

1        **BUD BREAK RESPONDS MORE STRONGLY TO DAYTIME THAN NIGHTTIME**  
2        **TEMPERATURE UNDER ASYMMETRIC EXPERIMENTAL WARMING**

3        **Running head:** bud break under asymmetric warming

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## ABSTRACT

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

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## INTRODUCTION

36 During the last century, mean surface temperature has risen by 0.7 °C at global scale and by 0.5-  
37 1.5 °C across North America (IPCC, 2013, Zhang *et al.*, 2000). Over the last 50 years the rate of  
38 warming has almost doubled attaining 0.13 °C decade<sup>-1</sup> (IPCC, 2013), with a warming of 0.26 °C  
39 decade<sup>-1</sup> estimated for the northern regions of North America (McKenney *et al.*, 2006). For  
40 North-eastern Canada, temperature increases are predicted to reach 3 °C over the next 50 years  
41 (Plummer *et al.*, 2006). Warming is geographically, seasonally and diurnally asymmetric, with  
42 changes being greater at the higher latitudes, during winter-spring, and at night (Donat &  
43 Alexander, 2012, Plummer *et al.*, 2006). Between 1950 and 1993, the minimum temperatures  
44 have increased at about twice the rate of maximum temperatures, leading to a less-cold, rather  
45 than warmer, climate (IPCC, 2013).

46 The carbon balance of terrestrial ecosystems is regulated by the CO<sub>2</sub> assimilation of plants.  
47 Growth reactivation in spring, defined by the phenology of buds and leaves, is mostly triggered  
48 by a cumulative effect of cold (autumn and winter) and warm (spring) temperatures, and  
49 photoperiod (Körner & Basler, 2010, Laube *et al.*, 2013). Responses to the environmental drivers  
50 are species specific, and, within the same species, genetically-different populations can exhibit  
51 divergent phenologies according to clinal variations in the environmental conditions (Rossi,  
52 2015). The timings of reactivation of meristem activity are the result of an evolutionary  
53 adaptation of plants to local climate, allowing all physiological processes to be synchronized with  
54 the period of the year more favourable for growth and reproduction. Plant development and  
55 switches to successive ontogenetic phases are based on chemical, enzyme-catalysed, and

56 temperature-dependent reactions. Accordingly, they occur earlier and faster at increasing  
57 temperatures (Badeck *et al.*, 2004).

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

72 To date, experimental manipulations on bud break have investigated responses under  
73 homogenous warming conditions (Rossi, 2015), despite evidence that plants respond differently  
74 to changes in minimum and maximum temperature (Alward *et al.*, 1999, Balducci *et al.*, 2015,  
75 Wan *et al.*, 2009). Moreover, the ongoing diverging increases in daytime and nighttime  
76 temperatures raise the question of whether daily-based models are still appropriate as prediction  
77 tools. Chronologies of spring bud phenology from Europe and the United States have recently

78 been compared to daytime and nighttime temperatures, finding that dates of leaf onset were more  
79 correlated to the former (Piao *et al.*, 2015). At the time of writing, the potential responses of bud  
80 break to asymmetric warming are basically unknown and need to be explored in nature and tested  
81 under experimental conditions.

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

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## MATERIAL AND METHODS

### 93 **Origin of the provenances**

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

99 The climate of the area is continental cold to boreal, with short and cool summers. Winters are  
100 very cold and harsh, with 173-241 frost days per year (Table 1). Absolute minimum temperatures  
101 are below -30 °C, reaching -48.8 °C in Parc Mistassini. Mean annual temperatures range between  
102 -0.9 °C in Parc des Laurentides and 5.4 °C in Forêt de Watopeka. Total precipitation is 856-1144  
103 mm, moderately decreasing with latitude and longitude across the study region (Table 1).  
104 Climatic data of the sites were assessed using BioSIM version 10.3 (Natural Resources Canada,  
105 Sainte-Foy), which contains historical and spatially-referenced daily weather records, and  
106 provided geographically adjusted long-term data according to the weather stations located near  
107 the stands (Régnière & St-Amant, 2007).

### 108 **Seedling production**

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

## 113 **Experimental design and bud break assessment**

114 In 2015, two warming experiments beginning on January 7<sup>th</sup> and February 20<sup>th</sup> were performed in  
115 three growth chambers (Conviron, Winnipeg) at different temperatures. Before each experiment,  
116 the seedlings were released from snow and transferred to 5 °C in the dark for 30 h to acclimate.  
117 Each treatment involved 10 seedlings per provenance distributed in 10 containers per growth  
118 chamber, which resulted in a total of 600 seedlings monitored per experiment. The treatments  
119 consisted in modifying either daytime or nighttime temperature by 4-8 °C according to the design  
120 in Table 2. These temperatures allowed to take into account the warming expected at the  
121 beginning of the growing season (Zhang *et al.*, 2000). This resulted in a daytime or nighttime  
122 warming, which in experiment 2 was associated to nighttime or daytime cooling. In both  
123 experiments, the control was set at day/night temperature of 12/12 °C. Twelve degrees Celsius  
124 represent the thermal conditions generally observed during bud break in the southern part of the  
125 black spruce distribution (Antonucci *et al.*, 2015, De Barba *et al.*, 2016). Photoperiod was  
126 maintained at 12 h in order to attain the same daily heat sum in the treatments, with a lighting  
127 condition set at 260-300  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . This intensity was chosen to avoid potential  
128 warming effects of lights on buds and needles (Rossi, 2015). RH was 75-85% and CO<sub>2</sub> 450-550  
129  $\mu\text{mol mol}^{-1}$ . Irrigation was supplied daily to maintain the soil conditions similar to those  
130 occurring in black spruce stands during spring.

131 Seedlings were examined daily for assessing the dates of apical bud break, which were reported  
132 as days from the beginning of the experiment. The six phases of bud break were: (1) open bud,  
133 with the scales starting to separate and a pale spot visible at the tip; (2) elongated bud, with  
134 lengthening scales; (3) swollen bud, with smooth and pale-coloured scales but no visible needle;

135 (4) translucent bud, with needles visible through the scales; (5) split bud, with open scales but  
136 needles still clustered; and (6) exposed shoot, with needles completely emerged from the  
137 surrounding scales and spreading outwards (Dhont *et al.*, 2010).

### 138 **Statistical analyses**

139 All analyses were performed by averaging data of the 5 seedlings growing in the same container,  
140 which resulted in two repetitions per provenance and growth chamber. The effect of the  
141 treatments on the timings of bud break was evaluated using type I sum of squares in Generalized  
142 Linear Models (GLM). The treatment represented a fixed factor and the annual temperature of the  
143 stands reported in Table 1 was used as independent variable for quantifying the effect of  
144 provenance on bud break. Phenological observations were data repeatedly collected on the same  
145 subjects. Thus, mixed models with repeated measurements were performed to assess the effects  
146 of the treatment on the time required to complete each phase of bud break. Multiple comparisons  
147 between control and treatments were tested by orthogonal contrasts. The distributions of raw data  
148 and residuals were checked before and after performing the models, respectively. Statistics were  
149 applied using SAS 9.4 (SAS Institute Inc., Cary, NC).

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## RESULTS

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

### 161 **Timings of bud break**

162 With the exception of open bud in experiment 1, the models were highly significant with  $F$ -  
163 values ranging between 5.32 and 23.64 ( $P < 0.001$ ) (Table 3). Due to the high variability within  
164 and between provenances (Fig. S2), the resulting  $R^2$  of significant models were low, at between  
165 0.19 and 0.52. Studentized residuals showed no trend and were well distributed around zero in  
166 both experiments and for all phases, suggesting that the analysis could be considered acceptable  
167 (Fig. S3). The studentized residuals exceeding the 95% confidence interval (the range between -2  
168 and 2) were less than 5%, except for phases 2-6 in experiment 1, where 6.0-6.9% of values  
169 exceeded the confidence interval.

170 In all significant models, the provenance, represented by the mean annual temperature of the  
171 provenance origin, affected bud break ( $P < 0.001$ ) (Table 3). As expected, the provenances

172 belonging to sites with higher annual temperatures had later bud breaks (Fig. 1). Overall, bud  
173 break was delayed by 0.63 days per additional degree of mean annual temperature of the site.  
174 With the exception of phase 1 in experiment 2, warming had a significant effect on the timings of  
175 bud break, with the lower probabilities being generally calculated for the latest phases of  
176 development. No interaction provenance  $\times$  treatment was observed, indicating that the treatment  
177 produced the same effect irrespective of the provenance of the seedlings (Table 3).

178 Contrasts revealed a different effect of daytime and nighttime warming, with more evident  
179 differences from phase 2 (Table 3). In experiment 1, both treatments differed significantly from  
180 the control for phases 2-6, but the higher advancements of bud break were observed with daytime  
181 warming from phase 3 (Fig. 1). As an example, exposed shoot (phase 6) occurred 3.8 and 5.6  
182 days earlier than the control with nighttime and daytime warming, respectively. In experiment 2,  
183 only daytime warming advanced bud break, while no difference was observed between nighttime  
184 warming and control (Table 3). Accordingly, seedlings warmed during the day exhibited an  
185 advancement of phase 6 of 3.4 days in respect to the control (Fig. 1).

## 186 **Duration of the phases**

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

193 A different number of days was required to complete each phase, as revealed by the significant *F*-  
194 values of 436.12 and 306.61 ( $P < 0.001$ ). On average, phases 3 (swollen bud) and 4 (translucent  
195 bud) lasted less than 4 days, while phase 5 (split bud) was the longest, requiring 9.2 days to be  
196 completed (Fig. 2). In comparison with the control, warming significantly modified the duration  
197 of the bud break phases, although the effect was not homogenous for all phases, as shown by the  
198 significant interaction phase  $\times$  treatment (Table 4). Nighttime warming differed from the control  
199 only in experiment 1, while the shortest durations were estimated with daytime warming. On  
200 average, the differences between this treatment and control were small, i.e. less than one day,  
201 although statistically significant.

202

203

## DISCUSSION

204 The effects of an asymmetric warming on bud break were investigated in black spruce by means  
205 of increases in daytime and nighttime temperatures. Two experiments were conducted consisting  
206 of a daytime or nighttime warming. In experiment 2, the daytime-nighttime warming was  
207 associated with nighttime-daytime cooling, which allowed similar daily heat sums to be  
208 maintained between treatments. The higher advancements of bud break and shorter times  
209 required to complete the phenological phases were observed with daytime warming in both  
210 experiments. Seedlings responded to nighttime warming only in experiment 1, but still with less  
211 advancement of bud break than under daytime warming. Our design, performed under controlled  
212 conditions, allowed to experimentally demonstrate at a very fine time scale the driving effect of  
213 the daily temperature on the bud break process. These findings confirmed the hypothesis that  
214 daytime warming would be more effective than nighttime warming in advancing bud break.

215 Higher temperatures speed up the leafing process, as also demonstrated by both long-term  
216 observations (Fu *et al.*, 2015, Park *et al.*, 2015), and experimental warmings (Prieto *et al.*, 2009,  
217 Wolkovich *et al.*, 2012). The advancement of bud break under climate warming can expose the  
218 developing meristems to frost damage during the long nights in early spring or create new  
219 opportunities for parasites (Thomson, 2010, Vitasse *et al.*, 2014). Our experiments were  
220 performed at temperatures representative of June, when growth reactivation is observed in black  
221 spruce (Antonucci *et al.*, 2015, De Barba *et al.*, 2016). At these thermal conditions, seedlings  
222 reactivate bud development promptly and quickly, even if the photoperiod is only 12 h. This  
223 demonstrated that photoperiod requirements are met in early spring, when days begin to be longer  
224 than nights. Thus, warmer temperatures could advance dormancy release of black spruce in May,

225 when the probability of nighttime frosts and the consequent damage to rehydrating buds is  
226 higher.

### 227 **Asymmetric warming and bud break**

228 Under warming conditions, the advancement of bud break is more evident and significant for the  
229 last phases. The increase in temperature seems to produce a cumulative effect on the sequential  
230 events of the bud break process, which are strictly connected together (Rossi, 2015, Rossi &  
231 Bousquet, 2014). When chilling and photoperiod requirements are met, temperature is the main  
232 driving force for spring phenology, and bud break can be estimated by thermal time calculated by  
233 heat units or growing degree days. Thermal time is commonly represented by the daily mean  
234 temperatures above a threshold of 0-6 °C (Antonucci *et al.*, 2015, Wang *et al.*, 2015, Wielgolaski,  
235 1999), but some studies demonstrate that hourly temperatures can produce more reliable  
236 predictions (Man & Lu, 2010). The diverging results found in this study under different  
237 (experiment 1) and similar (experiment 2) heat sums indicate that heat units are accumulated at  
238 hourly scales, and during daytime. This confirms the need to build phenological models based on  
239 hourly rather than daily temperatures, especially when predicting the timing of bud break under  
240 future climatic scenarios.

241 The ongoing changes in the global temperature are producing an asymmetric warming, with  
242 nighttime temperatures rising at faster rates than daytime ones. This will lead to a less-cold,  
243 rather than warmer, climate. Our study experimentally demonstrated that nighttime warming, if  
244 not associated to a daytime cooling, could significantly advance bud break by shortening the time  
245 required to complete the phenological phases, although the effects will be less than those  
246 expected under daytime warming. Piao *et al.* (2015) showed that leaf onset detected by remote

247 sensing in the northern hemisphere was mainly driven by maximum temperature, and proposed  
248 three possible explanations. First, minimum temperature is more likely than maximum to be  
249 below the thermal threshold of 0-6 °C (Wang *et al.*, 2015, Wielgolaski, 1999), thus potentially  
250 excluding nighttime hours being accounted for in growing degree days. Second, photoperiod  
251 interacts with temperature in forcing bud break (Körner & Basler, 2010), and its effect could  
252 modify the influence of daytime and nighttime temperatures on growth resumption. In this study,  
253 temperatures ranged between 8 and 16 °C, substantially exceeding the thermal threshold of most  
254 species (Wang *et al.*, 2015, Wielgolaski, 1999). Moreover, a constant photoperiod of 12 h was  
255 used for all treatments, allowing seedlings to experience similar periods of nighttime and daytime  
256 temperatures. Consequently, the first and second hypotheses could not applied in explaining the  
257 results for black spruce.

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

270 at different day lengths (Rossi, 2015). Despite the wide photoperiod range tested, from 14 to 22  
271 h, the treatment produced at best an advancement of only 3 days in the timings of exposed shoot,  
272 which was a marginal advancement with respect to the increase in day length (Rossi, 2015).  
273 Hormones such as gibberellins and auxin are also involved in bud break and growth resumption  
274 (Hansen *et al.*, 1999, Lavender & Silim, 1986). With respect to nighttime warming, daytime  
275 warming associated to higher contents of gibberellins and endogenous auxins resulted in greater  
276 enhancements of growth in *Arabidopsis thaliana* and *Pisum sativum*, respectively (Grindal *et al.*,  
277 1998, Thingnaes *et al.*, 2003). Further manipulation experiments are required to investigate the  
278 endogenous factors or physiological mechanisms affecting the asymmetric responses of bud  
279 break to daytime and nighttime warming.

## 280 **Population differentiation and bud break**

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

292 break to the temperature (Körner & Basler, 2010). Consequently, it is possible that the *in situ*  
293 responses of the provenances could diverge from those observed in our study.

#### 294 **Implications for prediction models**

295 The ecological effects of the increase in temperature at global scale require a better understanding  
296 of the response mechanisms of plants to environmental drivers to build reliable tools to predict all  
297 potential changes of bud phenology under climate warming. For technical reasons related to the  
298 historical datasets available, most existing phenological prediction models have been calibrated  
299 and run using degree days (Basler, 2015). The ongoing increases in minimum and maximum  
300 temperatures are temporally and spatially asymmetric, with the former rising at higher rates than  
301 the latter (Donat & Alexander, 2012, IPCC, 2013, Plummer *et al.*, 2006). These asymmetric  
302 changes in climate need to be associated with the diverging advancements of bud break to  
303 daytime and nighttime warming recently observed in the northern hemisphere (Piao *et al.*, 2015)  
304 and experimentally confirmed in this study. Based on our findings, the timings of bud break are  
305 expected to advance under nighttime warming, but to a lesser extent than under daytime  
306 warming. Prediction models using daily time scales could neglect the diverging influence of both  
307 asymmetric warming and biological responses of plants and should be recalibrated at higher  
308 temporal resolutions. The agreement of our observations with Piao *et al.* (2015) may indicate a  
309 similar response to asymmetric warming at all tree ages. However, based on the well-known  
310 ontogenic differences in phenology between young and mature trees (Vitasse, 2013), the results  
311 of this study should be carefully validated also on older trees.

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318

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- 422

423 **Table 1** Location and climatic characteristics of the stands where seeds were collected, and corresponding to the 20 provenances used  
 424 in the study. Stands are listed at increasing mean annual temperature. Climatic data were assessed using BioSIM version 10.3 (Natural  
 425 Resources Canada, Sainte-Foy).

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	Causapsca	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



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

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

430

431 **Table 3** GLM comparisons of the phases of bud break in black spruce resulting from the two warming experiments using temperature  
 432 of the site (TP), treatment (TT) and their interaction (TP×TT) as effects. Control was maintained at a constant temperature of 12 °C,  
 433 while treatments corresponded to nighttime and daytime warming. LS-means indicate the average day when a given phenological  
 434 phase was observed. Values with the same letters are not statistically different ( $p>0.05$ ).

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

Split bud	11.83 <sup>***</sup>	0.34	14.54 <sup>***</sup>	21.82 <sup>***</sup>	0.49	28.27 <sup>a</sup>	29.11 <sup>a</sup>	24.54 <sup>b</sup>
Exposed shoot	9.91 <sup>***</sup>	0.30	24.23 <sup>***</sup>	12.36 <sup>***</sup>	0.30	34.14 <sup>a</sup>	35.24 <sup>a</sup>	31.83 <sup>b</sup>

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One, two and three asterisks indicate  $p < 0.05$ ,  $0.01$  and  $0.001$ , respectively

436 **Table 4** Comparison of the durations of the six phases of bud break resulting from the two  
 437 warming experiments using mixed models with repeated measurements. Control was maintained  
 438 at a constant temperature of 12 °C, while treatments corresponded to nighttime and daytime  
 439 warming. LS-means indicate the predicted duration of the average interval. Values with the same  
 440 letters are not statistically different ( $p>0.05$ ).

Experiment	$\chi^2$	Effects			LS-means		
		Phase	Treatment	Interaction	Control	Nighttime warming	Daytime warming
1	79.06***	436.12***	24.43***	12.83***	5.78 <sup>a</sup>	5.13 <sup>b</sup>	4.83 <sup>c</sup>
2	114.50***	306.61***	7.23***	5.37***	5.87 <sup>a</sup>	5.69 <sup>a</sup>	5.30 <sup>b</sup>

Three asterisks indicate  $p<0.001$

441

442

## CAPTIONS OF THE FIGURES

443 **Figure 1** Days of occurrence of the six phases of bud break predicted for black spruce by the  
444 GLM models performed on data of the two warming experiments at different night (N) and day  
445 (D) temperatures. Dots represent the different provenances. The phases are reported as days from  
446 the beginning of the experiment.

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

453

454

## SUPPORTING INFORMATION CAPTIONS

455 **Fig. S1** Location of the stands corresponding to the 20 black spruce provenances from Eastern  
456 Canada.

457 **Fig. S2** Days of occurrence of the six phases of bud break observed in black spruce during the  
458 two warming experiments at different night (N) and day (D) temperatures. The phases are  
459 reported as days from the beginning of the experiment.

460 **Fig. S3** Studentized residuals vs predicted values resulting from the GLM models performed on  
461 data of the two warming experiments at different night and days temperatures. Dots with different  
462 colours represent the six phases of bud break. The range between -2 and 2 indicates the 95%  
463 confidence interval.

464 **Fig. S4** Studentized residuals vs predicted values resulting from the mixed models with repeated  
465 measurements on data of the two warming experiments at different night and days temperatures.  
466 The range between -2 and 2 indicates the 95% confidence interval.