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

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

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Abstract

Climate change is expected to increase winter temperatures in boreal climates. *Picea glauca* (white spruce) is vulnerable to spring frost damage due to its habit of early bud-break, which may be exacerbated or lessened with increasingly warm winters at its southern range-edge. We tested the effects of episodic warming during the quiescent stage on bud-break time and growth of seven seed sources grown in a common garden setting in Minnesota, USA. Treatment plots were warmed with infrared lamps for four days each in February, March, or February & March to simulate a mid-winter thaw. Control plots for each treatment and an overall control were included for comparison. Trees warmed in February experienced a slight delay in spring bud-break but differences in bud-break time were generally not significant. Terminal growth was significantly and negatively correlated with time of bud-break but not time to growth cessation. Our results suggest that white spruce is relatively resilient to the effects of intermittent warming, but that warming early in the season may delay bud-break time, which is expected to reduce terminal growth.

Keywords: climate change, conifer, winter warming, white spruce, bud-break, phenology
Introduction

White spruce (*Picea glauca* Moench Voss) is an important timber tree that is commercially planted across the lake states and Canada using a variety of seed sources (Nienstaedt 1981; Weng et al. 2010). In natural stands, white spruce occurs as a mid-successional species that thrives under an overstory of aspen (*Populus tremuloides* Michx) or birch (*Betula* spp.). The overstory protects seedlings from the deleterious effects of radiational cooling (Groot & Carlson 1996), which is especially beneficial for a species with a habit of early bud-break that may leaf out before the risk of frost passes. Genotypes that leaf out early can capitalize on early-season light availability and tend to grow more, as supported by positive genetic correlations between growth and bud-break time in the spring (Wilkinson 1977; Nienstaedt & King 1970).

Climate change is predicted to strongly influence boreal species like white spruce and is expected to force northward range shifts (Colombo 1998; Lesser & Parker 2006; Zhu et al. 2011) and/or changes in community assemblages (Hanberry et al. 2013). The persistence of white spruce will be a function of the severity and timing of enhanced warming, species’ response to warming, associated environmental changes (Sendall et al. 2015; Reich et al. 2015), and the presence of adaptive variation within stands located on range-edges (Savolainen et al. 2007). If climate warming hastens bud-break time and/or de-hardening of dormant evergreen leaves, then the likelihood of damage resulting from spring frost events may increase, with consequences for survival. Alternatively, bud-break may be delayed as a consequence of insufficient chilling (Laube et al. 2014; Søgaard et al. 2008), which could reduce growth. Our ability to forecast growth and make species recommendations for reforestation will hinge upon a successful understanding of the complex web of interactions among climate, phenology and growth.
White spruce is typical of other wind-pollinated conifers in possessing high genetic variation and large within-stand variation (Jaramillo-Correa et al. 2001; Cheliak et al. 1985; O’Connell et al. 2006; Crain & Tremblay 2014; Rweyongeza et al. 2007). In seedlings, shoot growth is likely related to both time of bud-break and time of bud-set because needle primorida for the growing season are placed prior to winter dormancy (Owens & Molder 1977). Thus, it is important to understand correlations between growth and climate-sensitive phenological traits such as bud-break to select species or genotypes that will be well adapted to a changing climate.

Phenological traits, for example timing of bud-break, bud-set, and growth rhythms, are indicators of synchrony between a plant and its environment. These traits are usually under strong genetic control, varying widely among provenances in common garden studies set in novel temperature, precipitation and/or photoperiodic conditions (Li et al. 1993; Hannerz et al. 1999; Oleksyn et al. 2001; Cannell & Willett 1975; Pollard & Ying 1979; Mimura & Aitken 2010; Rossi & Bousquet 2014). As seasonal temperatures change, the timing of phenological processes that initiate growth after dormancy are influenced. However, dormant trees may be insulated from novel winter weather depending on the severity and timing of warming relative to the stage of dormancy.

The annual phenological cycle of trees consists of two main periods: active growth and dormancy. Dormancy is a complex of stages with varying physiological underpinnings that is described using different models depending on taxa (Hänninen 2016). Each stage is characterized by a complex of physiologic and cellular activity largely governed by the combined effects of day-length and temperature. Dormancy in conifers has been described as a three- (Sarvas 1972; Sarvas 1974) or four-phase model (Kellomäki et al. 1992; Kellomäki et al. 1995; Hänninen & Kramer 2007), with each model including one stage of active growth.
two stages of dormancy in three-phase model are described as rest and quiescence. Rest is the
deepest state of dormancy. Chilling is a key signal for the initiation and cessation of rest; in the
absence of sufficient chilling, the cascade of events that lead to bud-break is broken, delaying it
(Søgaard et al. 2009; Campbell & Sugano 1979; Granhus et al. 2009; Heide 2003; Kriebel &
Wang 1962; Bailey & Harrington 2006).

The period after rest that precedes bud-break is known as quiescence. Quiescence begins
sometime in January for white spruce in Minnesota, and spans the vernal equinox until bud-
break in April or May (Nienstaedt 1966). Quiescence begins when symplastic pathways that
were closed during rest are re-opened in terminal buds, following sufficient chilling (Rinne et al.
2001; van der Schoot & Rinne 2011). During quiescence, metabolic changes occur that alter
sensitivity to ambient temperature from initiation to bud-break (Burr et al. 1993; Wisniewski et
al. 1997). Quiescence is notable as a phase change in the bud from a state of relative inactivity to
ontogenetic competence (Hänninen 2016), following some trigger. 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-break to occur (Rinne et al. 2011). Bud-break
occurs when the heat sum attains a critical value, $H_{\text{crit}}$, also referred to as the high temperature
requirement of growth onset (Hänninen & Kramer 2007).

The primary objective of this study is to observe whether white spruce phenology and
growth are altered following episodic warming applied during quiescence. Bud-break time,
leader growth, growing season length, and days to 90% terminal growth are considered as
response variables. We test two primary hypotheses: firstly, that warming treatments, applied
during quiescence, will hasten bud-break time in white spruce, and secondly that seasonal height
growth in white spruce is correlated with timing of bud-break in the spring.

Methods

Study site, climate and warming treatment description

The experiment was located at the Cloquet Forestry Center in Cloquet, Minnesota at
46°31’ N Lat and 92°30’ W Long 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 experiment: 13°C (four days in 1976) and
26°C (four days in 1946) for February and March respectively. The corresponding low
temperatures during these periods of warming ranged from -4°C to -1°C (February 1976) and
2°C to 4°C (March 1946). In 2010 and 2011, we applied warming treatments on white spruce
seedlings for four days each in February and March. The objective of the treatment was to
approximately match or exceed these historical maximum and minimum temperatures to test
whether these enhanced warming periods affect bud-break time, growth of the terminal leader,
length of the growing season, or the time when terminal growth ceased.

Seed source and propagation

Open-pollinated (OP) seed was obtained from seven, open-pollinated seed sources. Two
sources represent wild-collected seed bulked from each of two different seed zones originating
from approximately 100 kilometers north of the study site, because much of the white spruce
planted originates from, or is planted into, forests that are north of the study site. Open-pollinated
seed from five maternal genotypes, selected for having above-average height growth at a
progeny test (Pike & Montgomery 2015), was collected from a clonal seed orchard owned by
UPM Blandin in Grand Rapids, Minnesota at: 47°15' N Lat and 93°29' W Long, MAT= 3.9°C,
MAP= 627.4 mm. The combination of wild and selected seed sources was chosen to represent
the diversity of growth that is a hallmark of white spruce, but differences between wild and
selected sources are not the focus of this study. All seed was shipped to PRT greenhouse in
Dryden, Ontario in March 2008, germinated in 98-cubic mm styroblocks in peat moss and grown
to a target height of 20 cm and a target diameter of 2.7 mm. In October 2008, seedlings were
overwintered in their styroblocks in a 3°C cooler until transplanting into larger pots.

In spring 2009, 784 seedlings were planted into individual pots, each pot with a total
volume of 6.23 liters, 15 cm square and 41 cm tall (TPOT2, Stuewe and Sons, Corvallis OR).
The potting medium, chosen to best match growing requirements of white spruce trees, consisted
of 50% peat moss, 15% perlite, and 35% composted bark (Berger soil mix #BM7, JR Johnson
Supply, Roseville MN). The pots were positioned into six 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 (Figure 1). Only the
top 10 cm of the pots were above ground level. The beds were placed in an open area
approximately 10 meters from forest edge, along the West side of a building. A wooden support
rod, five-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 et al. 2006; Fraser 1962). The study was conducted twice, once each in 2010 and 2011
without re-randomizing treatments. We accept that some residual effects from treatments applied in 2010 might be observed the following year.

**Experimental design**

One tree from each of the seven seed sources was randomly placed in each short row of each nursery bed so that no seed source was represented disproportionately along the bed edges. Each of the six beds was divided into three plots, so that each plot contained seven columns and eight rows (56 trees) (Figure 1). Each treatment was replicated twice, once in each of two plots, and were assigned at random to the plots. A total of seven treatments were used, or 14 plots for this study. Three trees from each of the seven seed sources 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 all data collection.

Snow depth at the start of the February treatments was similar between 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. On the first day of treatments, approximately 30 cm of snow was carefully removed from treatment plots by hand, leaving a snow-depth of approximately 10 centimeters on each plot. Snow was removed to standardize exposure to heat and sunlight equally for all seedlings within each bed during the treatments. 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) in both years but snow removal for treatments was conducted similarly in both months.

Artificial warming was applied with one Kaglo® electric infrared lamp, hung from the support rod approximately 95 cm above the top of 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 plot and lamp to enhance warming and maintain uniform temperatures on the plots. Each lamp had a dial-up control to manually adjust the infrared output. Lamps were set to the lowest setting at night, and were adjusted during the day to ensure that the maximum temperature was achieved for two to three hours in the middle of each day.

Warming treatments were applied to two plots in February “Feb warm” and two different plots in March “Mar warm” (Table 1). Two additional plots were warmed twice, once each in February and March “Feb+Mar warm.” Snow was removed from two additional control plots per treatment “Feb cont,” “Mar cont,” and “Feb+Mar cont” to measure effects of snow removal alone. Two plots served as un-manipulated controls. The max and min temperatures that were applied during warming are shown in Table 2. After each warming treatment, all hardware was removed (lamps and plastic tarps). No significant snow fell in February or March of either year, so plots that experienced snow removal in February and March were 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 temperatures during warming treatments. 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. Hobos were left in place for the duration of the study.
In the final analysis, seven treatments were compared: three warming treatments (*Feb warm, Mar warm, Feb+March warm*) and four controls (*Feb cont, Mar cont, Feb+Mar cont, Control*).

### Assessment of bud development and terminal extension

In spring 2010 and 2011, we assessed bud-swelling and bud-break on the sub-sample of trees, 21 trees per plot (42 per treatment). Upon the first signs of bud-swelling, terminal buds on sub-sampled trees in all plots were assessed every three to four days until fully extended. Day of year to bud-break (*Days_{BB}*), was noted at the first date when the bud-cap was broken (Nienstaedt & King 1970), starting with January 1 as DOY=1.

We measured $T_{growth}$ as the length of the terminal shoot from the base of the current year growth to the distal tip approximately weekly after most trees had surpassed bud-break until the measurements had stabilized. Measurements were taken with a metal caliper for the first three weeks to the nearest 0.01 millimeter and with a ruler to the nearest millimeter for the last nine weeks.

### Statistical analyses

We calculated growing degree-days (GDD) using daily max and min temperatures from hobos, with $1^\circ C$ as a baseline temperature in each treatment (Man & Lu 2010) (Table 3). GDD are accumulated when the mean daily temperature (daily max temperature - daily min temperature) / 2; MDT) exceeded the base temperature of $1^\circ C$. If the MDT was less than the base temp, GDD = 0. We also calculated the number of chill days by counting the number of days from February 1 where the average daily temperature was lower than $1^\circ C$. Each day with MDT less than $1^\circ C$ counted as one chill day, and the summation of chill days was tabulated. Chill days were tabulated for the period from February 1 to April 1 to cover the period just before, during, and after treatments were applied.
We used nonlinear regression to estimate terminal growth for the season for the sub-sampled trees. We fit the weekly measurements of terminal lengths, which were sigmoidal, to a 4-parameter Richards function to estimate parameters (Venus & Causton 1979) with JMP software (JMP Pro, version 10.0, Cary NC) with the equation:

\[
\text{Terminal length (mm)} = a \times (1 - \exp^{-b-cT})^{1-d}
\]

Equation 1

Where \( T \) = day, \( a \) is the upper asymptote of terminal lengths (herein referred to as \( T_{\text{growth}} \)), \( c \) is the growth rate, \( b \) and \( d \) are parameters used to calculate the inflection point (Henderson et al. 2006). We used an inverse function to interpolate the dates when 90\% of growth (herein referred to as \( Days_{90} \)) was attained for each tree. We also calculated the number of days required for the terminal to complete its growth (growing season length; \( GSL \)), by calculating the time interval from \( Days_{BB} \) to \( Days_{90} \).

All data for each treatment and model was reviewed in Proc univariate in SAS (SAS Institute Inc., Cary NC) to assess normality and residuals. \( Days_{BB} \) was approximately normal for each year. \( T_{\text{growth}} \), estimated from the upper asymptote of the Richards function, \( Days_{90} \), and \( GSL \), were approximately normal with stable variances and required no further transformations.

A linear mixed model, “full model,” was employed in SAS (SAS/STAT, Proc mixed) to compare the overall effects of seed source (7), time of treatment (4; \( \text{Feb, Mar, Feb+Mar, none} \)), and warming (warmed or not warmed), including all two- and three-way interactions with dependent variables \( Days_{BB}, GSL \), and \( T_{\text{growth}} \) (Table 4). The year was set as a repeated measure with unstructured covariance matrix. Seed source, timing, and warming were set as fixed effects, with plot set as a random variable and residuals assumed to be N~(0,1). A Kenward Roger adjustment was applied to correct the covariance structure of upward biases (Kackar & Harville 1974).
In addition, we compared the seven treatments by year in a linear mixed model with treatment (7 levels) as a fixed effect, and plot as a random effect, “reduced model.” GSL and $T_{growth}$ were similar between years, so they were averaged across years. Bud-break varied widely between years, so the years were analyzed separately. Significance among treatments was tested at $p < 0.05$ with Tukey-Kramer’s test with SAS macro (Saxton 1998). Lastly, we used Pearson correlations to relate seasonal phenology ($Days_{BB}$, $Days_{90}$) with terminal growth ($T_{growth}$).

Because seed sources were significantly different and interacted with warming and timing effects for $Days_{90}$ in the full model, we used the least-squared means of source*treatment combination (Figures 4-5), with 49 data points (seven sources*seven treatments; n=14 per data point) to analyze the correlations.

**Results**

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. Snowfall was below average for all months except January and April 2011. No aberrant warming spells occurred between November and January in either year; 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 by 7.5°C and 5°C, respectively.

Maximum temperatures achieved in the Feb warm plots averaged a daytime high of 16°C and 13°C across the four-day period for 2010 and 2011, respectively, surpassing the historical average daily max temperature of -4 °C (Table 2). The max temperature in Feb warm exceeded...
the Feb cont by 14 (2010) and 9°C (2011), and the min by 10 (2010) and 4°C (2011). The Feb warm exceeded the Control by 5 (2010) and 2.5°C (2011) and the max temperature by 20 (2010) and 14°C (2011). The treatments in March exceeded the controls by similar margins (Table 2).

Growing degree-days accumulated more slowly in 2011 than 2010, due to the cooler ambient temperatures in the second year. By April 1, 2010, plots in the Feb+March warm had accumulated twice as many growing degree-days (156 GDD) than the un-manipulated Control (78 GDD)(Table 3). By April 1, 2011, the Feb+March warm plots had accumulated 53 GDD compared to 2.6 for the un-manipulated plots. In both 2010 and 2011, the un-manipulated plots had the highest number of chill days by April 1 and Feb+Mar warm had the fewest chill days in both years (Table 3). In both years, Feb warm accumulated the second fewest chilling days at 35.5 and 52 for 2010 and 2011, respectively.

Survival was 100% and no visible necrosis or chlorosis of needles was consistently associated with any treatment in either year, except for dead tops in beds warmed the second year that we attribute to their close proximity to the heat source.

Phenological and growth responses

Bud-swelling was first observed on April 2 (DOY = 92) and April 29 (DOY=119) in 2010 and 2011, respectively). Days_{BB} differed by Source and Time, with no significant interactions (Table 4). GSL differed by Source, but was unaffected by treatments. Days_{90} was significant for Source with significant Source*Warm and Source*Time interactions. T_{growth} was affected by source and warming with significant Time*Warm interaction. In the reduced model, Days_{BB} differed in 2011, but not in 2010 and was delayed in Feb warm vs Feb+Mar cont (Fig 2).

No differences in Days_{90} were found in 2010. T_{growth} was highest for Mar warm and generally
lower for *Feb warm* but significant differences between *Feb warm* and *Mar warm* treatments were not detectable (Fig 3).

We found a significant negative correlation ($r^2 = -0.503$) between *DaysBB* and $T_{growth}$ (Fig 4) so that earlier bud-break was associated with longer terminal growth. In contrast, there was no significant correlation ($r^2=0.06$) between *Days90* and $T_{growth}$ (Fig 5).

**Discussion**

Climate change is expected to disproportionately affect boreal rather than temperate regions, and be most pronounced during winter season. White spruce leaves emerge earlier than most other tree species, increasing its vulnerability to spring frosts and the effects of a changing climate, especially along its southerly range-edge in Minnesota. This study was undertaken to assess vulnerability of white spruce to the effects of intermittent warm temperatures applied artificially during the dormant winter months. In our study, the treatments induced subtle changes to phenology, but total seasonal height growth of the terminal ($T_{growth}$) was strongly, negatively correlated with bud-break time: trees with delayed bud-break grew less, across treatments. Bud-break was almost a full month earlier in 2010 than 2011, supporting the contention that the accumulation of growing degree days in late winter and early spring is the main trigger for initiation of active growth (Blum 1988; Hänninen et al. 2007; Granhus et al. 2009; Fu et al. 2012). In spite of natural differences in climate between years, the length of the growing season – the period during which the terminal was actively elongating – was relatively fixed, ca. 40 days, in both years of the study, illustrating the species’ resilience to extremes in spring temperatures. *Days90* was only weakly correlated with $T_{growth}$, highlighting the disproportionate role of bud-break time to underlying physiological processes that impact the annual height growth of white spruce.
Warming that was applied in mid-winter, represented by Feb warm treatments in our study, generally delayed bud-break time although the differences between Feb warm and Mar warm treatments were only weakly significant in the full model (Table 4), and not significant in the reduced for either year (Fig 2). In contrast, Man et al. (2014) reported differences in bud-break time between controls, and trees warmed for five and 10 days in a greenhouse to a max of 16° C and a minimum of 2° C for two northern hardwood species. White spruce might be more resilient to the effects of intermittent warming than northern hardwoods, but our warming treatments were not as lengthy in duration (four days instead of 5 or 10) though max temps were otherwise comparable. Differences in bud-break time of one to two days between Feb warm and Feb+Mar cont were significant in 2011 but have questionable biological significance. In Douglas fir, experiments that warmed intermittently during quiescence also reported small to nearly insignificant changes in bud-break time relative to untreated trees (Harrington et al. 2010).

In our study, the magnitude of significance for seed source effects on Days_{BB} (p<0.001) was considerably greater than the treatments, for example the time of warming (Feb vs March) was only significant at p=0.035 in the full model. The strong effect of seed source was also evident in the regression where treatments (indicated with different symbols) were not tightly clustered (Fig 4). As such, we conclude that the time of bud-break was conflated between treatments and seed source. The effects of our treatments would likely have been amplified had we extended the warming interval, or intensity, to a condition that may be expected in a future, warmer climate.

White spruce exhibited considerable plasticity in response to the twice-warmed treatments: delays in bud-break from Feb warm appear to be reversed by warming a second time in March. Even though trees in Feb+Mar warm accumulated two- to three-fold more growing degree days, the bud-break was not accelerated as a purely additive GDD model would predict:
delays that resulted from warming in February may have been offset by the modifying effects of warming in March. Instead, we posit that the threshold temperature may not be static, but gradually decreases during quiescence as postulated by Vegis (1964) and discussed at length in Hänninen (2016). By March, warmer ambient temperatures combined with a lower threshold temperature may have facilitated bud-development towards an advanced state of up-regulation that includes initiation of ATP-ase activity in the plasma membrane (van der Schoot & Rinne 2011; Laube et al. 2014), facilitating the onset of degree-day accumulation. A second hypothesis that rest may be completed around the vernal equinox, as suggested by Partanen et al (2005), is less likely in white spruce since Nienstaedt (1966) confirmed that chilling requirements can be satisfied with 4-6 weeks exposure to cool temperatures (2.2-4.4 °C). The Mar warm treatments in our study occurred just prior to the vernal equinox in both years, so we could not test effects of warming before and after the equinox, but the vernal equinox may prove a more reliable starting point for GDD accumulation to predict bud-break in white spruce.

Boreal trees, such as white spruce, are naturally resilient to the effects of warming and chilling events that are a hallmark of late winter season in high latitudes. The nearly average terminal growth and bud-break time of seedlings subjected to warming in February demonstrates the latent ability of white spruce to retain competent levels of frost hardiness to -28° C, the minimum air temperature we recorded in late February (2011), roughly 10 days after warming treatments had been applied. During quiescence, competency to withstand deep cold may be reduced after a warming event, relative to levels achieved during rest, because frost hardiness is not reset to nadir levels necessary for deepest dormancy (Leinonen et al. 1997). Essentially, release from dormancy to a state of ontogenetic competency may function as a physiological one-way track towards bud-break. In other words, the processes that awaken the trees after
sufficient chilling during rest are not reversed, increasing their vulnerability to extreme cold events. The timing that ontogenetic competence is attained varies with age: young trees tend to break bud earlier than mature, overstory trees (Nienstaedt & King 1970; Partanen et al. 2005). Additional research to quantify age-age correlations for bud-break and other phenological processes is warranted to assist forest managers in determining silvicultural prescriptions, e.g., what amount of residual overstory is necessary to enhance the survival of planted seedlings.

We could not attribute delays in bud-break following warming in February to any single physiological cause, but we hypothesize that stress from freezing, after de-hardening had been initiated, may have delayed bud-break as reported for other trees (Man et al. 2014; Søgaard et al. 2009; Heide 2003; Yu et al. 2010; Bailey & Harrington 2006; Leinonen et al. 1997; Repo 1991) and plants (Tahkokorpi et al. 2007). We did not test frost damage or stress levels in our seedlings, so the physiological cause of delay remains inconclusive. Others attributed delays in bud-break to insufficient chilling during rest (Søgaard et al. 2009; Heide 2003; Yu et al. 2010; Bailey & Harrington 2006), but we reject this hypothesis because our treatments were applied after rest was completed.

Our study demonstrated that white spruce is relatively resilient to the treatments we applied, but extreme winter freeze-thaw events can be catastrophic: in the northeast US, a natural January winter thaw produced widespread damage in red spruce but had little impact on the more boreal-occurring balsam fir (Strimbeck et al. 1995). In Ontario, freezing damage on spruce and pine was attributed to prolonged warming in March accompanied by hard-freezing in April (Man et al. 2013). The degree of damage to white spruce as a consequence of intermittent warming during quiescence will depend on the timing of warming, and subsequent temperatures that may re-harden, stress or damage tissues, prior to bud-break. Changes in climate that are manifest as
extreme swings in temperature during late winter, that are lengthier or more intense than the
treatments we applied or take place in late quiescence or after bud-break, are likely to devastate
trees and plants in northern boreal or temperate locales.

In conclusion, the effects of intermittent warming during the months of February and
March on bud-break were slight. Terminal growth was strongly negatively correlated with bud-
break time but was not correlated with late season ($Days_{90}$) effects, suggesting that physiological
processes that underpin growth in spruce are heightened in the early spring. We observed slight,
but insignificant, delays in bud-break associated with warming in February, and relative
advances among seedlings warmed in March. Our results suggest that quiescence may be best
modeled as a minimum of two physiological stages as suggested by an earlier study (Partanen et
al. 2005), but the deleterious effects of warming in early winter is challenging to model because
of underlying genetic variation among seed sources. Bud-break was more advanced for trees
warmed in March relative to those warmed in February and resulted in higher $T_{growth}$. Seasonal
growth in white spruce is strongly negatively correlated with bud-break time, so that genetics or
factors that delay bud-break are expected to have deleterious effects on growth, at least for
seedlings. Adult trees may break bud later, and therefore may prove to be more robust to
intermittent warming, but additional research is necessary to determine whether incremental
growth is altered by warming in the winter months.

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Table 1. Description of treatments applied during the study. The treatments were applied onto each of two plots (21 trees per plot; seven from each of three seed sources) in two consecutive years (2010-2011). Snow was removed to a depth of approximately one decimeter across the entire treatment plot just prior to warming with one infrared lamp.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Application</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warmed in February</td>
<td>Snow removed+ warmed 4 days in February</td>
<td>Feb warm</td>
</tr>
<tr>
<td>Control for February warming</td>
<td>Snow removed in February</td>
<td>Feb cont</td>
</tr>
<tr>
<td>Warmed in March</td>
<td>Snow removed+ warmed 4 days in March</td>
<td>Mar warm</td>
</tr>
<tr>
<td>Control for March warming</td>
<td>Snow removed in March</td>
<td>Mar cont</td>
</tr>
<tr>
<td>Warmed in February &amp; March</td>
<td>Snow removed + warmed in Feb and March</td>
<td>Feb+Mar warm</td>
</tr>
<tr>
<td>Control for February &amp; March</td>
<td>Snow removed in Feb and March</td>
<td>Feb+Mar cont</td>
</tr>
<tr>
<td>Control</td>
<td>Unmanipulated</td>
<td>Control</td>
</tr>
</tbody>
</table>
Table 2. Daily min and max temperatures (°C) averaged across two plots (n=21 trees per plot) and the four-day duration of the treatment for February and March in both years. Treatments are described in Table 1.

<table>
<thead>
<tr>
<th></th>
<th>February</th>
<th></th>
<th>March</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2010</td>
<td>2011</td>
<td>2010</td>
</tr>
<tr>
<td></td>
<td>Min</td>
<td>Max</td>
<td>Min</td>
</tr>
<tr>
<td>Feb warm</td>
<td>-1.7</td>
<td>16.7</td>
<td>0.6</td>
</tr>
<tr>
<td>Feb cont</td>
<td>-12.6</td>
<td>2.3</td>
<td>-3.7</td>
</tr>
<tr>
<td>Control</td>
<td>-6.7</td>
<td>-3.6</td>
<td>-1.9</td>
</tr>
<tr>
<td></td>
<td>2010</td>
<td>2011</td>
<td>2010</td>
</tr>
<tr>
<td>Mar warm</td>
<td>0.4</td>
<td>17.5</td>
<td>-2.7</td>
</tr>
<tr>
<td>Mar control</td>
<td>0.2</td>
<td>5.1</td>
<td>-8.4</td>
</tr>
<tr>
<td>Control</td>
<td>-0.3</td>
<td>2.5</td>
<td>-1.6</td>
</tr>
</tbody>
</table>
Table 3. Growing degree days (GDD) for February and March for the two years of the study.

GDD are calculated as the sum of days over threshold temperature 1 °C. Chill days are calculated as the number of days with average daily temperature < 1 °C. Treatments are described in Table 1.

<table>
<thead>
<tr>
<th>Year</th>
<th>Treatment</th>
<th>Growing degree days</th>
<th></th>
<th>Chill days</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Feb</td>
<td>March</td>
<td>Sum</td>
<td>Feb</td>
</tr>
<tr>
<td>2010</td>
<td>Feb warm</td>
<td>15.2</td>
<td>75.9</td>
<td>91.1</td>
<td>24.5</td>
</tr>
<tr>
<td></td>
<td>Feb cont</td>
<td>0.0</td>
<td>76.2</td>
<td>76.2</td>
<td>28.0</td>
</tr>
<tr>
<td></td>
<td>March warm</td>
<td>0.0</td>
<td>82.0</td>
<td>82.0</td>
<td>28.0</td>
</tr>
<tr>
<td></td>
<td>March cont</td>
<td>0.0</td>
<td>82.2</td>
<td>82.2</td>
<td>28.0</td>
</tr>
<tr>
<td></td>
<td>Feb+Mar warm</td>
<td>17.7</td>
<td>111.0</td>
<td>128.8</td>
<td>23.5</td>
</tr>
<tr>
<td></td>
<td>Feb+Mar cont</td>
<td>0.0</td>
<td>87.0</td>
<td>87.0</td>
<td>28.0</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.0</td>
<td>60.6</td>
<td>60.6</td>
<td>28.0</td>
</tr>
<tr>
<td>2011</td>
<td>Feb warm</td>
<td>15.0</td>
<td>4.6</td>
<td>19.6</td>
<td>24.0</td>
</tr>
<tr>
<td></td>
<td>Feb cont</td>
<td>0.3</td>
<td>5.5</td>
<td>5.8</td>
<td>27.5</td>
</tr>
<tr>
<td></td>
<td>March warm</td>
<td>0.0</td>
<td>12.0</td>
<td>12.0</td>
<td>28.0</td>
</tr>
<tr>
<td></td>
<td>March cont</td>
<td>0.0</td>
<td>4.9</td>
<td>4.9</td>
<td>28.0</td>
</tr>
<tr>
<td></td>
<td>Feb+Mar warm</td>
<td>10.1</td>
<td>15.9</td>
<td>26.0</td>
<td>24.5</td>
</tr>
<tr>
<td></td>
<td>Feb+Mar cont</td>
<td>0.7</td>
<td>4.8</td>
<td>5.5</td>
<td>27.0</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.0</td>
<td>1.6</td>
<td>1.6</td>
<td>28.0</td>
</tr>
</tbody>
</table>
Table 4. P-values for mixed model analysis of variance (full model) with independent variables seed Source (7 levels), Time (4 levels: February, March, Feb+Mar, None), Warm (2 levels: warmed vs not-warmed). Dependent variables included day of year to bud-break \((Days_{BB})\), day of year when 90% of terminal growth had occurred \((Days_{90})\), growing season length \((GSL\), the number of days from bud-break to 90% growth), and terminal growth extension \((T_{growth})\). Plot was set as random variable, and year as a repeated measure. Significant variables at \(\alpha < 0.05\) are boldfaced.

<table>
<thead>
<tr>
<th>Source</th>
<th>(Days_{BB})</th>
<th>(Days_{90})</th>
<th>(GSL)</th>
<th>(T_{growth})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source</td>
<td>(&lt;0.0001)</td>
<td>(0.010)</td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
</tr>
<tr>
<td>Time</td>
<td>(0.035)</td>
<td>0.523</td>
<td>0.585</td>
<td>0.104</td>
</tr>
<tr>
<td>Source*Time</td>
<td>0.653</td>
<td>(0.017)</td>
<td>0.132</td>
<td>0.103</td>
</tr>
<tr>
<td>Warm</td>
<td>0.483</td>
<td>0.558</td>
<td>0.928</td>
<td>(0.047)</td>
</tr>
<tr>
<td>Source*Warm</td>
<td>0.759</td>
<td>(0.005)</td>
<td>0.467</td>
<td>0.530</td>
</tr>
<tr>
<td>Warm*Time</td>
<td>0.225</td>
<td>0.119</td>
<td>0.059</td>
<td>(0.007)</td>
</tr>
<tr>
<td>Source<em>Warm</em>Time</td>
<td>0.177</td>
<td>0.671</td>
<td>0.589</td>
<td>0.481</td>
</tr>
</tbody>
</table>
Figure 1. Experimental design for warming study. Six beds were assembled and overlaid with cattle panels to support pots. Each bed was divided into three plots (18 plots, 56 pots per plot, one tree per pot). Seven seedlings, one from each seed source, were randomized to each row across the entire bed. Treatments were assigned to each of two plots at random. Several plots contained trees but are excluded from this study (indicated with *Skip*). The grey boxes show the time of warming: February (*Feb warm*), March (*Mar warm*), February and March (*Feb+Mar warm*). Controls plots, with snow removed, include *Feb cont*, *Mar cont*, and *Feb+Mar cont*.

Figure 2. Growing season length (*GSL*) by year for treatments reflecting day of year to bud-break (*Days_{BB}*), and day of year to 90% terminal growth (*Days_{90}*). Standard errors for *Days_{BB}* (left side of each bar) and *GSL* (right side of each bar) are shown. Significant differences for *Days_{BB}* with Tukey-Kramer test using reduced model, are indicated by different letters on the left of the bars. No significant differences among treatments were found for *GSL* or *Days_{90}*.

Figure 3. Average terminal growth (*T_{growth}* for each treatment (see Table 1 for descriptions), with year as repeated measure (full model). Terminal growth was measured weekly and estimated from the upper asymptote of weekly growth measurements using the Richards’ function. Significant differences using Tukey-Kramer method using full model are indicated with different letters.

Figure 4. Day of year to bud-break (*Days_{BB}* (year as repeated measure) by growth extension of the terminal (*T_{growth}* for each treatment (7) and seed source (7) (n=12 for each point). *T_{growth}* was estimated from the upper asymptote of weekly terminal growth measurements using the Richards function. Standard errors (SE) are shown for both variables. Pearson correlation (*R^2*) was significantly different from zero.
Figure 5. Day of year to 90% height ($Days_{90}$) by $T_{growth}$ with year as repeated measure for each treatment (7) and seed source (7). Terminal growth ($T_{growth}$) is described in Figure 4. Pearson correlation ($R^2$) did not differ significantly from zero.
Figure 2
Figure 3.
Figure 4.
Figure 5