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Research paper

Increased exposure to chilling advances the time to budburst in North American tree species

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The phenology of trees is highly susceptible to changing global temperatures. Leaf budburst advances with increasing spring temperatures, but can also be delayed when warmer winters reduce chilling exposure. Results from long-term observations show that increasing temperatures have triggered advanced budburst in the past decades, but some studies also show that budburst advance has slowed recently. Here, we conducted an experiment with five temperate deciduous tree species (*Acer rubrum* L., *Larix laricina* (Du Rois) Koch, *Populus tremuloides* Michx., *Quercus ellipsoidalis* E. J. Hill, *Betula papyrifera* Marsh.) and one invasive species (*Rhamnus cathartica* L.) in Minnesota, USA, to assess the impact of chilling on the timing of leaf budburst. We collected twigs over two winter seasons (2011/2012 and 2012/2013) on a biweekly basis and exposed them to spring-like temperatures of 21 °C/16 °C day and night, long day photoperiod (16 h). We found a significant relationship of advanced budbreak with increased chilling for all species tested ($P < 0.001$) and significant differences in the timing to budburst among all species ($P < 0.001$). *Acer rubrum* responded strongly to chilling, showing a very steep linear decline in days to budburst with increased exposure to chilling. On the other end of the spectrum, *L. laricina* responded least to increases in chilling. These results suggest that rising global temperatures will likely have diverse impacts on tree species with potential implications for species interactions such as competition.

Keywords: climate change, dormancy, North America, phenology, twig experiment.

Introduction

The annual development (phenology) of temperate trees, such as budbreak, flowering and fruiting, is an important component of the tree's fitness, realized niche and geographic range limits (Chuine and Beaubien 2001, Morin et al. 2009, Chuine 2010). Because temperature plays a major role in tree phenology, rising global temperatures are predicted to have a significant influence on the fitness and distribution of tree species (Chuine and Beaubien 2001, Cleland et al. 2007, Morin et al. 2009).

Tree budburst is a commonly studied phenophase that is activated by chemical changes within the shoot apical meristem (Rinne et al. 2001). In temperate regions, these biochemical processes are mainly stimulated by three external triggers: the length of daylight (photoperiod), chilling temperatures and spring forcing temperatures (Myking and Heide 1995, Körner and Basler 2010, Basler and Körner 2012, Laube et al. 2014).

Photoperiod seems to play a subordinate role in releasing dormancy and inducing budburst, although the importance of this cue likely varies among species (Basler and Körner 2012, Laube et al. 2014). In contrast, it is widely agreed that chilling (exposure to cold temperatures) and forcing (exposure to warm temperatures) are dominant triggers in the timing of budburst for many temperate trees (Myking and Heide 1995, Bennie et al. 2009, Polgar and Primack 2011, Laube et al. 2014). After the initiation of dormancy, chilling temperatures act as a cue that supports the increase of frost-hardiness in buds. Additionally, the accumulation of chilling units activates the movement of buds out of dormancy (Landsberg 1974, Hänninen 1990, 1996). According to the 'sequential' chilling model, the accumulation of forcing units will be initiated only after rest completion, i.e., the fulfillment of a specific chilling requirement (Sarvas 1972, Richardson et al. 1974, Hänninen and Kramer 2007),

while the 'parallel' model posits that chilling and forcing units can be accumulated simultaneously (Laube et al. 2014, Harrington and Gould 2015, Ford et al. 2016), and that the breaking of dormancy is a more dynamic process (Landsberg 1974, Cannell and Smith 1983). There are also several hybrid models (e.g., Hänninen 1990, 2016).

Common conventions exist for measuring the exposure of buds to temperature. Forcing is commonly measured in units of growing degree days (GDDs), the accumulated average daily temperature above a base threshold, that activates bud elongation and growth after dormancy release in the spring (Lechowicz 1984, Myking and Heide 1995). These forcing units or GDDs are also called thermal time. Chilling units are commonly measured as the number of days or hours a plant is exposed to cold temperatures below a threshold or within a temperature range as it prepares to end dormancy (Murray et al. 1989, Heide 1993, Caffarra and Donnelly 2011). The exact threshold and range at which chilling can be sensed by the buds has not been determined for most temperate forest tree species. While some argue that optimum chilling temperatures for temperate trees are typically above freezing (Heide 1993, Arora et al. 2003, Harrington and Gould 2015), much research is based on chilling ranges that include all temperatures below a threshold (Murray et al. 1989, Ashby et al. 1991, Wilson et al. 2002, Hawkings and Dhar 2012, Laube et al. 2014). Additionally, it is well established that species differ in their requirement for chilling, which has been attributed to successional status (Basler and Körner 2012, Laube et al. 2014), provenance or range (Thomas and Vince-Prue 1997, Harrington and Gould 2015), invasiveness (Laube et al. 2014) and differences in xylem properties (Lechowicz 1984).

Many experiments have demonstrated that a rise in temperatures leads to accelerated budburst (e.g., Heide 1993, Bradley et al. 1999, Caffarra and Donnelly 2011, Laube et al. 2014). However, species that require large amounts of mid-winter chilling to break bud ('high chill species') have shown a delay in budburst when winter temperatures are elevated and chilling requirements are met more slowly (Murray et al. 1989, Heide 1993, 2003, Myking and Heide 1995, Cleland et al. 2007). It is, therefore, reasonable to assume that global climate change may not result in linear advances in bud burst, but potentially in a delay for high chill species, if winter temperatures continue to increase as predicted (Morin et al. 2009, Schwartz and Hanes 2010, Pope et al. 2013). Such species might, therefore, experience a reduced growing season, putting them at a competitive disadvantage (Cannell and Smith 1986, Murray et al. 1989, Caffarra and Donnelly 2011). Reduced chilling, especially at southern margins, can also lead to abnormal leaf development with negative effects on productivity and survival (Morin et al. 2009, Harrington and Gould 2015). Long-term studies seem to support the theory of delayed budburst with reduced chilling. While the majority of such studies validate that increases in

global temperature have resulted in earlier flowering (Beaubien and Freeland 2000, Menzel 2001, Parmesan and Yohe 2003, van Vliet et al. 2003, Primack et al. 2009), there is new evidence that this initial trend towards earlier budburst has slowed down in the most recent decade in Northern latitudes (Yu et al. 2010, Jeong et al. 2011, Piao et al. 2011, Fu et al. 2014, 2015). Even though the exact mechanisms are not known, a lack of chilling has been suggested (Fu et al. 2015).

On the other hand, species with small mid-winter chilling requirements ('low chill species') might be better able to track climate change by leafing out earlier, potentially extending their growing season (Menzel 2001, Menzel et al. 2006, Schwartz et al. 2006, Bennie et al. 2009), but face an increased risk of frost damage (Cannell and Smith 1986, Hänninen 1991, 1996, Heide 1993) if the onset of budburst advances at a faster rate than the beginning of the frost-free season (Schwartz et al. 2006). However, new tissue of early flushing species has been shown to be more freeze-tolerant than that of late flushers (Vitasse et al. 2014a). While some studies found an increase in frost damage in the USA with global warming (Augspurger 2013), others suggest that for most species, frost damage will not be a common phenomenon (Morin and Chuine 2014, Ford et al. 2016). Schwartz et al. (2006) also found that, while both the date of budburst and the last frost have been earlier in North America, the relative rate of change was not the same for all areas, which complicates predictions.

The effect of chilling on tree phenology and its consequences for woody species has been studied extensively in Europe (c.f. Murray et al. 1989, Hänninen 1991, Myking and Heide 1995, Menzel 2002, Menzel et al. 2006, Ghelardini et al. 2009, Caffarra and Donnelly 2011, Basler and Körner 2012, Laube et al. 2014), but studies in North America are less common. There are some long-term phenological studies (e.g., Bradley et al. 1999, Beaubien and Freeland 2000, Augspurger 2013) and some experimental studies (e.g., Campbell and Sugano 1979, Ashby et al. 1991, Norby et al. 2003), and a fast-growing body of model-fitting approaches (e.g., Richardson et al. 1974, 2006, 2012, Schwartz et al. 2006, Morin et al. 2009, Clark et al. 2014a, 2014b, Morin and Chuine 2014, Harrington and Gould 2015, Ford et al. 2016). Despite this growing amount of research, there are still gaps in our knowledge of species-specific or even genotype-specific requirements for budburst, specifically how chilling requirements compare among Northern American tree species, and how increased chilling influences the timing of budburst.

Twig experiments offer the opportunity to isolate factors that influence budburst, such as chilling or forcing, and are increasingly used by scientists (Heide 1993, Laube et al. 2014), but we are aware of few such studies in North America (Miller-Rushing and Primack 2008, Polgar et al. 2013). Here, we conducted a twig experiment to examine the role of mid-to late winter chilling in the timing of bud burst for five native tree species from

Minnesota, USA, and one invasive species. We hypothesized that increased exposure to chilling would accelerate budburst for all species and reduce the thermal time to budburst. Slower budbreak was expected at initiation of the experiment because more forcing is required under low chilling. We also hypothesized that the time to budburst would differ among species.

Materials and methods

We conducted a greenhouse chilling experiment over two winter seasons (2011/2012 and 2012/2013) with twigs of adult tree samples (adapted from Heide 1993) from the Cedar Creek Ecosystem Science Reserve (CCESR) outside of East Bethel, Minnesota (N43°38'19.39", W116°14'28.86"). The climate at Cedar Creek is continental, with mean daily temperatures of −10 °C in the coldest (January) and 22 °C in the warmest month (July) (Data from 1 January 1985 to 31 December 2014). Average annual precipitation had a range of 59.2–116 cm between 1984 and 2014. This experiment was conducted over 2 years largely because we were interested in repeatability, i.e., if the general pattern of advanced budbreak with increased chilling would be the same for both years.

Suitability of twigs in phenological experiments

The use of twig cuttings or seedlings as proxies for adult trees has been widely established (Heide 1993, Basler and Körner 2012, Polgar et al. 2013, Laube et al. 2014), and is an easy and inexpensive way to isolate and study drivers of leaf phenology (Primack et al. 2015). Some studies have shown that the phenological development of seedlings can differ significantly from adult trees of the same species, complicating inferences (Augspurger and Bartlett 2003, Vitasse 2013). In contrast, the suitability of twig cuttings from adult trees for studies of tree phenology has been questioned because of their disconnection from hormonal signals from the parent plant. While it has been demonstrated that root temperature can have an influence on the timing of budburst in *Betula papyrifera* (Hawkings and Dhar 2012), triggers involved in dormancy and dormancy release, such as chilling, are targeted directly at the level of the bud apical meristem. Consequently, chemical changes that allow a bud to respond to forcing temperatures or daylengths are localized and integral to the bud (Metzger 1996, Rinne et al. 2001, Arora et al. 2003).

We are aware of only one study that directly compared the bud phenology of parent trees with that of their cuttings, including one species of the genus *Acer*. The authors found that the cuttings, which were kept in water under similar field conditions, reacted in a similar way phenologically to their parents, and they concluded that cuttings are better suited as surrogates in warming experiments than seedlings (Vitasse et al. 2014a, 2014b).

Year 1 We harvested twigs of four temperate tree species (*Acer rubrum*, *Larix laricina*, *Populus tremuloides*, *Quercus ellipsoidalis*)

within a small sampling area (<30 acres) on three dates between 2 February and 6 March of 2012. One dormant twig per tree was harvested on each collection date ($n = 5$ trees per species) for a total of 60 samples. Each twig had a minimum of six live buds and a length of at least 30 cm. We carefully examined all the twigs to ensure that each had a green tissue ring at the cut end, indicating they were alive and had the potential to break bud. Immediately after cutting, we put the twigs in an oversized cooler filled with snow and transported them to a greenhouse within 1.5 h. Once in the greenhouse, we immediately placed the twigs into 19-liter buckets (five twigs per bucket), filled with around 3 l of tap water each. To minimize obstruction of the xylem and to provide fresh water, we trimmed the end of the twigs, scrubbed the buckets, and changed the water on a weekly basis.

The greenhouse was kept at temperatures approximately simulating mid to late spring conditions for Minnesota: 21 °C day/16 °C night. We chose a constant long and late-season photoperiod (16 h) throughout the entire period of flushing to isolate the effect of forcing and chilling, and to exclude the effect of photoperiod on budbreak (see the methods of Polgar and Primack 2011). Because of large ambient temperature fluctuations, especially in the later spring, there were slight greenhouse temperature variations (on average, the temperature variation was around 1 °C, but some hours in March had a small number of hours with fluctuations as high as 3.95 °C). We calculated the cumulative GDDs as follows:

$$\text{GDD} = (T_{\max} + T_{\min})/2 - T_{\text{base}}$$

where T stands for temperature. We assumed T_{base} to be 0 °C (based on Heide 1993) and used the minimum and maximum temperature settings of the greenhouse for T_{\max} and T_{\min} . Based on this, twigs did not experience any GDDs prior to twig collection. Chilling hours were defined as the number of hourly temperature readings below 5 °C, starting November 1 when trees were assumed to be dormant (based on Murray et al. 1989). The choice of chilling threshold did not qualitatively change results. To calculate the amount of accumulated chilling hours prior to each twig collection, we used average hourly temperature data from the CCESR.

Phenological data was collected three times per week on all branches. We defined budbreak as a green leaf tip becoming visible at the end of the bud, but before the unfolding of a first leaf (USA-NPN). We are aware of studies that show a stronger effect of chilling on apical, compared with lateral buds (e.g., Falusi and Calamassi 1990). For this study, we assumed that all buds were in the same state of dormancy during collection, and we made the general observation that as soon as one bud broke, most other buds on the same twig were either breaking or close to breaking. In Year 1, branches that failed to break bud after 8 weeks were discarded. In both years, live branches were

removed from the experiment as soon as at least one of the buds had broken, or when the twigs were dead (see Table S1 available as Supplementary Data at *Tree Physiology* Online). Twigs were considered dead when they appeared dried out or no green tissue was present at the cut end. In both years, the experiment was terminated when outside temperatures began to regularly exceed the GDD threshold. As we were testing for the effect of chilling, we wanted to be able to control the amount and pattern of forcing temperatures.

Year 2 In the winter of 2012/2013, we repeated the sampling method and the greenhouse treatment, but with some adjustments: first, we increased the number of donor trees ($n = 10$ trees per species); second, two species were added (*B. papyrifera* and the Eurasian invasive shrub *Rhamnus cathartica*); third, twigs were harvest biweekly between 19 December 2012 and 28 March 2013, for a total of eight collections; finally, twigs were not discarded automatically after 8 weeks of observation. Instead, all twigs were kept until they either broke bud or were considered dead. Table 1 summarizes the accumulated chill hours for twigs from each collection during the 2 years of the project.

To explore how our results may have been affected by our definition of a chill hour ($<5\text{ }^{\circ}\text{C}$), we compared the accumulated chilling hours under this definition with three alternative scenarios, in which chill hours would accumulate at (i) $<3\text{ }^{\circ}\text{C}$, (ii) $<1\text{ }^{\circ}\text{C}$ or (iii) between $0\text{ }^{\circ}\text{C}$ and $7\text{ }^{\circ}\text{C}$, starting November 1. We chose these temperature ranges arbitrarily, but loosely based on thresholds used across the literature. For example, Caffarra and Donnelly (2011) used a constant $3\text{ }^{\circ}\text{C}$ in their chilling experiment, and according to some models and publications, temperatures in the range between 0 and $7\text{ }^{\circ}\text{C}$ have a chilling effect on fruit trees (Weinberger 1950, Arora et al. 2003).

To place our experiment in the context of climate change effects on our study area, we also looked at the trends of annual chilling hours between 1989 and 2017.

Statistical analysis

The data were analyzed with JMP® 13.1 (SAS Institute Inc.) and all graphs were produced with the R statistical package (R 3.3.2,

Crawley 2007). All values are mean \pm standard deviation. Our analysis met the model assumption of normally distributed residuals.

To test the hypothesis that the amount of chilling had an accelerating effect on the time to budburst, we performed a linear mixed-model analysis with year as a random effect. We treated year as a random effect to incorporate variation caused by abiotic factors, such as available moisture and soil temperature, and because we were interested in overall patterns of chilling hours vs days to budbreak. The mixed-model was run with species and chill hours and their interaction as the fixed effects and the number of days to leaf budbreak as the response variable.

Chill-hours were calculated as hours $<5\text{ }^{\circ}\text{C}$ accumulated in the field since November 1 (Murray et al. 1989, Polgar and Primack 2011, Laube et al. 2014). Because of fixed temperature conditions in the greenhouse, GDDs represent a transformation of the number of days to budbreak. Results are similar regardless of response variable, so we chose to present results with days to budbreak as the response variable.

Results

We found that species ($F_{5,463} = 193.24, P < 0.0001$), chilling hours ($F_{1,436} = 767.8514, P < 0.0001$) and their interaction ($F_{5,436} = 38.7976, P < 0.001$) were all significant predictors of leaf budburst. The adjusted R^2 value of the full model was 0.79. For all species, the number of cumulative chilling hours and the time to leaf budbreak were negatively associated, i.e., the more chilling hours the branches were exposed to, the less thermal time they required for budburst (Figure 1). The impact of chilling was most pronounced during the first collections, as can be observed in the sharp decline in the time to budburst for some species, especially *A. rubrum*. After short exposure to chilling, the twigs required a much longer thermal time to break bud than after large amounts of chilling.

The disparity in budburst timing among species was largely the result of differences in the slope of the chilling hour/budbreak relationship. The largest differences occurred with low levels of chilling and all converged towards similar numbers of days to budbreak after higher amounts of chilling (Figure 1).

Table 1. Cumulative chilling hours (average hourly temperature $<5\text{ }^{\circ}\text{C}$) for both winter seasons. Collections 1–3 in the winter 2011/2012 were paired with collections 4–6 from the winter 2012/2013 as they had comparable chilling hours. A graphic depiction of those data is shown in Figure 2.

2011/2012			2012/2013		
Collection	Cumulative chilling hours	Collection dates	Collection	Cumulative chilling hours	Collection dates
			1	945	19 December 2012
			2	1401	7 January 2013
			3	1665	18 January 2013
1	1924	2 February 2012	4	1977	31 January 2013
2	2252	16 February 2012	5	2313	14 February 2013
3	2682	6 March 2012	6	2649	28 February 2013
			7	2985	14 March 2013
			8	3308	28 March 2013

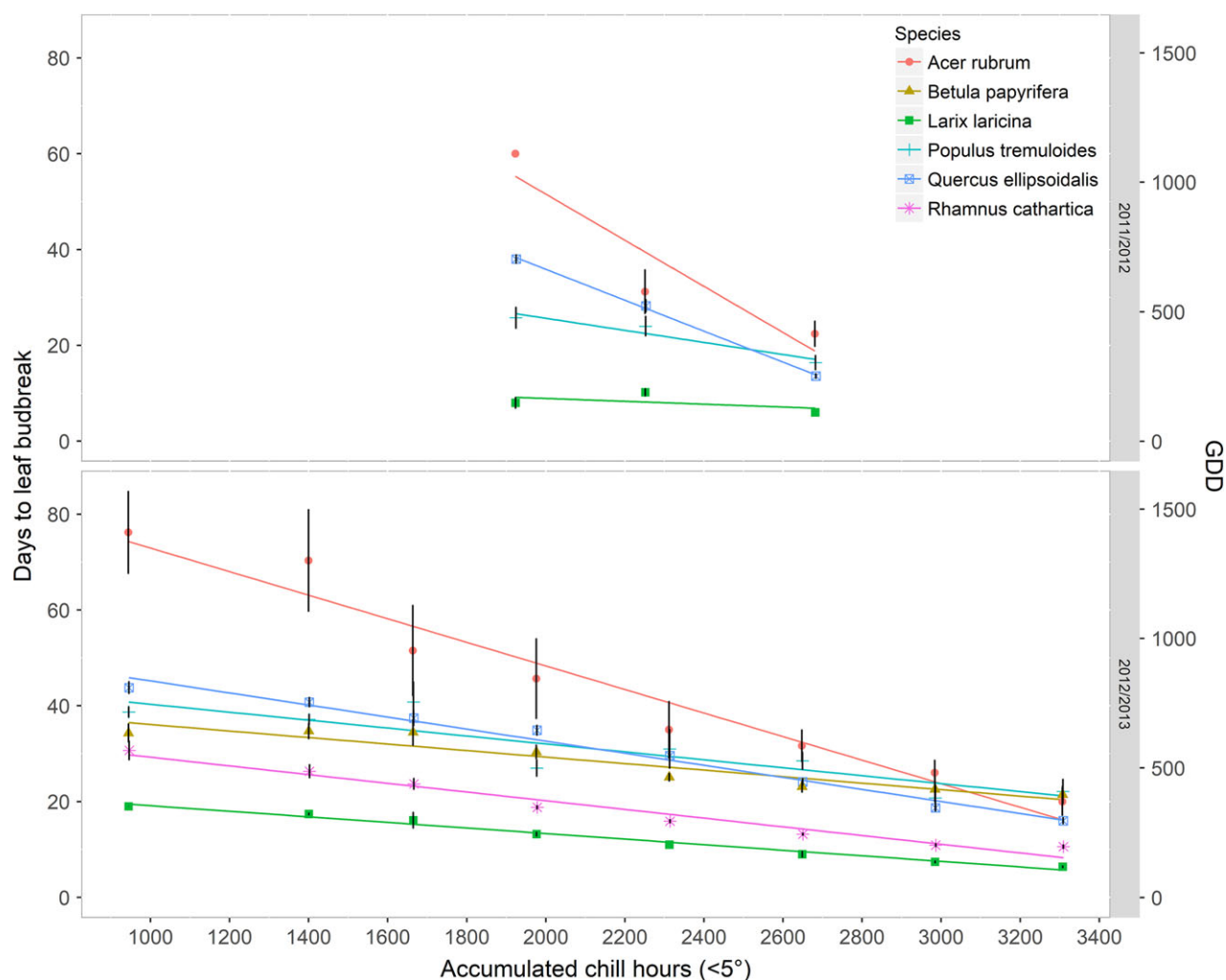


Figure 1. Time to leaf budbreak/thermal time (GDDs) plotted against accumulated ambient chilling hours (<5 °C, starting 1 November according to Murray et al. 1989) for two seasons (2011/2013 = Year 1, 2012/2013 = Year 2) at Cedar Creek, MN. Budbreak is defined as a green leaf tip becoming visible at the end of at least one bud. Points show the average days to leaf budbreak for each species and each collection, error bars show standard errors. The first y-axis shows the number of days in the greenhouse (21 °C /16 °C, 16 h photoperiod) required to break bud, the second y-axis shows thermal units (measured as GDDs). Data for both years were analyzed together.

Of all species, *A. rubrum* required the largest amount of thermal time to break bud, especially after a short exposure to chilling, and it responded most strongly to accumulations in chilling (Figures 1 and 2). For example, an increase of chilling from 945 to 3308 h decreased the requirement of thermal units from on average 1409.7 to only on average 370 GDDs, a 74% change (adj $R^2 = 0.65$, $P < 0.001$, $y = 100.39 - 6.86x$). On the other extreme, *L. laricina* responded fastest when exposed to thermal time among all species, even after short amounts of chilling (Figures 1 and 2). An increase of chilling from 945 to 3308 days led to on average 67% reduced need for thermal units (351.5 vs 118.4 GDDs) (adj $R^2 = 0.71$, $P < 0.0001$, $y = 24.47 - 0.88x$), but its thermal time requirements throughout the experiment were significantly lower than those of any other species. *Betula papyrifera* generally required more thermal time than *L. laricina* to break bud, but it showed the weakest response

of all species to increased chilling with 37.5% earlier budbreak (634.5 vs. 396.7 GDDs), which was still highly significant (adj $R^2 = 0.43$, $P < 0.001$, $y = 42.6 - 2.06x$). Finally, the response pattern of *R. cathartica* closely followed that of *L. laricina*, and in general the invasive species required the second to least amount of thermal time to budbreak after exposure to different amounts of chilling (adj $R^2 = 0.8$, $P < 0.001$, $y = 38.31 - 1.14x$).

Species differed in the percentage of branches that broke bud throughout the experiment (see Table S1 available as Supplementary Data at *Tree Physiology Online*). *Rhamnus cathartica* and *L. laricina* had the highest budbreak rate at 90–100% for all collections, while the budbreak rate for *A. rubrum* was as low as 20%. The fact that as early as 19 December (Year 2) most twigs had the capacity to develop leaves when forced suggests that exposure to chilling had initiated the progressive release of dormancy to a point where budbreak was possible already before our experiment was

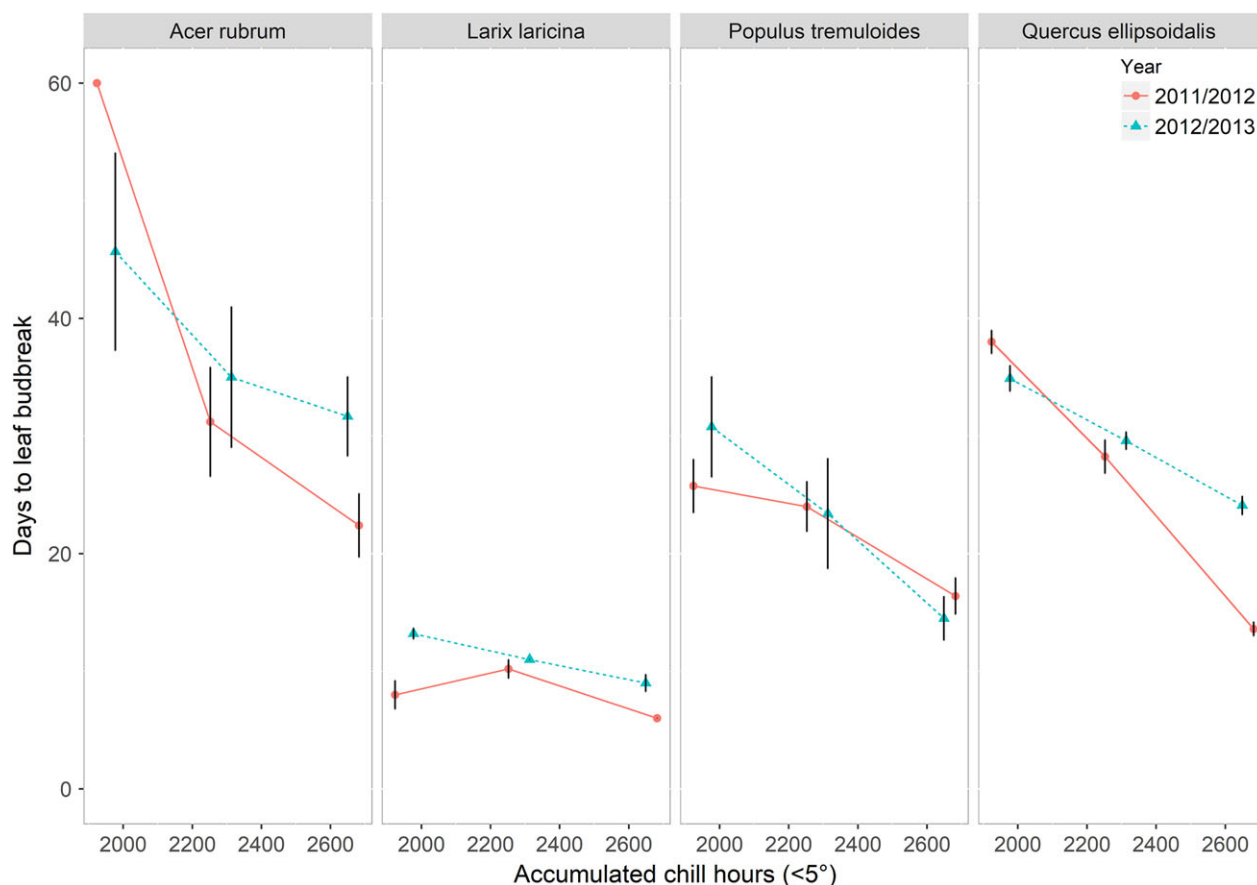


Figure 2. Comparison of days to budbreak with increased chilling between Year 1 (2011/2012) and Year 2 (2012/2013) for four overlapping species (*Acer rubrum*, *Quercus ellipsoidalis*, *Larix laricina*, *Populus tremuloides*). Points show the average days to leaf budbreak for each collection, bars are standard error bars. Chilling hours were accumulated starting November 1 of each year.

implemented (Landsberg 1974, Cannell and Smith 1983). The low budbreak rate of *A. rubrum* for most collections might suggest that *Acer* was prone to phloem or xylem damage during collection. On the other hand, on average 48% of the discarded *A. rubrum* twigs in Year 2 achieved flower budbreak prior to removal (unpublished results).

To see if changes in temperature have already started to affect our study site, we used hourly temperature data from the CCESR (Cedar Creek Ecosystem Science Reserve) and the WRCC (Western Regional Climate Center) to calculate the number of accumulated winter chill hours below 5 °C between 1989 and 2017, starting November 1 (Figure 3), and found that in the last 28 years, Cedar Creek has lost on average 12.7 chill hours annually. Even though this change is not statistically significant, and, due to the high scatter the adjusted R^2 value is very low (0.04), there is still an observable trend, which over time could influence the phenology of species at the site.

Our results are based on the supposition that all the tested species were capable of sensing and accumulating chilling at all temperatures below 5 °C, which we used to explain the significant decline of time to budburst with increased chilling exposure. By

this assumption, chill hours increase linearly throughout the coldest winter months in both years. Figure 4 shows our measure of chill hours (<5 °C) ($F_{9,1} = 19.7$, $P = 0.01628$) compared with three alternative models, in which buds are assumed to accumulated chilling at <3 °C ($F_{9,1} = 22.92$, $P < 0.001$), <1 °C ($F_{9,1} = 39.19$, $P < 0.001$) and at a range of 0–7 °C (not significant). The graph depicts the accumulation of chilling hours for both years, starting with the first collection and ending with the last collection. The lines do not start at a base of zero as ambient chilling units have already been accumulated before the first collection.

If we assume that twigs could sense and accumulate chilling at <3 °C or <1 °C, the pattern of chilling accumulation would still be linear and closely follow that of the <5 °C scenario. However, the pattern in a model where buds only responded to chilling temperatures of above freezing (0–7 °C) would differ substantially. Here, chilling hours would have mainly accumulated at the beginning of the winter season from in November, but the average daily below-freezing temperatures between December and February would not have significantly contributed to the accumulation of chilling. In that model, a linear decline in days to budbreak occurs despite no change in accumulated chill days.

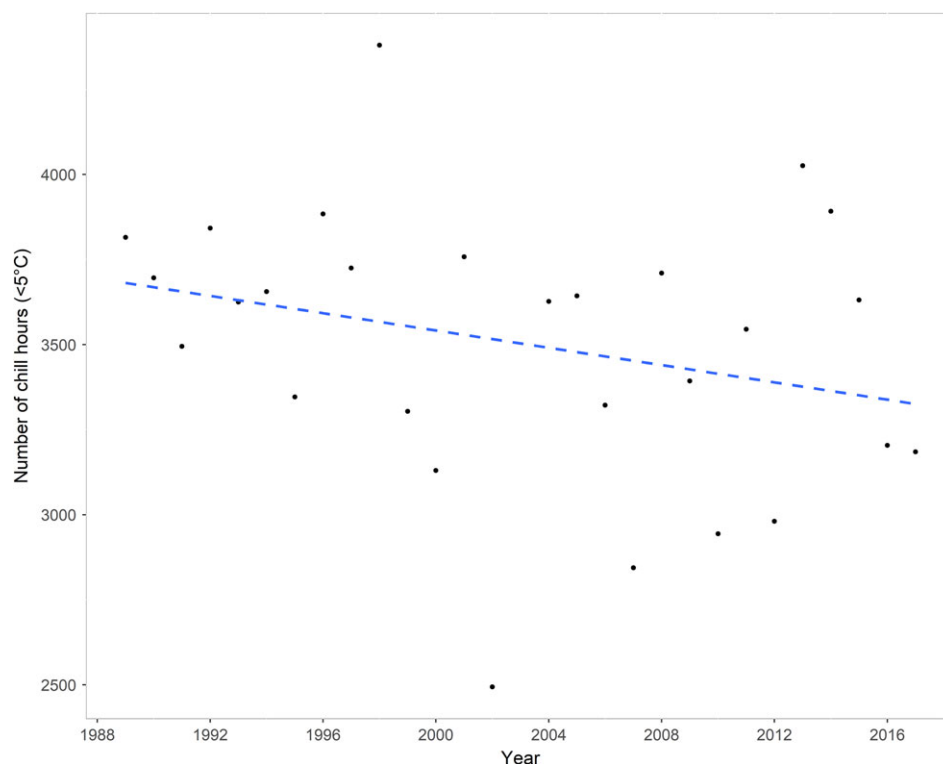


Figure 3. Accumulated chilling hours (<5 °C, 1 November–1 May), plotted by year between 1989 and 2017 at Cedar Creek, MN (data from 2003 was not available) ($y = 3694 - 12.7x$, adjusted $R^2 = 0.04$, not significant). Hourly temperature data were not available for earlier years. Hourly temperature data came from the CCSR, and missing hours were complemented with data from the Carlos Avery RAWS weather station.

Discussion

Increasing global temperatures have the potential to alter numerous aspects of ecological systems. One of the strongest signals of rising temperatures has been the change in plant phenology, especially spring budbreak and flowering. Much scientific work has focused on changing spring conditions, rather than the potential effect of warmer winters on phenology. Specifically, increasingly warm winters might influence the amount of winter chilling, potentially affecting timing of budbreak in high chill species. Our results confirm that chilling had a significant effect on the rate of budbreak for all species in our experiment, validating its crucial role in the phenology of temperate trees.

Species-specific responses to chilling

For all species in this experiment, increased chilling significantly reduced the amount of thermal time to reach budburst, and there were significant differences among the species. These differences were especially pronounced at the beginning of the experiment, but they became less strong with increased chilling.

Of all species, the low-chill *L. laricina* required the least amount of thermal time to break bud, regardless of chilling exposure, and it showed the weakest decrease in time to budburst between the first and last collection. These findings are in line with Harrington and Gould (2015). Consequently, *L. laricina* will most likely

experience earlier budbreak and potentially longer growing seasons with rising spring temperatures, possibly putting it at a competitive advantage over high-chill species (Polgar and Primack 2011). On the other hand, earlier budbreak might render it vulnerable to greater frost damage if advances in budbreak outpace advances in the beginning of the frost-free season (Cannell and Smith 1986).

On the other extreme, *A. rubrum* showed the strongest response to chilling and there was very steep reduction in thermal time to budbreak between the first and last collection. While *A. rubrum* twigs were initially much slower at developing leaves than other species, these differences became less substantial with increased chilling. The slow response of *A. rubrum* and some other species from the first collections suggests that chilling requirements were not yet fully met (Landsberg 1974). In the future, increasing winter temperatures in Minnesota could result in unfulfilled chilling requirements for genotypes of high-chill species, such as *A. rubrum*, potentially delaying instead of advancing budburst. As a consequence, *A. rubrum* might experience range contractions, especially in the south (see also Morin et al. 2008, 2009, Schwartz and Hanes 2010, Harrington and Gould 2015), and/or fail to benefit from the prolonged growing season compared with low-chill species, putting it at a competitive disadvantage. However, these assumptions need to be taken with care. For example, Marchin et al. (2015) found that after year-around warming of 5 °C, budburst was still advanced

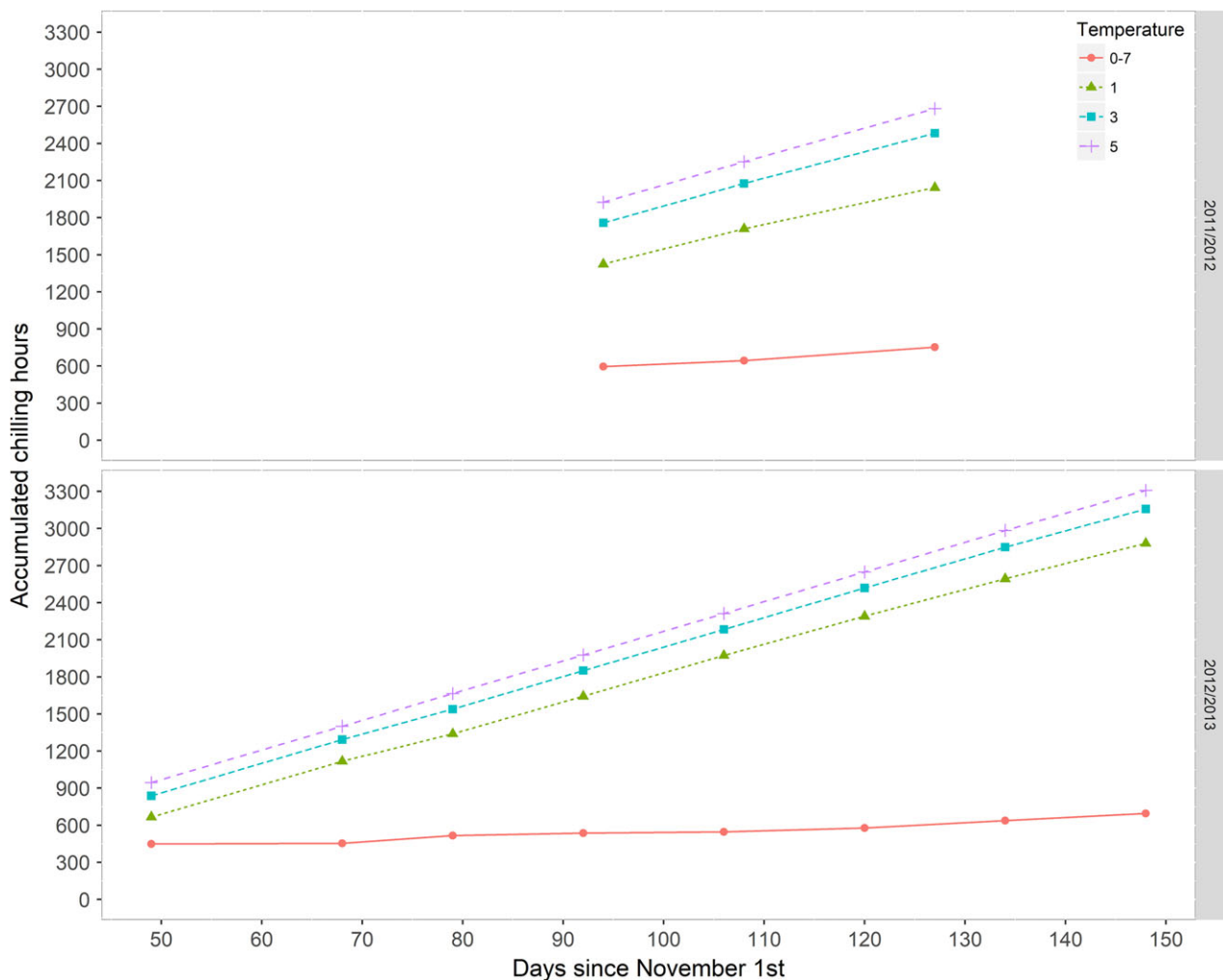


Figure 4. Comparison of winter chilling models with different temperature thresholds at Cedar Creek, MN (2011/2012 = Year 1, 2012/2013 = Year 2). Chilling hours were accumulated starting November 1. Points show accumulated chill hours for each collection. Temperatures thresholds are $<5^{\circ}\text{C}$ ($y = 1479.85 + 17.3x$, adj. $R^2 = 0.65$), $<3^{\circ}\text{C}$ ($y = 1337.22 + 17.38x$, $R^2 = 0.69$), $<1^{\circ}\text{C}$ ($y = 1068.19 + 17.56x$, $R^2 = 0.79$) and $0-7^{\circ}\text{C}$ (not significant). Data for both years were analyzed together.

in *A. rubrum* with increased spring temperatures. On the other hand, Caffarra and Donnelly (2011) reported failed budburst in *Tilia* and *Salix* with unfulfilled chilling. Additionally, range limits are also strongly determined by flowering and fruit maturation (Morin et al. 2008), and *A. rubrum* is one of the earliest species in Minnesota to produce flowers in the spring (Walters and Yawney 1990). Moreover, for the few twigs that flowered in our experiment, they appear not to show a strong relationship between chilling and thermal time to flowering (unpublished data). It is also possible that high chill species might experience faster budbreak with increasing winter temperatures, if chilling is accumulated more efficiently at higher temperatures. However, the pattern of a steep and linear decline of time to budburst for *A. rubrum* suggests that chilling at low temperatures is at least as effective at releasing dormancy as chilling at higher temperatures.

Because chilling affected all species in our experiment differently, it is reasonable to assume that rising global temperatures

will have a noticeable effect on ecological interactions among tree species (Forrest and Miller-Rushing 2010), especially competition for resources, such as light, with probable consequences for the composition of forests and woodlands (Augsburger 2013, Laube et al. 2014). It is also likely that a changing climate will alter the interactions between trees and wildlife, potentially leading to asynchronies if the phenology of wildlife is influenced by factors other than chilling (Both et al. 2010, Polgar and Primack 2011). These asynchronies could affect food and shelter for some species, especially long-distance migratory birds that overwinter in areas close to the equator where they are detached from changing climate cues of northern latitudes (Miller-Rushing et al. 2008), or for insects that primarily feed on young leaves (Polgar and Primack 2011).

Looking at a local context, we saw that the CCESR between 1989 and 2017 lost on average 12.7 h of chilling annually (Figure 3, hourly weather data from CCESR and WRCC). If this

trend was to continue linearly, the trees at the study site could experience on average 419 fewer chilling hours by the middle of this century compared with 2017, or even more with potential positive feedback effects of global warming. While low-chilling species, such as *L. laricina* would be less affected by this, species with higher chilling requirements, such as *A. rubrum*, might leaf out later in the season.

Possible influence of life history traits and range on phenology

To understand the different responses of species to chilling, it is useful to compare different characteristics and ranges. In common garden studies, higher-latitude species often leaf out before lower latitude ones (Myking and Heide 1995, Hänninen 1996), and for example *L. laricina* has a much more northern range than *A. rubrum*.

Several studies have shown that late successional species have a more conservative life-strategy than pioneers, requiring more chilling and leafing out later (Caffarra and Donnelly 2011, Laube et al. 2014). Our results support this, as shown in very low chilling requirements of the pioneers *L. laricina* and *R. cathartica*, followed by the pioneers *B. papyrifera* and *P. tremuloides*, and increasing need for chilling for the late successional *Q. rubra*. On the other hand, the mid-successional species *A. rubrum* requires more chilling to break bud than would be anticipated.

Another explanation could be the organization of xylem. With some exceptions (e.g., Davi et al. 2011), it has been shown that ring-porous species break bud later than diffuse-porous species (Lechowicz 1984, Wang et al. 1992, Körner and Basler 2010). Compared with small vessels, large ones more often get damaged by cavitation in the winter and lose their hydraulic conductivity (Sperry and Saliendra 1994), and new vessels need to be produced in the spring before leaves can be supported (Polgar and Primack 2011, Basler and Körner 2012). The results of our experiment are in general consistent with this, except for the diffuse-porous *A. rubrum*, which is much slower to break bud than the all other species, including the only ring-porous species, *Q. rubra*. As a conifer, the wood anatomy of *L. laricina* differs from that of the other species, but the chance of embolism mainly depends on vessel diameter in angiosperms and gymnosperms alike (Pitterman and Sperry 2003), and tracheids of *Larix* are medium-small (The Wood Database). On the other hand, Panchen et al. (2014) found that deciduous angiosperms leafed out earlier than deciduous gymnosperms.

The spring phenology of the invasive shrub *R. cathartica* closely followed that of the low-chill *L. laricina*, with only slightly larger forcing requirements to break bud at all levels of chilling. Non-native forest invaders often use phenology to create a competitive advantage over native species (Fridley 2012). It is believed that *R. cathartica*'s success in invading forest interiors in the USA is partially dependent on its ability to leaf out earlier

and keep leaves longer than native tree species, effectively increasing its growing season (Converse 1984, Knight et al. 2007, Becker et al. 2012, Pretorius 2015). The high nitrogen and chlorophyll levels in early-spring leaves of *R. cathartica* also enable it to utilize the short window before canopy closure in the spring to fix large amounts of carbon (Harrington et al. 1989). Our results suggest that forcing requirements might play a role in this phenological characteristic, as *R. cathartica* required comparably small amounts of thermal time to break bud, even after short exposure to chilling. Consequently, with increasing temperatures, *R. cathartica* might become more successful in invading forests in North America in the future.

Characterization of chilling

We found a significant decrease in time to budburst with increased chilling for all species in our experiment, based on a model that assumes uniform accumulation of chilling at average temperatures below 5 °C. However, this assumption is likely an oversimplification for several reasons, one of which is that chilling thresholds and ranges are thought to be species-specific (Heide 1993, Myking and Heide 1995, Morin et al. 2009, Basler and Körner 2012). As a consequence, it is likely that our species accumulated chilling units at different temperature ranges and that this effect was not incorporated into our calculations. To adjust for that requires knowledge of species-specific chilling requirements at the level of the bud. However, despite the existence of a large body of research about dormancy and budbreak, the underlying key mechanisms and genes involved in the interaction of chilling and dormancy release are still not fully understood (Rinne et al. 1997, Howe et al. 2003, Hänninen and Kramer 2007). While chilling is known to play a role in triggering bud-intrinsic chemical hormonal changes involving gibberellic acid, abscisic acid, cytokinins and auxins (see literature review Arora et al. 2003) and to regulate cell-to-cell signaling within the apical meristem (Rinne et al. 2001), these chemical interactions have only been tested for a fraction of temperate forest tree species. To determine species-specific chilling requirements, one must consider the stage of dormancy, environmental conditions, as well as the relative importance of different temperature ranges and durations. Compared with our very simple model that weighs chilling units at every level equally, based on the threshold temperature <5 °C, many more sophisticated models are used, but beyond the scope of this paper. For example, the fruit and viniculture industries have worked with more dynamic models (for example Utah Model) that are based on response curves that assign chilling units to temperature ranges (c.f. Richardson et al. 1974, Albuquerque et al. 2008), but not enough is known about temperate tree species to apply these models in our experiment. Many predictive models of leaf-unfolding have been developed for North American temperate tree species (e.g., Morin et al. 2009, Clark et al. 2014a, 2014b, Harrington and Gould 2015, Ford et al. 2016). Such models

incorporate complex calculations of interactions between the main triggers of budburst (chilling, forcing and photoperiod) and climate change, but often contain uncertainties regarding parameters, model structure and drivers (Migliavacca et al. 2012), leading to under- or over-estimations of the timing of budbreak and senescence (Richardson et al. 2012).

In this paper, we treat chilling as the main factor that is interacting with the amount of thermal time units needed to cue budburst. However, we also show that our interpretation about the role of chilling can be altered by adjusting the range of temperatures that contribute to chilling from $<5^{\circ}\text{C}$ to a range from 0 to 7°C (Figure 4). During the majority of December, January and February of both experimental years, the average daily and hourly temperatures were primarily below 0°C at Cedar Creek. If the trees in our experiment only sensed above-freezing temperatures, as is sometimes suggested in the literature (e.g., Rinne et al. 1997, Arora et al. 2003) and often applied in fruit tree research (Utah Models), the bulk of chilling accumulation would have occurred before the first collection in the fall, and in the spring. If this were the case, it would raise the question why we observed a steady decline in the timing to budburst with each subsequent collection.

One plausible explanation would be that the species we tested were indeed able to sense temperatures below 0°C . Alternately, the advance in budburst could be explained by a combination of internal and external cues that are partially temperature-independent, such as photoperiod. It is also plausible that endogenous mechanisms such as the circadian clocks allow trees to 'count' days (Ibanez et al. 2010), and that there is a relationship between number of days from some developmental milestone and the rapidity of budbreak (Yanovsky and Kay 2003). More research is needed to untangle the relative importance of various cues for temperate tree phenology.

Conclusion

Our study shows that an increase in chilling resulted in more rapid budburst after exposure to thermal time for five North American temperate tree species and an invasive shrub, and that all species reacted differently to an increase in chilling. These results can be used as a basis for predicting the response of the species used in our experiment to a change in global temperatures. For species with low chilling requirements, increasing temperatures will most likely lead to earlier budburst, potentially prolonging their growing season. High chill species, on the other hand, may not benefit from advanced budburst and a longer growing season if their winter chilling requirements are only partially met, but this prediction has to be taken with care. Our results highlight the need to assess species-specific responses to dominant cues for budburst. This includes determining the range of chilling that is actually relevant for ending dormancy. Moreover, given the widespread distributions of the study

species, future research should also focus on different provenances (Morin et al. 2009, Migliavacca et al. 2012). Improving our knowledge of chilling sensitivity contributes to better model predictions about possible vulnerabilities of species to global and local temperature increases.

Supplementary Data

Supplementary Data for this article are available at *Tree Physiology* Online.

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Conflict of interest

None declared.

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Authors' Contributions

C.R.B., C.N. and R.A.M. conceived the project, R.A.M. and C.R.B. developed methodology, A.M.P., C.R.B. and C.N. conducted fieldwork and performed statistical analyses, and C.N. and C.R.B. wrote the manuscript.

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