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ARTICLE

Trembling aspen, balsam poplar, and white birch respond differently to experimental warming in winter months

Rongzhou Man, Steve Colombo, Pengxin Lu, Junlin Li, and Qing-Lai Dang

Abstract: Climatic warming may increase temperature variability, especially in winter months, leading to increased risk of early loss of cold hardiness and therefore freezing damage. In this study, changes in cold hardiness (measured based on electrolyte leakage), budbreak, and survival were used to indicate the responses of seedlings of 3 boreal broadleaf species — trembling aspen (*Populus tremuloides* Michx.), balsam poplar (*P. balsamifera* L.), and white birch (*Betula papyrifera* Marsh.) — to experimental warming. Seedling responses were greater in winter (January) and spring (March) than fall (November), and were greater in trembling aspen and balsam poplar than white birch. Warming for 5 or 10 days at 16 °C day/–2 °C night with a 10-h photoperiod in winter and spring generally reduced cold hardiness. Combined with freezing temperatures in the postwarming ambient environment, this reduction increased seedling mortality and stem dieback and extended time to budbreak. Cold hardiness increased somewhat 10 days after seedlings were returned to the outdoor environment following warming in spring, when ambient temperatures were less damaging. The resistance of white birch to warming, likely because of its greater thermal requirement for budbreak and slower natural dehardening, suggests that this species is better suited to withstand increasing winter temperature variability that might occur under climate change. To improve the accuracy of phenological modelling, the effects of winter freezing on budbreak should be factored in.

Key words: cold hardiness, winter warming, dehardening, temperature, boreal broadleaves.

Résumé : Il est possible que le réchauffement climatique accentue les variations de température, particulièrement durant les mois d'hiver, augmentant ainsi les risques de perte hâtive de la résistance au froid et par conséquent de dommages dus au gel. Dans cette étude, les changements dans la résistance au froid (mesurée via la perte d'électrolytes), le débourrement et la survie ont été utilisés pour connaître la réaction des semis de trois espèces feuillues boréales, le peuplier faux-tremble (*Populus tremuloides* Michx.), le peuplier baumier (*P. balsamifera* L.) et le bouleau blanc (*Betula papyrifera* Marsh.), à un réchauffement expérimental. La réaction des semis était plus forte en hiver (janvier) et au printemps (mars) qu'à l'automne (novembre) et plus forte chez le peuplier faux-tremble et le peuplier baumier que chez le bouleau blanc. Un réchauffement pendant 5 à 10 jours à 16 °C le jour et –2 °C la nuit, avec une photopériode de 10 heures en hiver et au printemps, a généralement réduit la résistance au froid. Combinée à des températures sous le point de congélation dans le milieu ambiant après le réchauffement, cette réduction a augmenté la mortalité des semis ainsi que le dépérissement de la tige et a prolongé la durée de la période qui précède le débourrement. La résistance au froid s'est quelque peu intensifiée 10 jours après que les semis eurent été replacés à l'extérieur à la suite d'un réchauffement au printemps lorsque les températures ambiantes étaient moins dommageables. La résistance du bouleau blanc au réchauffement, probablement due à ses exigences thermiques plus grandes pour le débourrement et à son désendurcissement naturellement plus lent, indique que cette espèce est mieux adaptée pour résister aux variations de température hivernale qui pourraient survenir à cause des changements climatiques. On devrait tenir compte des effets du gel hivernal sur le débourrement pour améliorer la précision de la modélisation phénologique. [Traduit par la Rédaction]

Mots-clés : résistance au froid, réchauffement hivernal, désendurcissement, espèces feuillues boréales.

Introduction

In temperate and boreal regions, tree growth cessation in the fall and initiation in the spring is synchronized with annual cycles in environmental conditions (Saxe et al. 2001). In indeterminate species, bud formation and the termination of shoot elongation in late summer is thought to be induced by decreasing photoperiod, while initiation of growth in spring is temperature dependent (Kramer 1994; Saxe et al. 2001). Compared with photoperiod, temperatures on given dates can vary greatly from 1 year to another, resulting in variability in the timing of growth resumption in spring. On a seasonal basis, temperature shifts are not linear and unidirectional, often with episodes of warm days followed by periods of cold days. This variability in temperature may increase with climatic warming (Schär et al. 2004; Rigby and Porporato 2008), which is more pronounced in winter and spring (Bonsal et al. 2001; Robeson 2004) and could expose trees to growth temperatures during winter months (e.g., Man et al. 2009, 2013*a*).

As thermal sum accumulates in spring, trees become less hardy leading to budbreak (Glerum 1973). However, warmer and more variable spring temperatures can cause trees to lose cold hardiness earlier (Menzel 2000; Schaber and Baldeck 2005), making them susceptible to damage if temperatures decrease after a warm spell (Cannell and Smith 1986; Inouye 2000; Gu et al. 2008; Man et al. 2009, 2013*a*; Augspurger 2013). Spring frosts can damage open buds or newly flushed tissues, but dehardened trees can

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also be damaged by freezing temperatures that occur during fluctuations between thawing and freezing prior to budbreak (Cayford et al. 1959; Sakai and Larcher 1987; Perkins and Adams 1995; Man et al. 2009, 2013*a*). Compared with frost damage that is often local and affects small, regenerating trees (Krasowski and Simpson 2001), freezing damage in winter months is generally more extensive and affects trees of all sizes and species (Cayford et al. 1959; Hiratsuka and Zalasky 1993; Man et al. 2009, 2013*a*). In boreal forests, late spring and summer frosts during and after budbreak are not uncommon (Hiratsuka and Zalasky 1993; Dang et al. 1992), whereas freezing damage prior to budbreak is relatively rare and has been reported primarily on conifers (Cayford et al. 1959; van der Kamp and Worrall 1990; Hiratsuka and Zalasky 1993; Man et al. 2009, 2013*a*).

Trembling aspen (*Populus tremuloides* Michx.), balsam poplar (*P. balsamifera* L.), and white birch (*Betula papyrifera* Marsh.) are common broadleaf species in Canadian boreal forests (Rowe 1972). They occupy a wide range of climatic and site conditions and often grow in mixed stands with conifers or other broadleaf trees (Perala 1990; Safford et al. 1990; Zasada and Phipps 1990), contributing to stand and landscape diversity. Fast-growing and short-lived relative to conifers, these broadleaf species provide critical wildlife habitat, help to maintain ecosystem processes and function, and are used to produce various forest products. Compared with conifers, these broadleaf species require less thermal sum to break buds (Cayford et al. 1959) and therefore may be more vulnerable to freezing damage in the spring (Hiratsuka and Zalasky 1993; Lamontagne et al. 1998; Wolken et al. 2009). Although frost damage to flushing buds and elongating shoots of these boreal broadleaf species has been noticed (Cayford et al. 1959; Cole et al. 1999; Wolken et al. 2009; Vermont Forest Health Update 2010) and occasionally studied (Lamontagne et al. 1998; Man et al. 2013*b*), the effects of freezing temperatures before budbreak on their survival and growth are largely unknown. This is partially because of the lack of research on cold hardiness of broadleaf species and also because without leaves any damage they sustain during winter freezing events is not easily observed.

The general objective of this study was to evaluate the potential effects of winter warming on susceptibility of trembling aspen, balsam poplar, and white birch seedlings to freezing damage. Here we examined the responses of these species to different levels of experimental warming in fall, winter, and spring and compared the results with change in cold hardiness during natural dehardening later in the spring. We hypothesized that boreal broadleaf species of similar natural distribution (Perala 1990; Safford et al. 1990; Zasada and Phipps 1990) should respond similarly to warming in winter months in terms of dehardening and budbreak, and that the level of dehardening would differ seasonally, especially before (fall warming) and after (winter and spring warming) the fulfilment of chilling requirements for dormancy release. Finally, we hypothesized that dehardened trees would reacquire some of their prewarming hardiness when returned to the ambient environment after warming, as suggested by Kalberer et al. (2006).

Materials and methods

Seedlings

Seeds collected from single open-pollinated trees at the Petawawa Research Forest (46°00′N, 77°42′W) (white birch and trembling aspen) and at Kemptville (45°02′N, 75°39′W) (balsam poplar), Ontario, Canada, within the Great Lakes – St. Lawrence forest were provided by the National Tree Seed Centre at Fredericton, New Brunswick, Canada. Seeds were sown in late June 2010 in 3.8 cm × 21 cm SC-10 Super Cell tubes filled with 2:1 peat moss/vermiculite (*v*/*v*) mixture and grown in a greenhouse at the Ontario Forest Research Institute in Sault Ste. Marie, Ontario, Canada. The greenhouse was programmed to provide 26 °C (day)/18 °C (night) temperatures and a 16-h photoperiod. Seedlings were watered as required and fertilized weekly with 20-8-20 (N-P-K) (Plant Products Co Ltd, Brampton, Ont., Canada) at 100 ppm N. Beginning early September 2010, seedlings were exposed to natural photoperiods. Temperatures ranged from 3 to 9 °C during the day and 1 to 8 °C at night and fertilization was adjusted to 20-20-20 at 50 ppm N. Starting in mid-October, fertilization was discontinued but seedlings were watered as needed.

By the end of November, leaves had abscised in all seedlings. In mid-December seedlings were sealed in plastic bags, boxed, and stored in a freezer at –3 °C. In mid-March, after 3 months in frozen storage, seedlings were moved to refrigerated storage at 2 °C. In early May, seedlings were removed from the boxes and transplanted into 15-cm diameter pots. The transplanted seedlings were grown outside under natural environmental conditions for 2 years, and watered and fertilized as needed during the growing season. Seedlings in winter were fully covered in snow in the first year because of their small size. By fall 2012, heights ranged from 50 to 80 cm for balsam poplar and 120 cm to 150 cm for trembling aspen and white birch.

Warming treatments

In the first part of the study, experimental warming was applied to different groups of seedlings at 3 periods between November 2012 and March 2013, which we refer to as fall, winter, and spring warming periods. In the fall warming period (between November 10 and 20), seedlings were leafless and were not expected to have fully fulfilled the chilling requirement for budbreak. During the winter warming period (January 10 to 20), we anticipated that chilling requirements were largely met but that warm temperature (i.e., thermal) accumulation for budbreak would not yet have occurred. Finally, during the spring warming period (March 10 to 20), we expected seedlings to have begun to accumulate thermal units for budbreak and be entering a period of susceptibility to freezing damage as cold hardiness is progressively reduced during a period in which the risk of natural freezing events remains (Cayford et al. 1959; Man et al. 2009, 2013*a*; Augspurger 2013). At each artificial warming period, 3 treatments were applied: warming for 5 or 10 days, plus a control that remained outdoors (no artificial warming). Each warming treatment was applied to 6 seedlings and replicated 3 times, with different groups of seedlings brought in on different days. During winter and spring warming periods, seedlings were taken from the outside ambient environment into a greenhouse set at 16 °C (day)/–2 °C (night), a 10-h photoperiod at 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux density (PPFD), and 50% humidity; for the fall warming period, night temperature was set at 2 °C to reflect natural night temperatures at that time of the year. Greenhouse temperatures were monitored and controlled by a CMP6050 control system (Conviron, Winnipeg, Man., Canada). Warming treatments were chosen to provide approximately 25% and 50% of the thermal sum required for budbreak by these species for the 5- and 10-day warming treatments, respectively (Rousi and Pusenius 2005; Li et al. 2010). Seedlings in the 5-day warming treatment were placed in the greenhouse 5 days after the 10-day warming treatment, so that both warming treatments ended on the same day. Seedling pots were covered with fibreglass insulation during warming to reduce root warming for better emulating natural soil conditions (soil temperatures in pots during warming treatments were not monitored). In both winter and spring, seedlings in warming treatments were kept at 5 °C for 48 h after the end of the warming treatment to reduce temperature shock and risk of damage caused by a sudden return to potentially well below freezing ambient temperatures outdoors. During winter and spring warming periods, seedlings were partially covered by snow prior to warming treatments (less so in trembling aspen and white birch because of tall stems) and this cover was replaced when seedlings were returned outdoors after warming.

Table 1. Probability values from ANOVA for relative conductivity, time to budbreak, and stem dieback of trembling aspen, balsam poplar, and white birch.

(*a*) Relative conductivity[a]

Seasonal period	Species	Warming (W) (df = 2)	Temp (T) (df = 6)	W×T (df = 12)
Fall–immediately after warming	Aspen	0.967	<0.001	0.601
	Poplar	0.038	<0.001	0.681
	Birch	0.523	<0.001	0.950
Winter–immediately after warming	Aspen	0.135	<0.001	0.003
	Poplar	<0.001	<0.001	0.001
	Birch	0.412	<0.001	0.201
Winter–10 days outdoors after warming	Aspen	0.029	<0.001	0.159
	Poplar	0.001	<0.001	0.308
	Birch	0.060	<0.001	0.928
Spring–immediately after warming	Aspen	0.007	<0.001	<0.001
	Poplar	0.034	<0.001	<0.001
	Birch	0.710	<0.001	<0.001
Spring–10 days outdoors after warming	Aspen	0.062	<0.001	<0.001
	Poplar	0.145	<0.001	0.026
	Birch	0.948	<0.001	0.558

(*b*) Budbreak timing and stem dieback[b]

	Species	Seasonal period (S) (df = 2)	Warming (W) (df = 2)	S×W (df = 4)
Cumulative growing degree hours	Aspen	0.004	<0.001	0.029
	Poplar	0.004	<0.001	0.263
	Birch	<0.001	<0.001	0.097
Stem dieback (%)	Aspen	<0.001	0.006	0.005
	Poplar	0.057	0.067	0.020
	Birch	0.102	0.034	0.190

Note: Warming treatments at each seasonal period included 5- and 10-day warming for 10-h photoperiod at 16 °C (day)/+2 °C (night) for fall warming and 16 °C (day)/–2 °C (night) for winter and spring, in addition to control seedlings remaining outdoors. df, degrees of freedom.

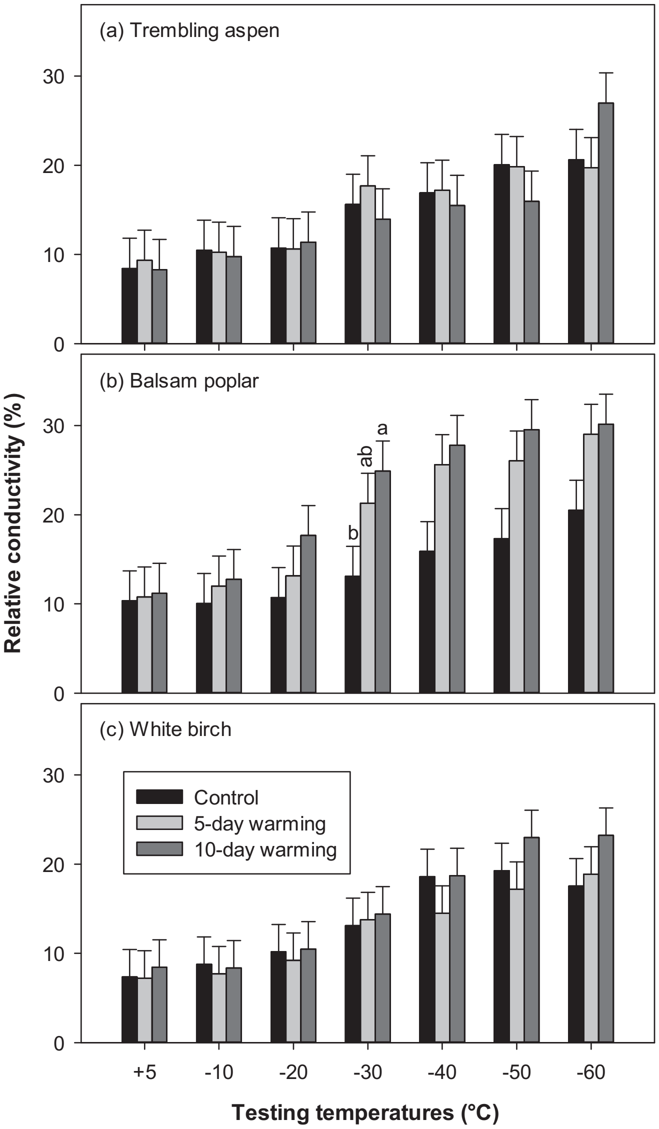
[a] Measured after freezing tests at different temperatures by seasonal period, species, and warming treatment.

[b] By species, seasonal period, and warming treatment.

Changes in seedling cold hardiness after warming were evaluated using freeze-induced electrolyte leakage measured using electrical conductivity of water in which stem tissues from 6 seedlings were immersed after freezing. Increased electrical conductivity indicates freezing damage (Colombo et al. 1984). Stem segments were frozen to 1 of 7 test temperatures, +5 (nonfreezing), –10, –20, –30, –40, –50, and –60 °C. Tests were conducted immediately after warming. To determine if seedlings regained hardiness that may have been lost during warming treatments, freezing tests were repeated 10 days after seedlings were returned to the outdoor environment during the winter and spring warming periods; seedlings were not retested after fall warming because relative conductivity was generally not significantly different among the treatments.

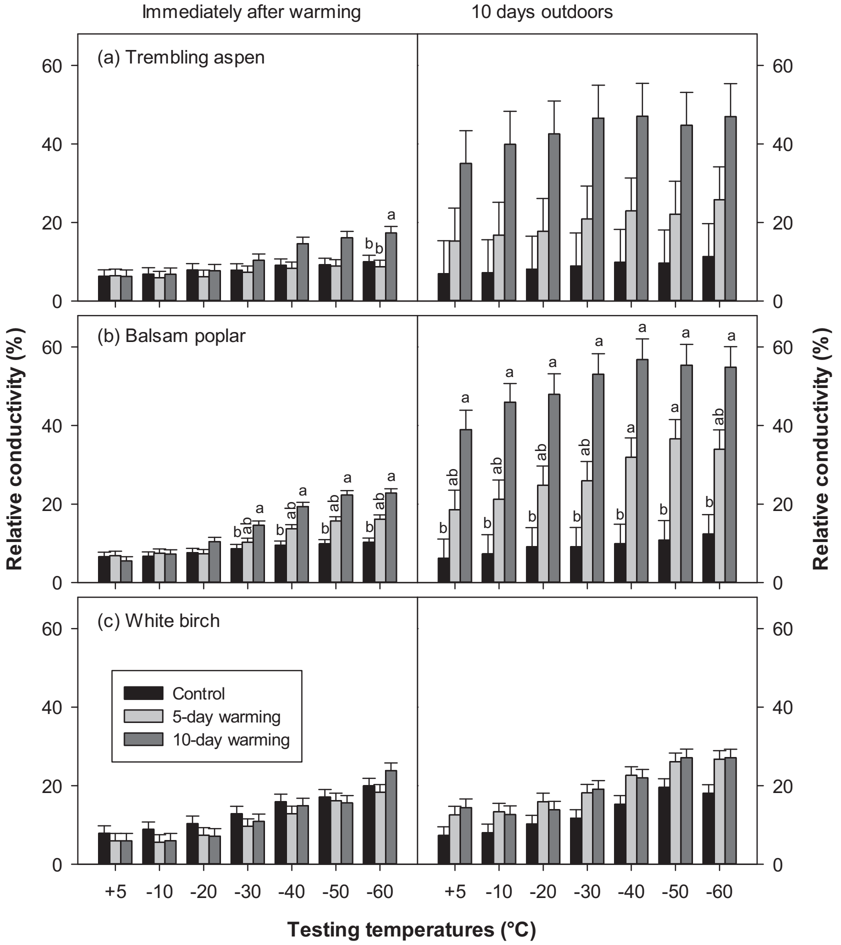
For each freezing test, a 7-cm-long twig collected from each of the 6 seedlings was kept at 5 °C overnight and then cut into 7 segments, each about 1 cm long. Stem segments were placed in 7 glass test tubes in a way that each tube had 6 shoot segments from 6 different seedlings and buds from 3 (trembling aspen and white birch) and 2 (balsam poplar) seedlings (2 buds were included per test for balsam poplar because of the number of shoots available). While the nonfreezing samples remained in the refrigerator at 5 °C for the duration of the freezing test, the samples to be frozen were transferred to a programmable freezer (Thermotron SM-32-C, Holland, Mich., USA). The temperature inside the freezer was reduced to 0 °C in the first hour, held there for 1 h for equilibration, and then lowered at a rate of 5 °C·h^{-1} between 0 °C and –20 °C and 10 °C·h^{-1} below –20 °C. Samples were removed from the freezer as soon as the shoot temperature, monitored with 2 thermocouples, reached the target value. Samples were thawed in a refrigerator at 5 °C overnight. After thawing, 20 mL of deionized water was added to each test tube, sufficient to immerse stem segments, and the tubes were mechanically shaken continuously for 24 h at 5 °C. The solution was measured to determine after-freezing electrical conductivity (ECF) at 20 °C using a Cole–Parmer conductivity meter (Cole–Parmer Instrument Company, Singapore). The solutions and samples were then placed in a hot water bath at 90 °C for 2 h, cooled to room temperature, and shaken at 5 °C for 24 h. The electrical conductivity after killing live tissues (ECK) was measured at 20 °C to calculate relative conductivity (Colombo et al. 1984).

Fig. 1. Relative conductivity (least square means ± SE) (measured after freezing tests at different temperatures) of trembling aspen (*a*), balsam poplar (*b*), and white birch (*c*) immediately after warming in the fall by warming treatments and testing temperatures. Means with different letters at a specific testing temperature differ significantly at 0.05.



Spring budbreak of the experimental seedlings after warming treatments was examined twice a day from late April; those not flushing by June 10 were considered dead (confirmed by cambium

Fig. 2. Relative conductivity (least square means ± SE) (measured after freezing tests at different temperatures) of trembling aspen (*a*), balsam poplar (*b*), and white birch (*c*) in the winter, immediately after warming (left), and 10 days after being returned to ambient environment (outdoors) (right). Means with different letters at a specific testing temperature differ significantly at 0.05.



discolouration). Time to budbreak of live seedlings was determined as thermal sum of cumulative growing degree hours above the 0 °C threshold (Snyder et al. 1999; Man and Lu 2010) since the beginning of the year (Colombo 1998) using Environment Canada data for Sault Ste. Marie. The growing degree hours that occurred during experimental warming were included in the thermal sum calculation of winter and spring treatments. Stem dieback of live seedlings was determined by the proportional death of main stems at the end of budbreak.

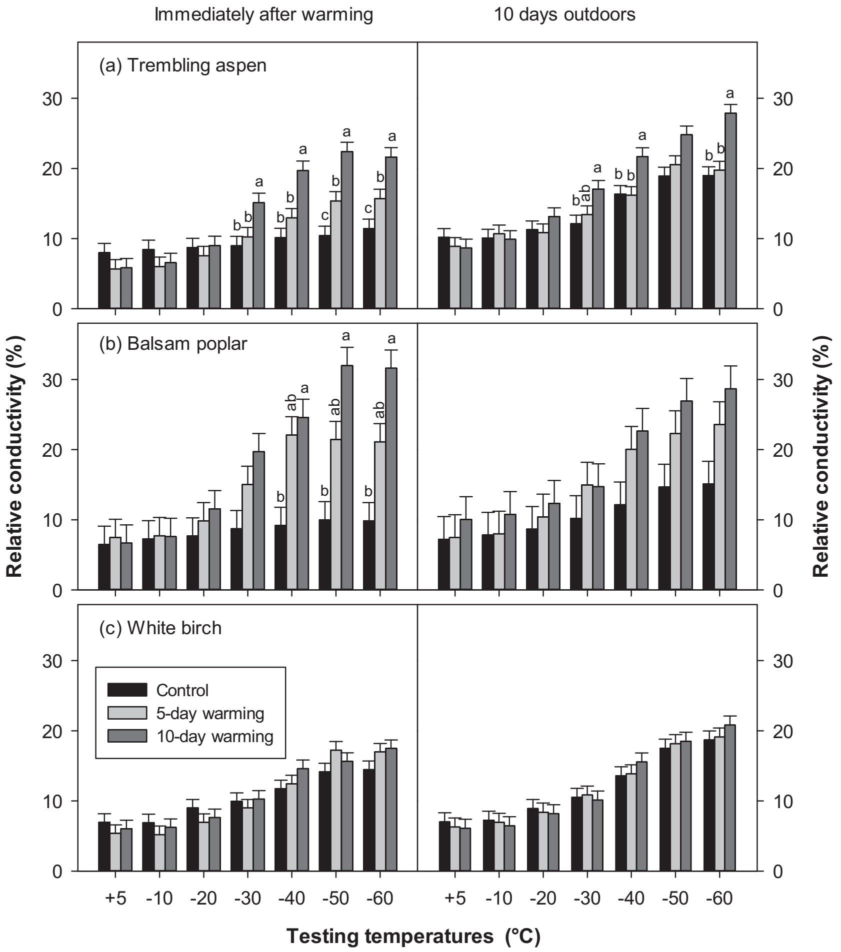
Cold hardiness during natural dehardening

In the second part of the study we tested cold hardiness of the seedlings from late winter to spring to determine natural dehardening. Seedling cold hardiness was assessed using 50 potted seedlings of each species kept outside over the 2012–2013 winter. The pots were sunk into the ground, level with the soil surface, to provide root temperatures similar to those of natural trees. Beginning in February 2013, shoot cold hardiness was periodically evaluated by visually assessing damage after shoot segments were exposed to 1 of 7 test temperatures, including a nonfreezing control (5 °C). Freezing temperatures varied with sampling date, ranging from −10 to −60 °C from February to early April, from −5 to −30 °C in late April, and from −2 to −12 °C in early May. On each assessment date, 7-cm shoot segments collected from individual seedlings were randomly assigned to each of 7 test tubes, such that each test tube contained either 4 shoot segments (for trembling aspen and white birch) or 2 segments for balsam poplar. After equilibrium at 5 °C overnight, one of the test tube remained at 5 °C and the rest were exposed to freezing temperatures in a programmable freezer with a cooling rate of 5 $°C \cdot h^{-1}$ before late April, followed by 2 $°C \cdot h^{-1}$ closer to the time of budbreak. After the freezing test, stem segments were thawed at 5 °C overnight and re-cut under water to reduce the risk of xylem embolism, which may affect tissue vigour and budbreak timing. Each test tube was then filled with 20 mL water and the top sealed with transparent plastic film to maintain high humidity during incubation in a growth chamber at 20 °C (day)/10 °C (night) and the 12-h photoperiod at 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD. Shoot segments were checked for budbreak twice daily. Segments were considered damaged when the thermal sum for budbreak (cumulative growing degree hours) was 50% greater than that of the nonfrozen control and were considered dead if buds did not open and the cambium turned black within 20 days. The cold hardiness of seedlings during and shortly after budbreak in May was determined from tissue discoloration after exposure to freezing temperatures as described above.

Statistical analyses

Analysis of cold hardiness after warming followed a split plot design with test temperatures nested within warming treatments for each species and seasonal period. Stem dieback (%), mortality (%), and time to budbreak based on thermal sum (cumulative growing degree hours) were analyzed using a 2-way factorial ANOVA with 3 warming treatments, 3 seasonal periods, and 3 replications. Time to budbreak of individual trees was averaged by warming treatment, seasonal period, and replication before sta-

Fig. 3. Relative conductivity (least square means ± SE) (measured after freezing tests at different temperatures) of trembling aspen (*a*), balsam poplar (*b*), and white birch (*c*) in the spring, immediately after warming (left), and 10 days after being returned to ambient environment (outdoors) (right). Means with different letters at a specific testing temperature differ significantly at 0.05.



tistical analysis. Normality was checked with graphical display and Shapiro–Wilks test on residuals. To achieve normality, relative conductivity data were log transformed, time to budbreak data were ranked using Proc Rank in SAS version 9.3, and square root transformation was applied to stem dieback data before statistical analyses. Multiple contrasts were conducted to examine differences among warming treatments at specific test temperatures or seasonal periods.

Results

Effects of warming on cold hardiness

A general increase in average relative conductivity by warming treatment was only observed in balsam poplar after fall warming (Table 1; Fig. 1; significant differences were detected only between the control and 10-day warming treatment at –30 °C). Immediately after warming treatments applied in winter and spring, relative conductivity increased significantly in trembling aspen and balsam poplar seedlings after freezing at test temperatures below –30 °C (Table 1; Figs. 2 and 3). For white birch, warming did not significantly increase relative conductivity after freezing in any seasonal period (fall, winter, or spring), even when seedlings were subject to 10 days of warming (Table 1; Figs. 1, 2, and 3). Relative conductivity increased markedly in aspen and balsam poplar seedlings moved outdoors in January after 5 and 10 days of winter warming, likely indicating damage that resulted from freezing after they were moved outdoors, a response not seen in seedlings moved outside in March (Table 1; Figs. 2 and 3).

Effects of warming on budbreak and mortality

On average, time to budbreak in the nonwarmed control varied little among species, ranging from 2567 cumulative growing degree hours (>0 °C) in balsam poplar to 2888 in trembling aspen and 2953 in white birch. Warming in winter delayed time to budbreak in all species compared with trees not receiving the warming treatments (Table 1; Fig. 4). The same was true for spring warming of trembling aspen and balsam poplar seedlings, but spring warming did not significantly delay white birch budbreak.

Overall, 9% mortality was observed in balsam poplar, occurring primarily in seedlings that received a warming treatment, especially warming during the winter, even though the overall treatment effect was not significant ($p = 0.084$). Mortality in trembling aspen and white birch seedlings was negligible (<1%). Among the surviving trees, stem dieback was greater for trembling aspen and balsam poplar (9%) than for white birch (4%), occurring mainly in warming treatments applied during winter, with significant seasonal period by warming interactions (Table 1).

Cold hardiness during natural dehardening

Trembling aspen and white birch seedlings were not damaged by temperatures as low as –60 °C until 1 April, by which time about 20% of cumulative growing degree hours required for these

Fig. 4. Time to budbreak in spring (least square means ± SE, measured by cumulative growing degree hours (>0 °C)) of (*a*) trembling aspen, (*b*) balsam poplar, and (*c*) white birch by seasonal period and warming treatment. Means with different letters at a specific seasonal period differ significantly at 0.05.

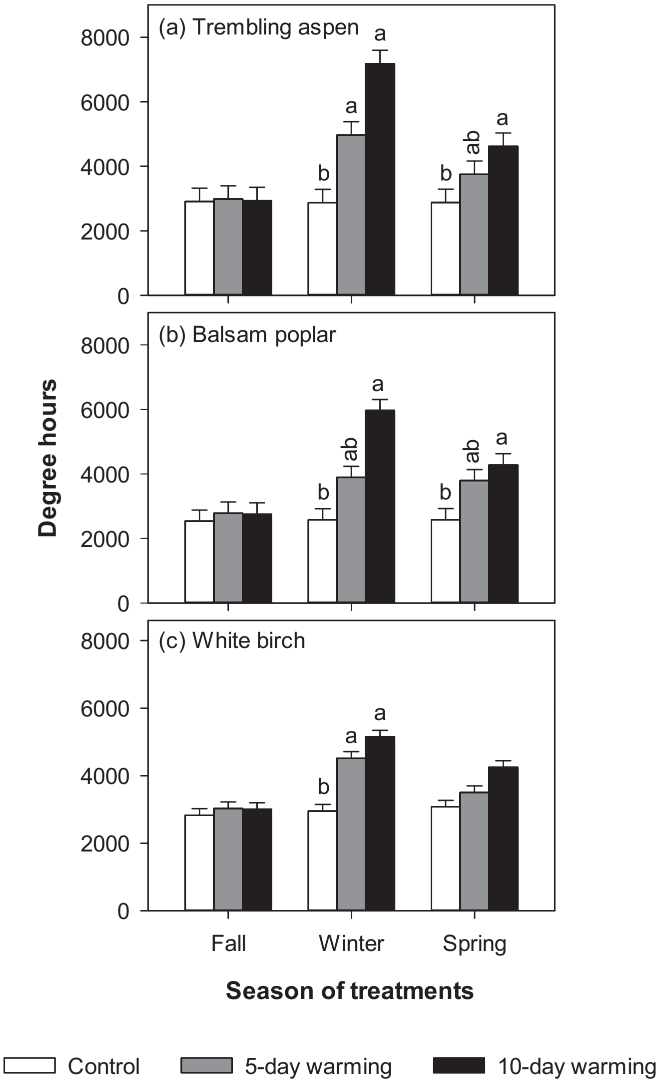
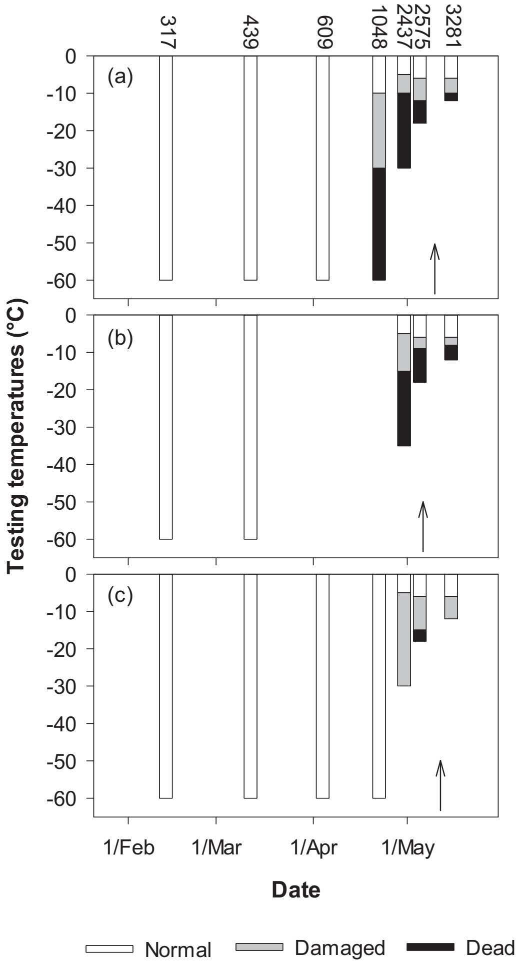


Fig. 5. Cold hardiness as indicated by level of tissue damage (white–normal, grey–damaged, and black–dead) for trembling aspen (*a*), balsam poplar (*b*), and white birch (*c*) shoots during natural dehardening. Numbers above bars indicate cumulative growing degree hours (>0 °C) at each assessment date. At a given testing temperature shoot segments were considered damaged when time to budbreak was 50% greater than that of shoots held in a nonfreezing environment (+5 °C). Balsam poplar was not assessed in April because of the lack of suitable plant material. Arrows indicate the time of budbreak.



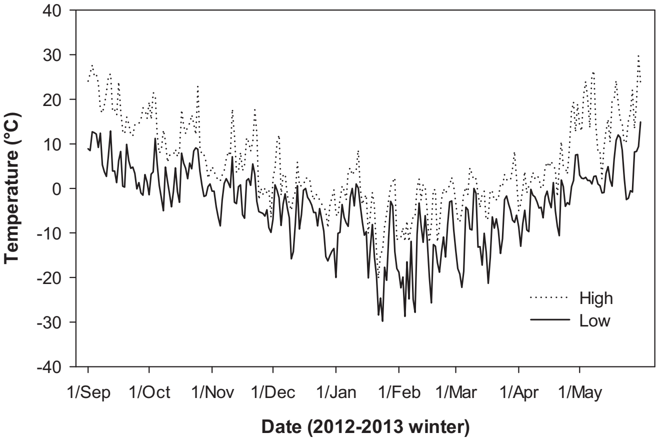
species to break bud had been received (Fig. 5; balsam poplar was not assessed in April because of lack of available shoots). After a further 2 weeks, by which time an additional 15% of the degree hours needed for budbreak were received, white birch was still hardy to −60 °C, while cold hardiness of trembling aspen had decreased substantially, as shown by the levels of shoot damage and mortality. Further accumulation of growing degree hours in late April brought all species close to budbreak and reduced cold hardiness to −4 to −6 °C. White birch seedlings were more tolerant of freezing than trembling aspen and balsam poplar both during natural dehardening and after budbreak (Fig. 5).

Discussion

Significant species differences were observed in response to a period of warming in winter months. White birch was consistently less responsive to warming than trembling aspen and balsam poplar, consistent with field observations by Cayford et al. (1959) after a winter freezing damage event in central Canada in 1958. The lack of response of white birch to winter warming may be partially due to its slightly greater thermal requirement for budbreak (the untreated control differed significantly between birch and balsam poplar, but not between aspen and birch, see Fig. 4), slower rate of dehardening in the spring (Fig. 5), or sensitivity of budbreak to photoperiod (Hawkins and Dhar 2012). In a separate experiment, white birch was observed to take longer to break its buds than trembling aspen and balsam poplar (Man et al. 2013*b*). Among the 3 species, balsam poplar was most sensitive to warming, likely because of its lower thermal requirement for budbreak.

As expected, the responses of boreal broadleaves to warming were much less in the fall than in the winter or spring (before and after fulfilment of chilling requirements). The general response pattern in cold hardiness, however, was similar among seasonal periods. Surprisingly, warming in the winter and early spring did not lead to earlier budbreak, which would be expected if growing degree hours are cumulative after chilling requirements are met (Colombo 1998; Hannerz 1999; Tanja et al. 2003; Søgaard et al. 2008); instead, the thermal sum required for budbreak was higher after winter and spring warming. A possible reason was that the trees dehardened by warming were damaged by cold after seed-

Fig. 6. Daily (high and low) temperatures for Sault Ste Marie, Ontario, Canada, from September 2012 to May 2013 (source: Environment Canada online archive at http://climate.weather.gc.ca).



lings were moved outside after the warming treatment, because of reduction of cold hardiness as indicated by the changes in relative conductivity after warming (Figs. 2 and 3). Combined with freezing temperatures in the postwarming ambient environment (–30 °C in winter and –15 °C in spring warming, see Fig. 6), this reduction resulted in increased mortality and stem dieback. Natural occurrences of winter warming-induced damage may have occurred recently over large areas of the boreal forest (Man et al. 2009, 2013*a*).

Given the broadleaf responses to warming and their thermal requirements for budbreak, we believe that broadleaves would have been damaged during the recent warming–freezing events in the boreal forest that caused wide spread damage to conifers (Man et al. 2009, 2013*a*). The average time to budbreak in the nonwarmed control of the 3 broadleaf species was close to 50% to 60% of the values found for white spruce (Man and Lu, 2010), which is one of early flushing boreal conifers (O'Reilly and Parker 1982; Man et al. 2009). According to our results, postwarming freezing damage varies not only with warming (dehardening) level and species, but also with season (i.e., temperature environment). In our study, the postwarming winter period was colder and had larger changes in temperature when seedlings were returned to the outdoor ambient environment than the fall and spring periods (from +5 to –12 °C in winter vs. +16 to +6 °C in fall and +5 to –3 °C in spring).

Compared with winter warming, seedlings showed some recovery of cold hardiness following spring warming (as shown by the reduced differences in relative conductivity among treatments after 10 days outdoors) when postwarming ambient temperatures were less damaging. Dehardened seedlings may better recover cold hardiness lost to warming if temperatures are milder following warming treatment (Kalberer and Arora 2007; Kalberer et al. 2007).

The results of this study have 2 major implications. First, winter and spring warming can deharden boreal broadleaf species, predisposing them to freezing damage in subsequent cold periods. Among the 3 broadleaf species we examined, white birch was most resistant to warming and balsam poplar was least resistant. If the incidence of mid-winter warming increases in nature, white birch may be better able to avoid dehardening and winter freezing damage than trembling aspen and balsam poplar. Second, our quantitative data on thermal sum for budbreak, response to warming, and natural dehardening can be used in studies requiring such information for budbreak predictions and phenological modelling of these species. Furthermore, the delayed budbreak caused by freezing temperatures after warming suggests that winter dehardening should be considered in future efforts to refine budbreak and phenology models, especially for a climate with increasing temperature variability (Schär et al. 2004; Rigby and Porporato 2008), as frosts have been found to delay budbreak and affect modelling accuracy (Bailey and Harrington 2006).

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