oleksyn, J., Modrzynski, J., Tjoelker, M. G., Zytkowiak, R., Reich, P. B., & Karolewski, P. (1998). Growth and physiology of *Picea abies* populations from elevational transects: common garden evidence for altitudinal ecotypes and cold adaptation. *Functional Ecology*, *12*(4), 573–590.

Oleksyn, J., Reich, P. B., Chalupka, W., & Tjoelker, M. G. (1999). Differential above-and below-ground biomass accumulation of European *Pinus sylvestris* populations in a 12-year-old provenance experiment. *Scandinavian Journal of Forest Research*, *14*(1), 7–17.

Oleksyn, J., Reich, P. B., Tjoelker, M. G., & Chalupka, W. (2001). Biogeographic differences in shoot elongation pattern among European Scots pine populations. *Forest Ecology and Management*, *148*(1-3), 207–220. doi:10.1016/S0378-1127(00)00537-5

Oleksyn, J., Tjoelker, M. G., & Reich, P. B. (1998). Adaptation to changing environment in Scots pine populations across a latitudinal gradient. *Silva Fennica*, *32*(2), 129–140.

Ottander, C., Campbell, D., & Oquist, G. (1995). Seasonal changes in photosystem II organisation and pigment composition in *Pinus sylvestris*. *Planta*, *197*, 176–183.

Owens, J. N., & Molder, M. (1977). Bud development in *Picea glauca*. I. Annual growth cycle of vegetative buds and shoot elongation as they relate to date and temperature sums. *Canadian Journal of Botany*, *55*, 2728–2745.

Partanen, J., Hänninen, H., & Häkkinen, R. (2005). Bud burst in Norway spruce (*Picea abies*): preliminary evidence for age-specific rest patterns. *Trees-Structure and Function*, *19*(1), 66–72. doi:10.1007/s00468-004-0364-5

Pike, C., Warren, J., & David, A. (2002). Annual Report of the Minnesota Tree Improvement Cooperative. Department of Forest Resources, College of Natural Resources, University of Minnesota.

Pike, C., Warren, J., & David, A. (2006). Annual Report of the Minnesota Tree Improvement Cooperative. Department of Forest Resources, College of Food, Agricultural and Natural Resource Sciences, University of Minnesota.

Polge, P.H.; Illy, G. (1967). Heritabilite de la densite du bois et correlations avec la croissance etudiees a l'aide de tests non destructifs sur plants de Pins maritimes de quatre ans. *Silvae Genetica*, 173–181.

Pollard, D., & Ying, C. C. (1979). Variation in response to declining photoperiod among families and stands of white spruce in southeastern Ontario. *Canadian Journal of Forest Research*, *9*(4), 443–448.

Poorter, H., & Nagel, O. (2000). The role of biomass allocation in the growth response of plants to different levels of light, CO2, nutrients and water: a quantitative review. *Australian Journal of Plant Physiology*, *27*, 595–607.

Poorter, H., Niklas, K. J., Reich, P. B., Oleksyn, J., Poot, P., & Mommer, L. (2012). Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. *New Phytologist*, *193*(1), 30–50. doi:10.1111/j.1469-8137.2011.03952.x

Poorter, H., & Remkes, C. (1990). Leaf area ratio and net assimilation rate of 24 wild species differing in relative growth rate. *Oecologia*, *83*(4), 553–559. doi:10.1007/BF00317209

Radsliff, W., Mohn, C. A., & Cromell, W. (1983). Variation among white spruce provenances in Minnesota test plantings. *Minnesota Forestry Research Notes*, *No. 283*.

Reich, P. B. (2002). Root-Shoot relations: Optimality in acclimation and adaptation or the "emperor's new clothes"? In *Plant Roots: the Hidden Half* (pp. 205–220). Marcel Dekker, New York.

Reich, P. B., & Oleksyn, J. (2004). Global patterns of plant leaf N and P in relation to temperature and latitude. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(30), 11001–6. doi:10.1073/pnas.0403588101

Reich, P. B., Oleksyn, J., & Tjoelker, M. G. (1994). Seed mass effects on germination and growth of diverse European Scots pine populations. *Canadian Journal of Forest Research*, *24*(2), 306–320.

Reich, P. B., Tjoelker, M. G., Walters, M. B., Vanderklein, D. W., & Buschena, C. (1998). Close association of RGR, leaf and root morphology, seed mass and shade tolerance in seedlings

of nine boreal tree species grown in high and low light. *Functional Ecology*, *12*(3), 327–338.

Reich, P. B., Wright, I. J., Cavender-Bares, J., Craine, J. M., Oleksyn, J., Westoby, M., & Walters, M. B. (2003). The evolution of plant functional variation: traits, spectra, and strategies. *International Journal of Plant Sciences*, *164*(S3), S143–S164. doi:10.1086/374368

Repo, T. (1991). Rehardening potential of Scots pine seedlings during dehardening. *Silva Fennica*, *25*(1), 13–21.

Rinne, P. L., Kaikuranta, P. M., & van der Schoot, C. (2001). The shoot apical meristem restores its symplasmic organization during chilling-induced release from dormancy. *The Plant Journal*, *26*(3), 249–64.

Rinne, P., Welling, A., Vahala, J., Ripel, L., Ruonala, R., Kangasjärvi, J., & van der Schoot, C. (2011). Chilling of dormant buds hyperinduces FLOWERING LOCUS T and recruits GA-inducible 1,3-beta-glucanases to reopen signal conduits and release dormancy in Populus. *The Plant Cell*, *23*(1), 130–46. doi:10.1105/tpc.110.081307

Roa-Fuentes, L., Campo, J., & Parra-Tabla, V. (2012). Plant Biomass allocation across a precipitation gradient: an approach to seasonally dry tropical forest at Yucatán, Mexico. *Ecosystems*, *15*, 1234–1244. doi:10.1007/s10021-012-9578-3

Rweyongeza, D. M., Yeh, F. C., & Dhir, N. K. (2005). Heritability and correlations for biomass production and allocation in white spruce seedlings. *Silvae Genetica*, *54*(4-5), 228–234.

Savolainen, O., Pyhäjärvi, T., Knürr, T., & Knurr, T. (2007). Gene flow and local adaptation in trees. *Annual Review of Ecology, Evolution, and Systematics*, *38*(1), 595–619. doi:10.1146/annurev.ecolsys.38.091206.095646

Saxe, H., Cannell, M. G. R., Johnsen, Ø., Ryan, M. G., & Vourlitis, G. (2001). Tree and forest functioning in response to global warming. *New Phytologist*, *149*(3), 369–400.

Schaberg, P. G., Shane, J. B., Hawley, G. J., Strimbeck, G. R., DeHayes, D. H., Cali, P. F., & Donnelly, J. R. (1996). Physiological changes in red spruce seedlings during a simulated winter thaw. *Tree Physiology*, *16*(6), 567.

Shipley, B., & Meziane, D. (2002). The balanced-growth hypothesis and the allometry of leaf and root biomass allocation. *Functional Ecology*, *16*(3), 326–331.

Simpson, W. T. (1993). Specific gravity, moisture content, and density relationship for wood. *USDA Forest Service*, (Gen. Tech. Rep. FPL-GTR-76), 13 pp.

Smith, D. M. (1954). Maximum moisture content method for determining specific gravity of small wood samples. *USDA Forest Service*, (Rept. No. 2014), 8.

Smith, H., & Kefford, N. P. (1964). The chemical regulation of the dormancy phases of bud development. *American Journal of Botany*, *51*(9), 1002–1012.

Søgaard, G., Granhus, A., & Johnsen, Ø. (2009). Effect of frost nights and day and night temperature during dormancy induction on frost hardiness, tolerance to cold storage and bud burst in seedlings of Norway spruce. *Trees*, *23*(6), 1295–1307. doi:10.1007/s00468-009-0371-7

Stellrecht, J. W., Mohn, C. A., & Cromell, W. (1974). Productivity of white spruce seed sources in a Minnesota tree planting. *Minnesota Forestry Research Notes*, *No. 251*.

Strimbeck, G. R., Kjellsen, T. D., Schaberg, P. G., & Murakami, P. F. (2007). Cold in the common garden: comparative low-temperature tolerance of boreal and temperate conifer foliage. *Trees*, *21*(5), 557–567. doi:10.1007/s00468-007-0151-1

Strimbeck, G. R., Schaberg, P. G., DeHayes, D. H., Shane, J. B., & Hawley, G. J. (1995). Midwinter dehardening of montane red spruce during a natural thaw. *Canadian Journal of Forest Research*, *25*(12), 2040.

Tharakan, P. J., Volk, T. A., Nowak, C. A., & Abrahamson, L. P. (2005). Morphological traits of 30 willow clones and their relationship to biomass production. *Canadian Journal of Forest Research*, *431*, 421–431. doi:10.1139/X04-195

Van der Schoot, C., & Rinne, P. L. H. (2011). Dormancy cycling at the shoot apical meristem: transitioning between self-organization and self-arrest. *Plant Science*, *180*(1), 120–31. doi:10.1016/j.plantsci.2010.08.009

Venus, J. C., & Causton, D. R. (1979). Confidence limits for Richards functions. *The Journal of Applied Ecology*, *16*(3), 939–947. doi:10.2307/2402867

Wahid, N., Rainville, A., Lamhamedi, M. S., Margolis, H. A., Beaulieu, J., & Deblois, J. (2012). Genetic parameters and performance stability of white spruce somatic seedlings in clonal tests. *Forest Ecology and Management*, *270*, 45–53. doi:10.1016/j.foreco.2012.01.003

Weng, Y., Tosh, K., & Fullarton, M. (2010). Determining and projecting realised genetic gains: Results from early-stage spruce improvement programmes in New Brunswick, Canada. *New Zealand Journal of Forestry Science*, *40*, 5–17.

Westin, J., Sundblad, L.-G., Strand, M., & Hällgren, J.-E. (2000). Phenotypic differences between natural and selected populations of *Picea abies* I. frost hardiness. *Scandinavian Journal of Forest Research*, *15*(5), 489–499. doi:10.1080/028275800750173393

Wilkinson, R. C. (1977). Inheritance of budbreak and correlation with early height growth in white spruce (*Picea glauca*) from New England. *USDA Forest Service, Northeastern Experiment Station*, *Res.Pap.*(NE-391), 7.

Wisniewski, M., Sauter, J., Fuchigami, L., & Stepien, V. (1997). Effects of near-lethal heat stress on bud break, heat-shock proteins and ubiquitin in dormant poplar (*Populus nigra* Charkowiensis x *P. nigra* incrassata). *Tree Physiology*, *17*(7), 453–60.

Wright, J. W. (1976). *Introduction to Forest Genetics* (p. 463). Academic Press: New York.

Yousry, A., & Barclay, H. J. (1992). Cost of reproduction in Douglas fir. *Canadian Journal of Botany*, *70*, 1429–1432.

Yu, H., Luedeling, E., & Xu, J. (2010). Winter and spring warming result in delayed spring phenology on the Tibetan Plateau. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(51), 22151–6. doi:10.1073/pnas.1012490107

Zhang, S. Y. (1995). Effect of growth rate on wood specific gravity and selected mechanical properties in individual species from distinct wood categories. *Wood Science and Technology*, *29*(6), 451–465. doi:10.1007/BF00194204

Zhang, S. Y., & Morgenstern, E. K. (1995). Genetic variation and inheritance of wood density in black spruce (*Picea mariana*) and its relationship with growth: implications for tree breeding. *Wood Science and Technology*, *30*(1), 63–75. doi:10.1007/BF00195269

Zhang, S. Y., Simpson, D., & Morgenstern, E. K. (1996). Variation in the relationship of wood density with growth in 40 black spruce (*Picea mariana*) families grown in New Brunswick. *Wood and fiber science*, *28*(1), 91–99.

Zhang, S. Y., Yu, Q., & Beaulieu, J. (2004). Genetic variation in veneer quality and its correlation to growth in white spruce. *Canadian Journal of Forest Research*, *34*(6), 1311–1318. doi:10.1139/X04-015

Appendix

A-Table 1. Genetic (above the diagonal) and phenotypic (Pearson) correlations (below the diagonal) between trait pairs for chapter 1. Significant correlations ($p < 0.05$) are indicated in italics / boldfaced print. Variables that were log-transformed are indicated with t-subscript.

	20-year volume	^{t}Spec. Grav.	^{t}Percent N	^{t} N / cm2	^{t}N / g	^{t}SLA	^{t}LAR	^{t}LMR
20-year volume	---	***-0.58***	.	***-0.92***	***-0.66***	***0.99***	***0.76***	***0.96***
^{t}Spec. Grav.	***-0.25***	---	***-0.90***	0.24	0.16	**.**	-0.09	***-0.59***
^{t}Pct N	0.09	***-0.16***	---	***0.99***	***0.45***	**.**	-3.3	-0.29
^{t}N / cm2	-0.05	0.00	***0.47***	---	***0.97***	-0.35	***-0.76***	***-0.70***
^{t}N / g	-0.89	0.01	***0.39***	***0.98***	---	***-0.48***	***-0.84***	-0.47
^{t}SLA	***0.18***	-0.04	***0.41***	***0.24***	0.02	---	***0.95***	.
^{t}LAR	***0.36***	-0.08	0.12	-0.27	***-0.41***	***0.56***	---	***0.86***
^{t}LMR	***0.34***	-0.09	-0.05	-0.44	***-0.50***	***0.22***	***0.93***	---

A-Table 2. Seed sources used in the warming experiment, chapter 3. Numeric seed sources represent individual families selected for fast-growth.

Year Collected	Seed Source	Origin	Orchard / Provenance
2006	482	Ontario	Blandin College
2006	484	Ontario	Blandin College
2006	485	Ontario	Blandin College
2007	508	Maine	St Louis County
2007	392	Ontario	St Louis County
2007	Wild collection	DNR Seed Zone 104	Hibbing
2007	Wild collection	DNR Seed Zone 101	Baudette

A-Table 3. Average daily temperature (°C) by month for 2009-2011 compared with 100-year data.

Temperature(°C)	2009	2010	2011	100-year Average
January	-16.16	-12.04	-13.84	-13.04
February	-9.11	-9.05	-9.31	-10.46
March	-3.33	3.49	-4.25	-3.92
April	5.26	8.13	5.04	4.37
May	11.15	12.06	10.54	10.97
June	15.39	16.37	15.22	16.00
July	16.83	20.73	21.34	19.28
August	16.99	20.77	19.30	18.18
September	16.20	11.69	13.52	13.15
October	4.21	8.08	8.75	6.83
November	2.85	-1.09	0.06	-1.86
December	-11.08	-10.79	-5.88	-9.78

A-Table 4. Total monthly precipitation (mm liquid) in 2009-2011 compared with 100-year average.

Precipitation(mm)	2009	2010	2011	100-year Average
January	0.45	0.88	0.96	0.90
February	0.95	0.28	0.25	0.75
March	3.12	0.69	0.70	1.31
April	0.76	0.65	3.01	1.83
May	1.28	1.83	2.14	2.77
June	1.85	3.73	2.90	3.51
July	5.12	4.04	3.98	3.16
August	6.06	5.83	4.15	3.07
September	1.30	3.34	0.94	2.88
October	4.20	4.42	1.32	2.00
November	0.74	1.81	0.47	1.51
December	2.03	1.60	0.44	0.97

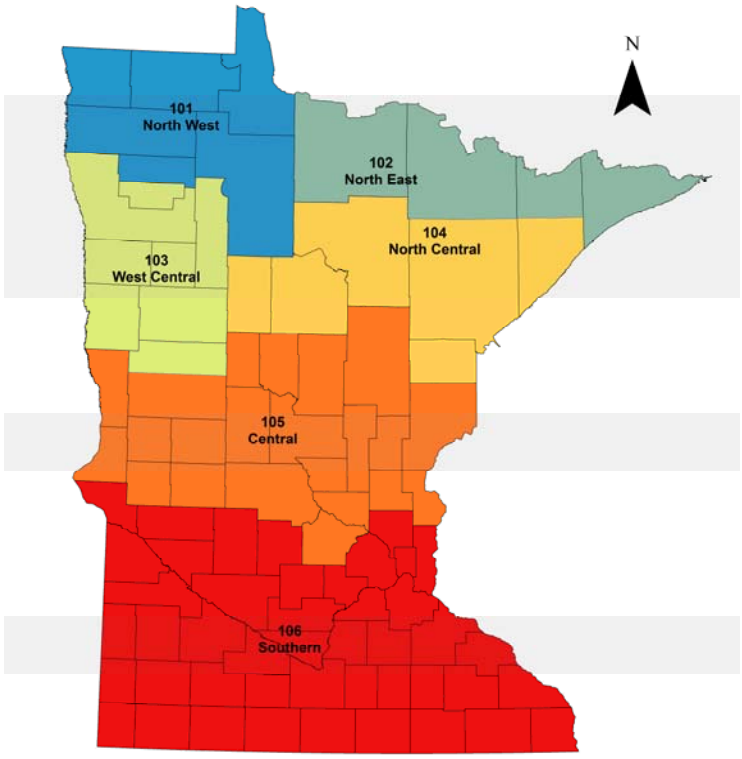
A-Table 5. Monthly snowfall for 2009-2011, and 100-year average for each month (cm).

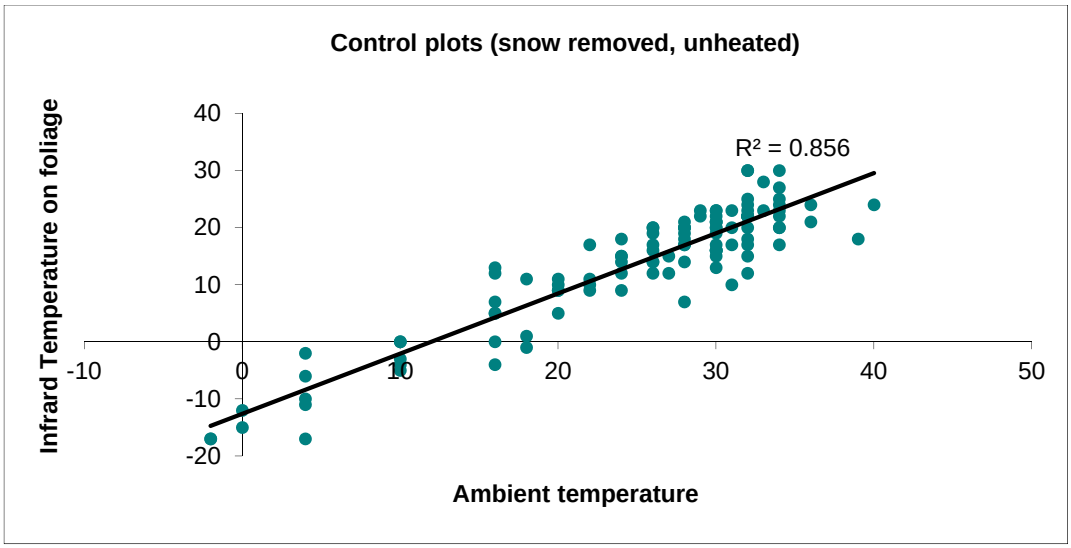
Snowfall (cm)	2009	2010	2011	100-year Average
January	0.75	0.74	1.85	1.16
February	0.70	0.61	0.30	0.94
March	1.11	0.00	0.60	0.90
April	0.14	0.00	0.80	0.37
May	0.03	0.18	0.00	0.02
June	-	-	-	-
July	-	-	-	-
August	-	-	-	-
September	0.00	0.00	0.00	0.00
October	0.19	0.49	0.00	0.13
November	0.02	1.94	0.00	0.69
December	2.87	1.29	0.00	1.04

A-Table 6. Average daily ambient minimum and maximum temperatures (°C) for February, March and April in 2009, 2010 and 2011.

	February		March		April	
Year	Min	Max	Min	Max	Min	Max
2009	-15.10	-3.08	-9.68	3.05	-1.41	11.80
2010	-15.69	-2.32	-3.44	10.29	-0.06	16.43
2011	-15.58	-3.10	-10.16	1.70	-1.04	11.09

A-Figure-1. Seed zones used by the Minnesota Department of Natural Resources in Minnesota, referenced in chapters 2 and 3.





A-Figure 2. Infrared temperature readings of foliage in control plots regressed on ambient temperatures in February 2010 for chapter 3.