

Bud break responds more strongly to daytime than night-time temperature under asymmetric experimental warming

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Abstract

Global warming is diurnally asymmetric, leading to a less cold, rather than warmer, climate. We investigated the effects of asymmetric experimental warming on plant phenology by testing the hypothesis that daytime warming is more effective in advancing bud break than night-time warming. Bud break was monitored daily in *Picea mariana* seedlings belonging to 20 provenances from Eastern Canada and subjected to daytime and night-time warming in growth chambers at temperatures varying between 8 and 16 °C. The higher advancements of bud break and shorter times required to complete the phenological phases occurred with daytime warming. Seedlings responded to night-time warming, but still with less advancement of bud break than under daytime warming. No advancement was observed when night-time warming was associated with a daytime cooling. The effect of the treatments was uniform across provenances. Our observations realized under controlled conditions allowed to experimentally demonstrate that bud break can advance under night-time warming, but to a lesser extent than under daytime warming. Prediction models using daily timescales could neglect the diverging influence of asymmetric warming and should be recalibrated for higher temporal resolutions.

Keywords: bud burst, climate change, ecotype, maximum temperature, minimum temperature, phenology, *Picea mariana*

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Introduction

During the last century, mean surface temperature has risen by 0.7 °C at global scale and by 0.5–1.5 °C across North America (Zhang *et al.*, 2000; IPCC, 2013). Over the last 50 years, the rate of warming has almost doubled attaining 0.13 °C decade⁻¹ (IPCC, 2013), with a warming of 0.26 °C decade⁻¹ estimated for the northern regions of North America (McKenney *et al.*, 2006). For north-eastern Canada, temperature increases are predicted to reach 3 °C over the next 50 years (Plummer *et al.*, 2006). Warming is geographically, seasonally and diurnally asymmetric, with changes being greater at the higher latitudes, during winter–spring and at night (Plummer *et al.*, 2006; Donat & Alexander, 2012). Between 1950 and 1993, the minimum temperatures have increased at about twice the rate of maximum temperatures, leading to a less cold, rather than warmer, climate (IPCC, 2013).

The carbon balance of terrestrial ecosystems is regulated by the CO₂ assimilation of plants. Growth reactivation in spring, defined by the phenology of buds and leaves, is mostly triggered by a cumulative effect of cold (autumn and winter) and warm (spring) temperatures and photoperiod (Körner & Basler, 2010; Laube *et al.*, 2013). Responses to the environmental drivers are species specific, and, within the same species, genetically different populations can exhibit divergent phenologies according to clinal variations in the environmental conditions (Rossi, 2015). The timings of reactivation of meristem activity are the result of an evolutionary adaptation of plants to local climate, allowing all physiological processes to be synchronized with the period of the year more favourable for growth and reproduction. Plant development and switches to successive ontogenetic phases are based on chemical, enzyme-catalysed and temperature-dependent reactions. Accordingly, they occur earlier and faster at increasing temperatures (Badeck *et al.*, 2004).

Phenological responses to recent climate changes are widely documented, showing marked

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advancements of bud break and flowering due to the worldwide increase in temperature (Menzel *et al.*, 2006; Fu *et al.*, 2015; Piao *et al.*, 2015). On the one hand, lengthening of the growing season may potentially enhance carbon uptake and net ecosystem productivity of forests (Randerson *et al.*, 1999). On the other, the earlier phenology increases the risk of frost damage to leaves and reproductive structures in temperate and cold biomes and could mismatch the synchronisms with parasite or mutualistic species (Bennie *et al.*, 2010; Thomson, 2010). In addition, such delays or advancements of phenology could create new host-parasite synchronisms between species that were previously mismatched (Nealis & Régnière, 2004). These phenological changes are expected to affect the ecological fitness of species and adaptation of local populations to the climatic conditions. Thus, the challenge is to predict to what extent future climate will modify bud phenology. For this purpose, several phenological prediction models are available, mostly based on species-specific algorithms using thermal degree days as a spring forcing unit (Basler, 2015).

To date, experimental manipulations on bud break have investigated responses under homogenous warming conditions (Rossi, 2015), despite evidence that plants respond differently to changes in minimum and maximum temperature (Alward *et al.*, 1999; Wan *et al.*, 2009; Balducci *et al.*, 2015). Moreover, the ongoing diverging increases in daytime and night-time temperatures raise the question of whether daily based models are still appropriate as prediction tools. Chronologies of spring bud phenology from Europe and the United States have recently been compared to daytime and night-time temperatures, finding that dates of leaf onset were more correlated to the former (Piao *et al.*, 2015). At the time of writing, the potential responses of bud break to asymmetric warming are basically unknown and need to be explored in nature and tested under experimental conditions.

In this study, we investigated the effects of asymmetric experimental warming on bud phenology in a species with wide geographical distribution. Timing and duration of bud break were monitored daily in black spruce [*Picea mariana* (Mill.) BSP] seedlings belonging to 20 provenances from eastern Canada and subjected to different daytime and night-time temperatures. Two experiments were conducted in growth chambers under constant photoperiod at temperatures between 8 and 16 °C. We hypothesized that daytime warming would be more effective than night-time in advancing bud break. The experimental design also allowed the potential differences to be assessed in the sensitivity of bud break to warming across provenances.

Materials and methods

Origin of the provenances

Seedlings were produced from lots of black spruce seeds representing 20 provenances from Eastern Canada. The seeds were collected in mature stands between the 45th and 51st parallels, which mark the latitudinal distribution of the species within the commercial forest of Quebec (Table 1 and Fig. S1). The stands were located in the bioclimatic domains belonging to the mixed forest of the northern temperate zone and the coniferous forest of the boreal zone.

The climate of the area is continental cold to boreal, with short and cool summers. Winters are very cold and harsh, with 173–241 frost days per year (Table 1). Absolute minimum temperatures are below –30 °C, reaching –48.8 °C in Parc Mistassini. Mean annual temperatures range between –0.9 °C in Parc des Laurentides and 5.4 °C in Forêt de Watopéka. Total precipitation is 856–1144 mm, moderately decreasing with latitude and longitude across the study region (Table 1). Climatic data of the sites were assessed using BIOSIM version 10.3 (Natural Resources Canada, Sainte-Foy, QC, Canada), which contains historical and spatially referenced daily weather records and provided geographically adjusted long-term data according to the weather stations located near the stands (Régnière & St-Amant, 2007).

Seedling production

In May 2014, the seeds were sown in plastic containers and the resulting seedlings were grown until October to develop appropriately until completing the first growing season. Each container had 20 seedlings belonging to four different provenances. The seedlings were maintained in an open field covered by a thick layer of snow during winter.

Experimental design and bud break assessment

In 2015, two warming experiments beginning on January 7th and February 20th were performed in three growth chambers (Convion, Winnipeg, MB, Canada) at different temperatures. Before each experiment, the seedlings were released from snow and transferred to 5 °C in the dark for 30 h to acclimate. Each treatment involved 10 seedlings per provenance distributed in 10 containers per growth chamber, which resulted in a total of 600 seedlings monitored per experiment. The treatments consisted in modifying either daytime or night-time temperature by 4–8 °C according to the design in Table 2. These temperatures allowed to take into account the warming expected at the beginning of the growing season (Zhang *et al.*, 2000). This resulted in a daytime or night-time warming, which in experiment 2 was associated with night-time or daytime cooling. In both experiments, the control was set at day/night temperature of 12/12 °C. Twelve degrees Celsius represent the thermal conditions generally observed during bud break in the southern part of the black spruce distribution (Antonucci *et al.*, 2015; De Barba *et al.*, 2016). Photoperiod was maintained at 12 h to attain the same daily heat

Table 1 Location and climatic characteristics of the stands where seeds were collected and corresponding to the 20 provenances used in the study

ID	Provenance	Latitude (°N)	Longitude (°W)	Altitude (m a.s.l.)	Annual temperature (°C)			Frost days (days)	Total precipitation (mm)
					Mean	Absolute maximum	Absolute minimum		
1	Parc des Laurentides	47°52'	72°47'	892	−0.9	27.5	−45.7	241	1144
2	Parc Mistassini	50°27'	74°22'	408	−0.9	30.5	−48.8	222	869
3	Manicouagan 5	50°40'	69°13'	437	−0.1	28.7	−43.3	213	952
4	Rivière Portneuf	48°30'	71°56'	591	0.5	30.1	−41.6	217	988
5	Nicabau Chibougamau	49°13'	75°55'	405	0.5	31.0	−46.4	214	860
6	Péribonka	49°36'	72°42'	336	0.6	30.8	−48.4	213	885
7	Canton Chaste Abitibi	49°01'	78°25'	300	0.7	31.8	−47.4	210	856
8	Senneterre	48°22'	77°02'	362	0.9	31.6	−49.2	208	859
9	Port Cartier	50°07'	68°50'	113	1.7	27.0	−36.0	196	1016
10	Ile Anticosti	49°37'	64°37'	198	1.7	26.1	−33.9	203	1012
11	Mars Ha! Ha!	48°12'	71°04'	319	1.8	31.6	−43.8	204	886
12	Causapscal	48°30'	68°52'	349	1.9	30.7	−37.6	204	1078
13	Forestville	48°55'	70°52'	168	2.0	31.7	−39.5	199	947
14	Parc de la Vérendrye	47°04'	77°27'	395	2.2	30.9	−46.1	205	904
15	Gravier Bonaventure	48°34'	67°34'	329	2.2	30.0	−35.6	202	1065
16	Lac Pimbina	46°55'	77°30'	387	2.4	31.3	−44.9	204	902
17	Chandler	48°23'	65°07'	193	2.9	29.1	−33.3	195	1077
18	Perthuis	46°55'	73°54'	283	3.2	30.9	−40.7	195	1129
19	Station Valcartier	46°32'	72°42'	130	4.7	32.4	−36.3	176	1102
20	Forêt de Watopeka	45°22'	72°31'	274	5.4	31.9	−36.6	173	1048

Stands are listed at increasing mean annual temperature. Climatic data were assessed using BIOSIM version 10.3 (Natural Resources Canada, Sainte-Foy, QC, Canada).

sum in the treatments, with a lighting condition set at 260–300 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. This intensity was chosen to avoid potential warming effects of lights on buds and needles (Rossi, 2015). RH was 75–85% and CO_2 450–550 $\mu\text{mol mol}^{-1}$. Irrigation was supplied daily to maintain the soil conditions similar to those occurring in black spruce stands during spring.

Seedlings were examined daily for assessing the dates of apical bud break, which were reported as days from the beginning of the experiment. The six phases of bud break were the following: (i) open bud, with the scales starting to separate and a pale spot visible at the tip; (ii) elongated bud, with lengthening scales; (iii) swollen bud, with smooth and pale-coloured scales but no visible needle; (iv)

translucent bud, with needles visible through the scales; (v) split bud, with open scales but needles still clustered; and (vi) exposed shoot, with needles completely emerged from the surrounding scales and spreading outwards (Dhont *et al.*, 2010).

Statistical analyses

All analyses were performed by averaging data of the five seedlings growing in the same container, which resulted in two repetitions per provenance and growth chamber. The effect of the treatments on the timings of bud break was evaluated using type I sum of squares in generalized linear models (GLM). The treatment represented a fixed factor, and the annual temperature of the stands reported in Table 1 was used as independent variable for quantifying the effect of provenance on bud break. Phenological observations were data repeatedly collected on the same subjects. Thus, mixed models with repeated measurements were performed to assess the effects of the treatment on the time required to complete each phase of bud break. Multiple comparisons between control and treatments were tested by orthogonal contrasts. The distributions of raw data and residuals were checked before and after performing the models, respectively. Statistics were applied using SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

Table 2 Thermal conditions of control and treatments set during the two experiments in growth chambers. Photoperiod was maintained at 12 h to attain the same daily heat sum between treatments during each experiment

Experiment	Day/night temperatures (°C)		
	Control	Daytime warming	Night-time warming
1	12/12	16/12	12/16
2	12/12	16/8	8/16

Results

In control seedlings, phase 1 occurred 5.6 and 8.8 days after the beginning of observations in experiments 1 and 2, respectively. The comparisons indicated that the phases of bud break in experiment 2 were significantly delayed by ca. 3 days in respect to experiment 1, although seedlings had previously been submitted to the same environmental conditions and acclimation. This lag was maintained for phases 2–4, but disappeared for phases 5–6. As the two controls were not equivalent, all models and comparisons were performed separately for experiments 1 and 2. Experiments 1 and 2 lasted for a total of 52 and 62 days, respectively, until all seedlings showed their needles completely emerged from the surrounding scales (phase 6), revealing that the process of bud break had concluded.

Timings of bud break

With the exception of open bud in experiment 1, the models were highly significant with *F*-values ranging between 5.32 and 23.64 ($P < 0.001$) (Table 3). Due to the high variability within and between provenances (Fig. S2), the resulting R^2 of significant models was low, at between 0.19 and 0.52. Studentized residuals showed no trend and were well distributed around zero in both experiments and for all phases, suggesting that the analysis could be considered acceptable (Fig. S3). The

studentized residuals exceeding the 95% confidence interval (the range between -2 and 2) were $<5\%$, except for phases 2–6 in experiment 1, where 6.0–6.9% of values exceeded the confidence interval.

In all significant models, the provenance, represented by the mean annual temperature of the provenance origin, affected bud break ($P < 0.001$) (Table 3). As expected, the provenances belonging to sites with higher annual temperatures had later bud breaks (Fig. 1). Overall, bud break was delayed by 0.63 days per additional degree of mean annual temperature of the site. With the exception of phase 1 in experiment 2, warming had a significant effect on the timings of bud break, with the lower probabilities being generally calculated for the latest phases of development. No interaction provenance \times treatment was observed, indicating that the treatment produced the same effect irrespective of the provenance of the seedlings (Table 3).

Contrasts revealed a different effect of daytime and night-time warming, with more evident differences from phase 2 (Table 3). In experiment 1, both treatments differed significantly from the control for phases 2–6, but the higher advancements of bud break were observed with daytime warming from phase 3 (Fig. 1). As an example, exposed shoot (phase 6) occurred 3.8 and 5.6 days earlier than the control with night-time and daytime warming, respectively. In experiment 2, only daytime warming advanced bud break, while no difference was observed between night-time warming

Table 3 Generalized linear model (GLM) comparisons of the phases of bud break in black spruce resulting from the two warming experiments using temperature of the site (TP), treatment (TT) and their interaction (TP \times TT) as effects

Experiment	Phase	Model		Effects			LS-means		
		<i>F</i> -value	R^2	TP	TT	TP \times TT	Control	Night-time warming	Daytime warming
1	Open bud	2.11	0.08	0.00	5.13**	0.16	5.66 ^a	5.40 ^a	6.21 ^b
	Elongated bud	6.12***	0.21	13.72***	7.97***	0.46	11.89 ^a	10.79 ^b	10.01 ^b
	Swollen bud	9.60***	0.30	20.79***	13.02**	0.60	15.49 ^a	14.22 ^b	12.86 ^c
	Translucent bud	8.92***	0.29	14.00***	15.07***	0.23	18.10 ^a	16.82 ^b	15.09 ^c
	Split bud	23.64***	0.52	17.73***	49.76***	0.48	28.93 ^a	25.85 ^b	22.98 ^c
	Exposed shoot	22.30***	0.50	19.67***	45.58***	0.34	34.69 ^a	30.85 ^b	29.05 ^c
2	Open bud	5.32***	0.19	16.19***	2.71	2.49	8.30 ^a	8.87 ^a	7.74 ^a
	Elongated bud	9.90***	0.30	30.31***	8.40***	1.20	12.79 ^a	13.74 ^a	11.54 ^b
	Swollen bud	9.73***	0.30	26.01***	10.40***	0.92	16.26 ^a	17.37 ^a	14.45 ^b
	Translucent bud	9.91***	0.30	27.91***	10.19***	0.64	19.41 ^a	20.61 ^a	17.40 ^b
	Split bud	11.83***	0.34	14.54***	21.82***	0.49	28.27 ^a	29.11 ^a	24.54 ^b
	Exposed shoot	9.91***	0.30	24.23***	12.36***	0.30	34.14 ^a	35.24 ^a	31.83 ^b

Control was maintained at a constant temperature of 12 °C, while treatments corresponded to night-time and daytime warming. LS-means indicate the average day when a given phenological phase was observed. Values with the same letters are not statistically different ($P > 0.05$).

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

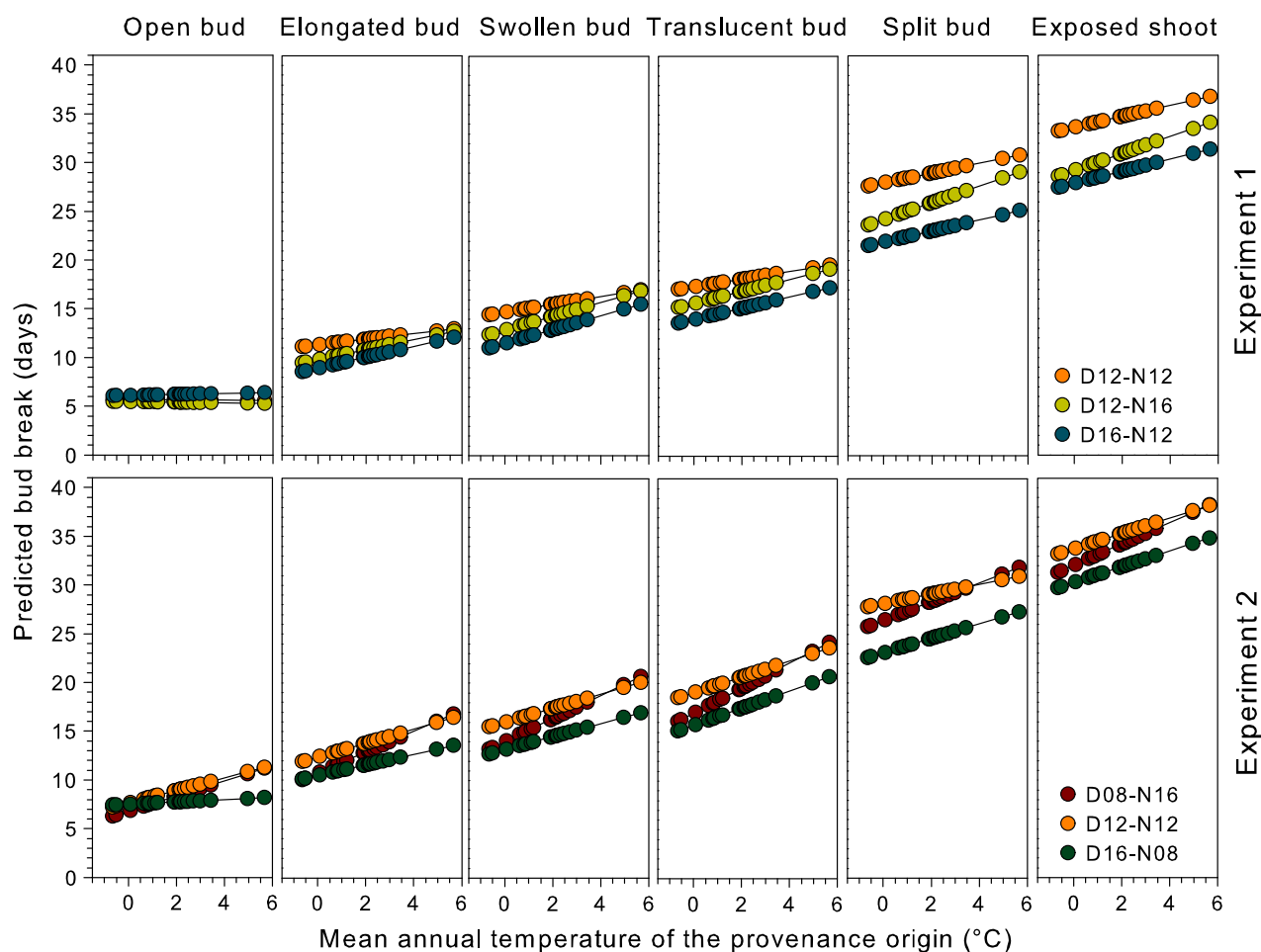


Fig. 1 Days of occurrence of the six phases of bud break predicted for black spruce by the generalized linear model (GLM) performed on data of the two warming experiments at different night (N) and day (D) temperatures. Dots represent the different provenances. The phases are reported as days from the beginning of the experiment.

and control (Table 3). Accordingly, seedlings warmed during the day exhibited an advancement of phase 6 of 3.4 days in respect to the control (Fig. 1).

Duration of the phases

The duration of each phase of bud break was analysed using mixed models with repeated measurements (Table 4). Both models were significant ($P < 0.001$) and exhibited a χ^2 of 79.06 and 114.50 for experiments 1 and 2, respectively. Studentized residuals were homogeneously distributed around zero in both experiments, and <5% of them exceeded the 95% confidence intervals (the range between -2 and 2) (Fig. S4). The models could be considered to correctly fit the data.

A different number of days were required to complete each phase, as revealed by the significant F -values of 436.12 and 306.61 ($P < 0.001$). On average, phases 3 (swollen bud) and 4 (translucent bud) lasted less than

4 days, while phase 5 (split bud) was the longest, requiring 9.2 days to be completed (Fig. 2). In comparison with the control, warming significantly modified the duration of the bud break phases, although the effect was not homogenous for all phases, as shown by the significant interaction phase \times treatment (Table 4). Night-time warming differed from the control only in experiment 1, while the shortest durations were estimated with daytime warming. On average, the differences between this treatment and control were small, that is <1 day, although statistically significant.

Discussion

The effects of an asymmetric warming on bud break were investigated in black spruce by means of increases in daytime and night-time temperatures. Two experiments were conducted consisting of a daytime or night-time warming. In experiment 2, the daytime–night-time

Table 4 Comparison of the durations of the six phases of bud break resulting from the two warming experiments using mixed models with repeated measurements

Experiment	χ^2	Effects			LS-means		
		Phase	Treatment	Interaction	Control	Night-time warming	Daytime warming
1	79.06***	436.12***	24.43***	12.83***	5.78 ^a	5.13 ^b	4.83 ^c
2	114.50***	306.61***	7.23***	5.37***	5.87 ^a	5.69 ^a	5.30 ^b

Control was maintained at a constant temperature of 12 °C, while treatments corresponded to night-time and daytime warming. LS-means indicate the predicted duration of the average interval. Values with the same letters are not statistically different ($P > 0.05$).

*** $P < 0.001$.

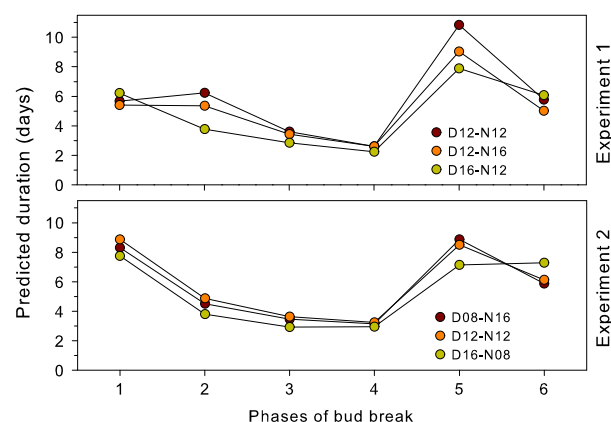


Fig. 2 Duration of the phases of bud break predicted by the mixed models with repeated measurements performed on data of the two warming experiments at different night (N) and day (D) temperatures. Interpolations aim to make the interpretation easier, without suggesting any indication of continuity between the categories shown on the horizontal axis. The six phases are represented by open bud (1), elongated bud (2), swollen bud (3), translucent bud (4), split bud (5) and exposed shoot (6).

warming was associated with night-time–daytime cooling, which allowed similar daily heat sums to be maintained between treatments. The higher advancements of bud break and shorter times required to complete the phenological phases were observed with daytime warming in both experiments. Seedlings responded to night-time warming only in experiment 1, but still with less advancement of bud break than under daytime warming. Our design, performed under controlled conditions, allowed to experimentally demonstrate at a very fine timescale the driving effect of the daily temperature on the bud break process. These findings confirmed the hypothesis that daytime warming would be more effective than night-time warming in advancing bud break.

Higher temperatures speed up the leafing process, as also demonstrated by both long-term observations (Fu *et al.*, 2015; Park *et al.*, 2015) and experimental warming

(Prieto *et al.*, 2009; Wolkovich *et al.*, 2012). The advancement of bud break under climate warming can expose the developing meristems to frost damage during the long nights in early spring or create new opportunities for parasites (Thomson, 2010; Vitasse *et al.*, 2014). Our experiments were performed at temperatures representative of June, when growth reactivation is observed in black spruce (Antonucci *et al.*, 2015; De Barba *et al.*, 2016). At these thermal conditions, seedlings reactivate bud development promptly and quickly, even if the photoperiod is only 12 h. This demonstrated that photoperiod requirements are met in early spring, when days begin to be longer than nights. Thus, warmer temperatures could advance dormancy release of black spruce in May, when the probability of night-time frosts and the consequent damage to rehydrating buds is higher.

Asymmetric warming and bud break

Under warming conditions, the advancement of bud break is more evident and significant for the last phases. The increase in temperature seems to produce a cumulative effect on the sequential events of the bud break process, which are strictly connected together (Rossi & Bousquet, 2014; Rossi, 2015). When chilling and photoperiod requirements are met, temperature is the main driving force for spring phenology, and bud break can be estimated by thermal time calculated by heat units or growing degree days. Thermal time is commonly represented by the daily mean temperatures above a threshold of 0–6 °C (Wielgolaski, 1999; Antonucci *et al.*, 2015; Wang *et al.*, 2015), but some studies demonstrate that hourly temperatures can produce more reliable predictions (Man & Lu, 2010). The diverging results found in this study under different (experiment 1) and similar (experiment 2) heat sums indicate that heat units are accumulated at hourly scales, and during daytime. This confirms the need to build phenological models based on hourly rather than daily

temperatures, especially when predicting the timing of bud break under future climatic scenarios.

The ongoing changes in the global temperature are producing an asymmetric warming, with night-time temperatures rising at faster rates than daytime ones. This will lead to a less cold, rather than warmer, climate. Our study experimentally demonstrated that night-time warming, if not associated with a daytime cooling, could significantly advance bud break by shortening the time required to complete the phenological phases, although the effects will be less than those expected under daytime warming. Piao *et al.* (2015) showed that leaf onset detected by remote sensing in the northern hemisphere was mainly driven by maximum temperature and proposed three possible explanations. First, minimum temperature is more likely than maximum to be below the thermal threshold of 0–6 °C (Wielgolaski, 1999; Wang *et al.*, 2015), thus potentially excluding night-time hours being accounted for in growing degree days. Second, photoperiod interacts with temperature in forcing bud break (Körner & Basler, 2010) and its effect could modify the influence of daytime and night-time temperatures on growth resumption. In this study, temperatures ranged between 8 and 16 °C, substantially exceeding the thermal threshold of most species (Wielgolaski, 1999; Wang *et al.*, 2015). Moreover, a constant photoperiod of 12 h was used for all treatments, allowing seedlings to experience similar periods of night-time and daytime temperatures. Consequently, the first and second hypotheses could not be applied in explaining the results for black spruce.

The third hypothesis proposed by Piao *et al.* (2015) involved another physiological process of plants. In particular, a higher availability of photosynthates to the developing buds may be expected to accelerate leafing. In evergreen conifers, carbohydrates and photosynthates from the previous year's needles support shoot growth until new needles develop (Hansen & Beck, 1994). As photosynthesis occurs during the day, and the rate of carbon fixation is related to temperature (Turnbull *et al.*, 2002; Kolari *et al.*, 2007; Tan *et al.*, 2015), daytime warming could have enhanced photosynthesis, and, in turn, the bud development rate. The significant effects of night-time warming on bud phenology during experiment 1 could also be related to an indirect effect on photosynthesis. In the temperature steppe of northern China, night warming stimulated plant respiration and carbohydrate consumption, producing a compensatory enhancement of photosynthesis during the subsequent days (Wan *et al.*, 2009). However, the supposed influence of photosynthetic rate on bud break conflicts with the results of a previous experiment performed at different day lengths (Rossi, 2015).

Despite the wide photoperiod range tested, from 14 to 22 h, the treatment produced at best an advancement of only 3 days in the timings of exposed shoot, which was a marginal advancement with respect to the increase in day length (Rossi, 2015). Hormones such as gibberellins and auxin are also involved in bud break and growth resumption (Lavender & Silim, 1986; Hansen *et al.*, 1999). With respect to night-time warming, daytime warming associated with higher contents of gibberellins and endogenous auxins resulted in greater enhancements of growth in *Arabidopsis thaliana* and *Pisum sativum*, respectively (Grindal *et al.*, 1998; Thingnaes *et al.*, 2003). Further manipulation experiments are required to investigate the endogenous factors or physiological mechanisms affecting the asymmetric responses of bud break to daytime and night-time warming.

Population differentiation and bud break

In this study, the provenances were represented by the annual temperature of the provenance origin, which covered an interval of temperature exceeding 6 K, from –0.9 to 5.4 °C, representing the wide range of thermal conditions experienced by black spruce across most of its latitudinal distribution. Seedlings originating from the colder sites had an earlier bud break, which indicated that less heat or accumulation of degree days was needed for resuming growth and confirmed the ecotypic differentiation of local populations in black spruce (Blum, 1988; Rossi, 2015). In colder sites, thermal conditions for growth are reached in late spring, when nights are short, and the risk of damage due to frost events is unlikely. The interaction provenance × treatment was not significant, suggesting that the provenances exhibited similar responses to the asymmetric warming. However, the growth of ecotypes located along a latitudinal gradient resumes under different photoperiods, which could affect the response of bud break to the temperature (Körner & Basler, 2010). Consequently, it is possible that the *in situ* responses of the provenances could diverge from those observed in our study.

Implications for prediction models

The ecological effects of the increase in temperature at global scale require a better understanding of the response mechanisms of plants to environmental drivers to build reliable tools to predict all potential changes of bud phenology under climate warming. For technical reasons related to the historical datasets available, most existing phenological prediction models have been calibrated and run using degree days (Basler,

2015). The ongoing increases in minimum and maximum temperatures are temporally and spatially asymmetric, with the former rising at higher rates than the latter (Plummer *et al.*, 2006; Donat & Alexander, 2012; IPCC, 2013). These asymmetric changes in climate need to be associated with the diverging advancements of bud break to daytime and night-time warming recently observed in the northern hemisphere (Piao *et al.*, 2015) and experimentally confirmed in this study. Based on our findings, the timings of bud break are expected to advance under night-time warming, but to a lesser extent than under daytime warming. Prediction models using daily timescales could neglect the diverging influence of both asymmetric warming and biological responses of plants and should be recalibrated at higher temporal resolutions. The agreement of our observations with Piao *et al.* (2015) may indicate a similar response to asymmetric warming at all tree ages. However, based on the well-known ontogenic differences in phenology between young and mature trees (Vitasse, 2013), the results of this study should be carefully validated also on older trees.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Location of the stands corresponding to the 20 black spruce provenances from Eastern Canada.

Figure S2. Days of occurrence of the six phases of bud break observed in black spruce during the two warming experiments at different night (N) and day (D) temperatures.

Figure S3. Studentized residuals vs. predicted values resulting from the generalized linear model (GLM) performed on data of the two warming experiments at different night and days temperatures.

Figure S4. Studentized residuals vs. predicted values resulting from the mixed models with repeated measurements on data of the two warming experiments at different night and days temperatures.