

1 **SYNCHRONISMS BETWEEN BUD AND CAMBIUM PHENOLOGY IN**  
2 **BLACK SPRUCE: EARLY FLUSHING PROVENANCES EXHIBIT EARLY**  
3 **XYLEM FORMATION**

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17

18 **ABSTRACT**

19 Bud and cambial phenology represent the adaptation of species to the local environment  
20 that allows the growing season to be maximized while minimizing the risk of frost for  
21 the developing tissues. The temporal relationship between the apical and radial  
22 meristems can help in the understanding of tree growth as a whole process. The aim of  
23 this study was to compare cambial phenology in black spruce (*Picea mariana* (Mill.)  
24 B.S.P) provenances classified as early and late bud flushing. The different phases of  
25 cambial phenology were assessed on wood microcores sampled weekly from April to  
26 October in 2014 and 2015 from 61 trees growing in a provenance trial in Quebec,  
27 Canada. Trees showing an early bud flush also exhibited early reactivation of xylem  
28 differentiation, although an average difference of 12 d for buds corresponded to small  
29 although significant differences of 4 d for xylem. Provenances with early bud flush had  
30 an early bud set and completed xylem formation earlier than late bud flush provenances.  
31 No significant difference in the period of xylem formation and total growth was  
32 observed between the flushing classes. Our results demonstrate that the ecotype  
33 differentiation of black spruce provenances represented by the phenological adaptation  
34 of buds to the local climate corresponds to specific growth dynamics of the xylem.

35

## 36 INTRODUCTION

37 Phenology is one of the most important aspects of plant adaptation that defines the  
38 seasonal timings of biological events, balancing between optimal growth and  
39 minimizing risks of damage (Kaennel and Schweingruber 1995). In temperate and cold  
40 climates, phenology represents the sequential phases occurring from growth reactivation  
41 in spring to the beginning of dormancy in autumn (Chuine and Beaubien 2001).

42 Investigations on bud phenology help to assess the ability of species to adapt to local  
43 environmental conditions (Chmura and Rozkowski 2002) and predict changes in the  
44 timings of growing season under current global warming (Bronson et al. 2009).

45

46 The timings and dynamics of apical (i.e. bud phenology) and radial (i.e. phenology of  
47 wood formation) growth are quantitative traits influenced by both environmental  
48 conditions and endogenous factors (Klug et al. 2006). The dates of bud flush and bud  
49 set have a strong impact on cold hardiness, which in turn plays a key role in tree  
50 distribution (Bannister and Neuner 2001). Environmental impacts on the two meristems,  
51 buds and cambium, have recently been investigated in forest trees (Basler and Korner  
52 2014; Bosio et al. 2016; Gricar et al. 2014). Results showed that external factors such as  
53 temperature, photoperiod and precipitations are key drivers of these processes.

54 However, studies looking at the different impact of genetic variability and environment  
55 on secondary growth are still scarce (Deslauriers et al. 2015).

56

57 Previous studies on conifers using provenance trials have shown some variation among  
58 tree populations for bud phenology. In black spruce, the variation among provenances

59 represented 11-17% of the variability in flushing date (Parker et al. 1994). Søgaard et al.  
60 (2008) observed no significant differences in bud phenology among Norway spruce  
61 provenances, but interactions between provenances and thermal conditions during  
62 dormancy induction and release. For cambium, studies on Norway spruce showed a  
63 small effect of provenance (Dieset 2011; Kalliokoski et al. 2011; Søgaard et al. 2008).  
64 However, a question remains as to whether differences observed in bud phenology  
65 could be echoed or mirrored at the cambial level. To our knowledge, it is not clear if the  
66 phenological traits observed in buds correspond to specific timings of cambial activity  
67 and wood formation. In particular, we raise the question as to whether provenances with  
68 different bud phenology exhibit a similar pattern of xylem phenology.

69

70 In black spruce [*Picea mariana* (Mill.) B.S.P], differences in specific traits, for instance  
71 timing of bud flush and bud set, have indicated some genetic differentiation among  
72 provenances (Beaulieu et al. 2004; Morgenstern 1978). We used the common garden  
73 described in Beaulieu et al. (2004) to assess phenology of wood formation and  
74 determine the relationships with bud phenology. As phenology reflects the adaptation of  
75 plants to their environment, it is of great importance to understand how provenances  
76 originating from different ecoregions respond under the same environment. In this  
77 study, we compared the phenology of wood formation (xylogenesis) among seven black  
78 spruce provenances represented by two bud flushing classes (early and late). We  
79 assessed the variation among and within provenances in bud and cambium phenology.  
80 We tested the hypothesis that provenances with an earlier bud flush exhibit earlier  
81 cambial reactivation and secondary growth.

82

## 83 MATERIAL AND METHODS

### 84 Site and provenance selection

85 The study site is located at the experimental station Forêt Montmorency (47°19'20.1" N,  
86 71°08'49.6" W, 663 m a.s.l.), which belongs to the balsam fir-white birch bioclimatic  
87 domain. The climate is cold and humid. Mean annual temperature over the last 30 years  
88 is 0.5 °C, with a mean monthly temperature of -15.9 °C in January and 14.6 °C in July.  
89 On average, there are 132 days with a minimum temperature >0 °C. Annual  
90 precipitation is 1583 mm, of which 964 mm falls in form of rain. Snow is present from  
91 the end of October to mid-May.

92  
93 The study was conducted in a provenance trial established in 1999, after harvesting the  
94 previous black spruce stand. Several black spruce provenances were planted, each one  
95 represented by three half sib-families (i.e., seeds having a common and known mother  
96 tree, but unknown father tree) (Beaulieu et al. 2004). In the text, the half-sib families  
97 will be called families for simplicity. For this study, seven provenances, originated from  
98 the coniferous boreal forest of Quebec, Canada, were chosen with divergent bud  
99 flushes, i.e. early- and late-flushing class, according to Beaulieu et al. (2004) and  
100 confirmed by observations made in 2013 (Table 1). On average, 15 days of difference  
101 were observed between the two flushing classes. Three families per provenance and 2-3  
102 trees per family were sampled, resulting in a total of 61 trees analysed.

### 103 Bud phenology

104 During 2014 and 2015, bud phenology was evaluated weekly from April to October  
105 according to Dhont et al. (2010). In spring, six phases of bud flush were defined: open

106 bud (BB1), with the scales starting to separate and a pale spot visible at the tip;  
107 elongated bud (BB2), with lengthening scales; swollen bud (BB3), with smooth and  
108 pale-coloured scales but no visible needles; translucent bud (BB4), with needles visible  
109 through the scales; split bud (BB5), with open scales but needles still clustered; and  
110 exposed shoot (BB6), with needles completely emerging from the surrounding scales  
111 and spreading outwards. In summer, five phases of bud set were defined: white bud  
112 (BS1), initiation of the apical bud that is still hidden by the needles; beige bud (BS2),  
113 with the bud increasing in size and showing beige scales; brownish bud (BS3), with the  
114 bud increasing in volume and being enveloped by brown-turning scales; brown bud  
115 (BS4), with a visible bud and needles starting to spread outwards; spread needles (BS5),  
116 with an opaque brown, clearly visible bud, well developed, concave scales and needles  
117 spreading completely outwards.

### 118 **Phenology of wood formation**

119 Wood micro-cores were collected weekly from April to October on the stem with a  
120 Trephor (Rossi et al. 2006) The samples were stored in ethanol solution (30% in water)  
121 at 5 °C. The microcores were dehydrated, embedded in paraffin, and cut in sections 7  
122 µm thick with a rotary microtome (Rossi et al. 2006). After removing the paraffin and  
123 rehydrating the sections, the samples were stained with cresyl violet acetate (0.15% in  
124 water) and examined under an optic microscope with visible and polarized light.  
125 Cells of different development stages were identified, and cambium phenology defined  
126 as: first enlarging cell (C1), first wall-thickening cell (C2), first mature cell (C3), last  
127 enlarging cell (C4), and last wall-thickening cell (C5). Enlarging cells showed a radial  
128 diameter at least twice that of cambial cells. The polarized light was used to  
129 discriminate wall-thickening cells. This was because, due to the arrangement of

130 cellulose microfibrils, the developing secondary walls shine when observed under  
131 polarized light. The colorant reacts with lignin, producing a colour ranging from violet,  
132 for wall-thickening cells, to blue, for mature cells (Rossi et al. 2014). Three radial rows  
133 of cells were measured and averaged for each sample. The radial thickness of the zones  
134 with enlarging, wall-thickening, and mature cells was measured at  $\times 100-400$   
135 magnifications according to the size of the tree ring using the Leica Application Suite  
136 (Leica Microsystems, Switzerland).

### 137 **Statistical Analysis**

138 The variance in the date of occurrence of the different phenological phases was  
139 partitioned between years, provenances, families within provenances and individuals.  
140 The sum of these effects resulted in 100% of the variance, as the partitioning was forced  
141 between these factors.

142

143 The measurements were transformed into binary data (0-1) according to the presence of  
144 each phenological phase. Generalized Linear Models (GLM) based on logistic functions  
145 were performed

$$146 \quad y = \frac{1}{1 + e^{-t}}$$

147 by solving the regression

$$148 \quad t = \beta_0 + \beta_1 x + \beta_2 y + \beta_3 z$$

149 according to the explanatory variable  $t$  and the factors flushing class ( $x$ ), year ( $y$ ), and  
150 Day of the Year (DOY,  $z$ ).

151

152 The annual growth in terms of tree-ring width was compared between flushing classes  
153 using a two-way ANOVA after checking the assumption of normality and  
154 homoscedasticity of variances. All statistical analyses were performed in R, using the  
155 packages *hier.part* and *stats*.  
156

## 157 RESULTS

### 158 Variance partitioning

159 The factor tree explained most of the variance for the timing of bud flush, but its effect  
160 slightly decreased throughout the growing season (Fig. 1). Forty-five percent of  
161 variation was explained by the differences among trees for BB1, decreasing to 30% for  
162 exposed shoot (BB6). Family and provenance accounted for a similar proportion of the  
163 variance, which ranged between 19 and 31%. A marked increase of the variance  
164 accounting for year was observed, from less than 5% for BB1-BB5 to 28% for BB6  
165 (Fig. 1).

166

167 Year explained most of the variance in the first two phases of bud set (65% for BS1,  
168 and 46% for BS2), and decreased to 1-11% for the successive phases (BS3-BS5).  
169 Conversely, the effect of family and provenance increased from 6% to 25%. A similar  
170 pattern was observed to variance accounting for individual tree, whose effect increased  
171 from 18% for BS1 to 60% for BS5 (Fig. 1).

172

173 Variance partitioning for cambium phenology showed different patterns than those  
174 observed for bud phenology. For the phases C1-C3, which occur in spring, family and  
175 provenance had marginal effects (1-5%), while year explained most of the variance (61-  
176 74%). The proportion of variance due to the tree ranged between 21 and 31%. For C4  
177 and C5, tree explained between 56 and 76% of the variance while the effect of year was  
178 reduced to 4 and 24%, respectively. Family and provenance explained 18 and 2% of the  
179 variance in C4 and C5, respectively (Fig. 1).

180 **Phenological variability between classes and years**

181 The GLM produced significant models for all phases of bud flush, bud set and wood  
182 formation. The factor DOY was highly significant ( $p < 0.001$ ). As expected, bud flush  
183 started first in early-flushing provenances in both years. A small but significant effect of  
184 the year was observed in bud flush, but only for BB1 and BB5 (Fig. 2). For bud set,  
185 BS1, BS2 and BS3 showed significant differences between years, with 2015 being the  
186 earliest. The result for BS1 phase should be interpreted with care because data were  
187 missing for some trees at the beginning of this phase (Fig. 3). C1-C5 showed significant  
188 differences between years, confirming that 2015 was also the earliest year for wood  
189 formation (Fig. 4 a. and b.). Similar results were obtained by using heat sums (results  
190 not shown).

191

192 The flushing classes significantly affected all phases of bud set and cambium  
193 phenology, with the exception of C2 (Table 2). Differences between flushing classes for  
194 bud flush ranged from 20.0 d for BB1 to 9.0 d for BB3, with an average of 12 d (Fig. 2).  
195 Differences between flushing classes were similar across years. On average, 5.4 d  
196 separated the phases of bud set between flushing classes, ranging between 0 from BS3  
197 in 2014 to 10.0 d in 2015 for BS1 (Fig. 3). For wood formation, the difference in days  
198 between flushing classes was 4.0 d on average, ranging from 1.0 d for C2 to 5.0 d for  
199 C1 (Fig. 4).

200

201 On average, early provenances had tree rings of 2169.8  $\mu\text{m}$  in thickness, while in late  
202 provenances they were 2092.8  $\mu\text{m}$ . The difference in growth between early and late  
203 provenances was not significant (ANOVA,  $p < 0.05$ ) (Table 3). Significant differences in

204 tree-ring width were observed between 2014 and 2015. No interaction class×year was  
205 detected by the ANOVA ( $p>0.05$ ).  
206

207 **DISCUSSION**

208 **Bud and cambium phenology**

209 In this study, we compared the phenology of bud and cambium in seven black spruce  
210 provenances representing two flushing classes. We verified whether differences in bud  
211 flush were mirrored during wood formation. The assignment of provenances to either  
212 early or late flushing class, which was based on observations conducted at the seedling  
213 stage (Beaulieu et al. 2004), was confirmed in both years of the study. Thus, the  
214 flushing traits in young trees were maintained over time, which was anticipated since  
215 conifers generally show high age-to-age correlations for bud flush (Aitken and Hannerz  
216 2001).

217

218 Cambium phenology showed similar patterns (early vs late) to bud flush phenology  
219 (compare the timings of bud and cambium phenology between the two flush classes  
220 summarized in Fig. 5). We confirmed the hypothesis that provenances with early bud  
221 flush have an early reactivation of cambial activity, and therefore start xylem cell  
222 differentiation earlier. Indeed, we observed differences of up to 7 d in cambial  
223 reactivation between early and late flushing classes. A similar trend was observed at the  
224 end of the growing season, with a difference of 6 d between flushing classes in the  
225 ending of cell-wall lignification. Differences in bud set were found between flushing  
226 classes, but to a lesser extent. For the beginning (white bud and beige bud) a difference  
227 of 10 d was observed between early and late flushing class, but the end of bud set  
228 (spread needles) showed almost no difference (0 to 1 days).

229

230 As early flushing provenances started and completed their growth earlier than late ones,  
231 the flushing classes chosen a priori were confirmed for all processes. In a precedent  
232 study on black spruce, Antonucci et al. (2015) showed a fairly high degree of  
233 correlation between the phenology of cambium and bud. However that study analysed  
234 the phenophases occurring at the beginning of the growing season. Our observations  
235 confirm these previous findings but add new information on the dynamics of xylem  
236 formation and bud set at the end of the growing season. Our results diverge from those  
237 reported for Norway spruce, which showed no difference among provenances in  
238 tracheid formation in terms of onset, cessation and duration of xylem formation  
239 (Kalliokoski et al. 2011). This discrepancy could be due to the tree age, as we measured  
240 younger trees than those authors (13 vs 80 year-old trees). It has already been shown  
241 that older trees could be more adapted to their planting site (Beuker 1994) or less  
242 responsive to environmental variations. In fact, young trees have a different radial  
243 growth pattern (highly influenced by the environment) from older trees, with ring areas,  
244 ring density and ring latewood proportion changing over the lifetime of an individual  
245 (Koubaa et al. 2004).

246

247 For its wide geographical distribution, black spruce has developed the ability to endure  
248 large ranges of environmental conditions, which was revealed by the genetic variation  
249 in phenology observed among provenances in this study. However, there is evidence  
250 that trees and families account most genotypic variation of several adaptive traits of  
251 boreal conifer species (Li et al. 1993; Prunier et al. 2011; Rossi and Bousquet 2014).  
252 Our results confirmed this pattern, as the variance in phenology was mainly detected  
253 within rather than among provenances. Such a wide genetic variation within populations

254 represent the surviving potential of species to adapt the future generations in response  
255 under quickly changing environments.

256

### 257 **Duration of the growing season**

258 In our study, the provenances assigned to the early class of bud flushing remained in the  
259 same class for other phenological traits. This result is in agreement with the literature on  
260 conifers (Blum 1988; Johnsen and Seiler 1996b). Thus, the provenances classified as  
261 early in spring also completed their growth early, which led to a similar duration of the  
262 growing season. Xylem cell production in black spruce is mainly affected by the  
263 duration of the growing season rather than the rate of cell production (Rossi et al. 2014).  
264 Accordingly, there was no significant difference between the annual tree-ring growth  
265 and the two classes of trees produced the same amount of xylem, regardless of their  
266 flushing timings.

267

268 The pattern of radial growth was similar in the provenances under study despite the shift  
269 in phenology observed between flushing classes. It has been suggested that an earlier  
270 growth resumption would lengthen the growing season in conifers of the northern  
271 hemisphere, leading to more cells being produced by the cambium (Kalliokoski et al.  
272 2011; Rossi et al. 2015). Our results did not confirm this hypothesis, because the shift in  
273 cambium reactivation did not correspond to an extended period of xylem production.  
274 The incongruence observed in comparison with results from other studies (trees  
275 beginning earlier having a wider annual ring) could be related to our experimental  
276 design. We characterized only provenances from the boreal forest region, as identified  
277 by Morgenstern (1978). Although these boreal provenances showed a large variation for

278 timing of budburst, the inclusion of southern provenances might have produced a  
279 pattern more consistent with the expected results. Southern provenances are generally  
280 more productive than northern ones (Morgenstern 1978). This raises new questions  
281 about the existing genetic variability in xylem phenology within both species and  
282 populations. Our two bud flushing classes represented geographically distinct  
283 provenances from boreal regions with different climatic conditions (mean annual  
284 temperature of -0.21 and 2.16 °C for early and late flushing class, respectively, Table 1)  
285 showing different phenotypes, despite their similar growth. Significant allelic variations  
286 on genes involved in climate adaptation were found in these populations (Prunier et al.  
287 2011), which indicate the effect of significant selection pressures.

#### 288 **Factors influencing cambial activity**

289 Our study demonstrated that cambium phenology is controlled by both endogenous  
290 (genetic) and environmental drivers. However, the contribution of these factors  
291 affecting the successive phases of xylem cell differentiation changes over the growing  
292 season. In spring (i.e. timings of first enlarging, first wall thickening and first mature  
293 cell), the environmental component, in this study represented by the year factor, was the  
294 most important. Differences accounted for by years correspond to the weather  
295 conditions that vary between years. The reactivation of xylem growth therefore seems to  
296 be more dependent on environmental conditions, as previously shown by Dufour and  
297 Morin (2010). However, the differences in cambial phenology found between the early  
298 and late flushing classes indicated that adaptive component likely plays a significant  
299 role, albeit in interaction with the environment.

300

301 In this study, we were able to quantify the differences in cambium phenology between  
302 two flushing classes of trees growing under the same environmental conditions. This  
303 suggests that xylem growth exhibits specific responses to the weather signals depending  
304 on the provenance. Thus, early flushing trees need a lower temperature to start growth  
305 than late flushing trees, because of their adaptation to the local weather conditions in  
306 their place of origin (Rossi and Isabel 2016).

307

308 The timings of the last enlarging and wall-thickening cell are potentially under a  
309 stronger genetic control than other phases. We observed that the factors provenance,  
310 family, and tree explained a high proportion of the variance, which indicated that  
311 endogenous factors are likely involved in growth cessation. In cambium phenology,  
312 dormancy is a continuation of other tree phenophases in the canopy, going into frost  
313 hardiness. Studies showed that cold hardiness is an adaptive trait controlled by  
314 numerous genes or genomic regions (Pelgas et al. 2011), involved in several molecular  
315 mechanisms (El Kayal et al. 2011), which are in turn influenced by environmental  
316 drivers. Cooke et al. (2012) evaluated the mechanisms inducing dormancy in Norway  
317 spruce concluding that growth cessation was mainly under endogenous control, and that  
318 meteorological cues had a minor effect.

### 319 **Factors influencing bud phenology**

320 In black spruce, temperature and photoperiod are the two main environmental drivers  
321 that affect tree growth responses (Morgenstern 1978). In our study, we assumed that the  
322 year reflects the environmental variation. For bud flush, only two phases (open bud and  
323 exposed shoot) were affected by the factor year. However in the case of the open bud,  
324 the year explained a marginal proportion of the variance (0.1%). On average 2014 and

325 2015 showed only a slight variation in temperature and precipitations. Except for the  
326 exposed shoot, most of the variation was explained by genetic factors (provenance,  
327 family, and tree). Previous findings showed that thermal time requirement (mean  
328 degree-day accumulation needed to start flushing) differed among provenances (Bennie  
329 et al. 2010). According to the criteria used for selecting trees in our experimental  
330 design, it seems logical that the influence of the environment on the beginning of bud  
331 flush is lower than that of genetics.

332

333 The effects of provenance, family, and tree decrease according to the phases: the  
334 beginning of bud flush (the first two phases) appears to be under stronger genetic  
335 control than the subsequent phases. These results are new and surprising, and can be  
336 explained by the phase classification used, which varies from the literature. Indeed, a  
337 systematic assessment of all phenological phases is rare, as most studies describe closed  
338 and open buds, and bud flush is considered to occur when the shoots are exposed  
339 (Søgaard et al. 2008). Moreover, the difficulty in reaching some sites in very early  
340 spring, due to snow cover for instance, might have restricted such observations in  
341 previous studies.

342

343 Differences in bud flush among provenances were also found in red and white spruce  
344 (Blum 1988) and sessile oak along an altitudinal gradient (Alberto et al. 2011) but  
345 disagree with the finding of Vitasse et al. (2009) in temperate deciduous trees. Vitasse  
346 et al. (2009) studied only two populations within a reduced latitudinal gradient. Local  
347 adaptations to climate might need a wider range of conditions. Indeed, in black spruce  
348 seedlings, boreal provenances were found to initiate their growth (timing of bud flush)

349 and bud formation earlier than southern provenances (Johnsen and Seiler 1996a;  
350 Morgenstern 1978). Some studies observing older trees (23 years-old) found similar  
351 results (Johnsen and Seiler 1996b). With our trees being 16 and 17 years old when the  
352 observations were conducted, our results fall within the same range as those already  
353 found (Johnsen and Seiler 1996a; Johnsen and Seiler 1996b).

354

355 Two main tendencies were found for bud set, with white and beige bud (beginning of  
356 bud set) under different controlling factors than the other phases (ending of bud set).  
357 Thus, the beginning of bud set was principally under environmental control. Indeed,  
358 environmental cues such as temperature and photoperiod are essential to start formation  
359 of the winter bud and the subsequent dormancy (Delpierre et al. 2015). As photoperiod  
360 is constant between years, the observed differences between 2014 and 2015 are  
361 expected to be related to the temperature and growing conditions in the previous year  
362 (which impact the number of primordia formed in the bud).

363

364 The last three phases of bud set were mostly under the control of the tree, family and  
365 provenance, which means that the end of bud formation and growth cessation is mainly  
366 under genetic control. Indeed, growth in conifers is determinate: the growth cessation is  
367 genetically pre-determined and environmental effects on the phenology are only  
368 marginal (Cooke et al. 2012; Junttila 1976).

369

370 **CONCLUSIONS**

371 In this work, we compared timings of bud phenology and cambium phenology among  
372 black spruce provenances assigned to early and late bud flushing class to verify whether  
373 a divergent timing of bud flush corresponded to a different cambial phenology. Our  
374 results showed that trees from different locations but growing under the same  
375 environmental conditions can have different dynamics of wood formation, with the  
376 timings of phenological events shifted throughout the growing season. Consequently,  
377 early and late flushing trees also experience different periods of wood growth. Bud and  
378 xylem development are complex growth processes, regulated by several interacting  
379 factors and their relationship requires deeper investigations. Our study raises the need to  
380 identify the environmental factors involved in growth resumption and their interaction  
381 with the endogenous factors.

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512 **CAPTION LIST**

513 **Table 1** Location and climatic characteristics of studied provenances

514 **Table 2** Z scores and associated significances resulting from the GLM based on logistic  
515 functions

516 **Table 3** F-value of the two-way ANOVA comparing the tree-ring width in early- and  
517 late-flushing black spruce

518 **Figure 1** Variance partition for the different phases of bud and cambial phenology in  
519 black spruce provenances

520 **Figure 2** Proportion of trees reaching each phase of bud flush in early- and late-flushing  
521 black spruce

522 **Figure 3** Proportion of trees reaching each phase of bud set in early- and late-flushing  
523 black spruce

524 **Figure 4** Proportion of trees reaching each phase of cambium phenology in early- and  
525 late-flushing black spruce during spring (A) and autumn (B)

526 **Figure 5** Timings of bud and cambium phenology summarized for early- and late-  
527 flushing black spruce. Horizontal error bars represent the standard deviation between  
528 trees.

**TABLE 1**

<b>Provenance</b>	<b>Latitude (°N)</b>	<b>Longitude (°W)</b>	<b>Altitude (m a.s.l.)</b>	<b>Annual temperature (°C)</b>	<b>Total precipitation (mm)</b>	<b>Bud flushing class</b>	<b>Family</b>	<b>Number of trees</b>
<b>Nicabau Chibougamau</b>	49.23	74.08	392	0.09	1026	Early	1	3
							2	3
							3	2
<b>Parc des Laurentides</b>	47.87	-71.2	861	-1.19	1340	Early	1	3
							2	3
							3	3
<b>Manicouagan</b>	50.67	-68.77	437	-0.59	1093	Early	1	3
							2	3
							3	3
<b>Rivière Portneuf</b>	48.5	-70.06	424	0.87	1207	Early	1	3
							2	3
							3	2
<b>Parc de la Vérendrye</b>	47.08	-76.55	394	1.81	1090	Late	1	3
							2	3
							3	3
<b>Station Valcartier</b>	46.54	-71.29	129	4.32	1284	Late	1	3
							2	3
							3	3
<b>Senneterre</b>	48.37	-76.95	363	0.35	1018	Late	1	3
							2	3
							3	3

**TABLE 2**

	<b>Phase</b>	<b>DOY</b>	<b>Year</b>	<b>Flushing class</b>
<b>Bud flush</b>	BB1 - Open bud	12.93***	3.87***	11.81***
	BB2 - Elongated bud	13.33***	0.59	10.29***
	BB3 - Swollen bud	12.11***	1.29	9.78***
	BB4 - Translucent bud	11.55***	0.23	10.26***
	BB5 - Split bud	11.53***	2.58**	10.90***
	BB6 - Exposed shoot	11.33***	1.44	9.77***
<b>Bud set</b>	BS1 - White bud	7.53***	2.05*	6.20***
	BS2 - Beige bud	13.31***	1.03	8.46***
	BS3 - Brownish bud	12.95***	8.37***	2.68**
	BS4 - Brown bud	15.01***	3.03**	5.45***
	BS5 - Spread needles	16.18***	0.04	1.28***
<b>Cambium</b>	C1 - First enlarging cell	15.19***	4.67***	6.03***
<b>phenology</b>	C2 - First wall thickening cell	21.79***	3.56***	0.73
	C3 - First mature cell	18.37***	5.67***	6.51***
	C4 - Ending of cell enlargement	31.02***	5.66***	8.87***
	C5 - End of cell wall lignification	30.56***	6.74***	6.36***

**One, two, and three asterisks correspond to  $p < 0.05$ ,  $p < 0.01$ , and  $p < 0.001$ , respectively**

**TABLE 3**

	<b>Df</b>	<b>F-value</b>
<b>Class</b>	1	0.600
<b>Year</b>	1	54.134***
<b>Class×Year</b>	1	0.673
<b>Residuals</b>	236	

**Three asterisks corresponds to  $p < 0.001$**

FIGURE 1

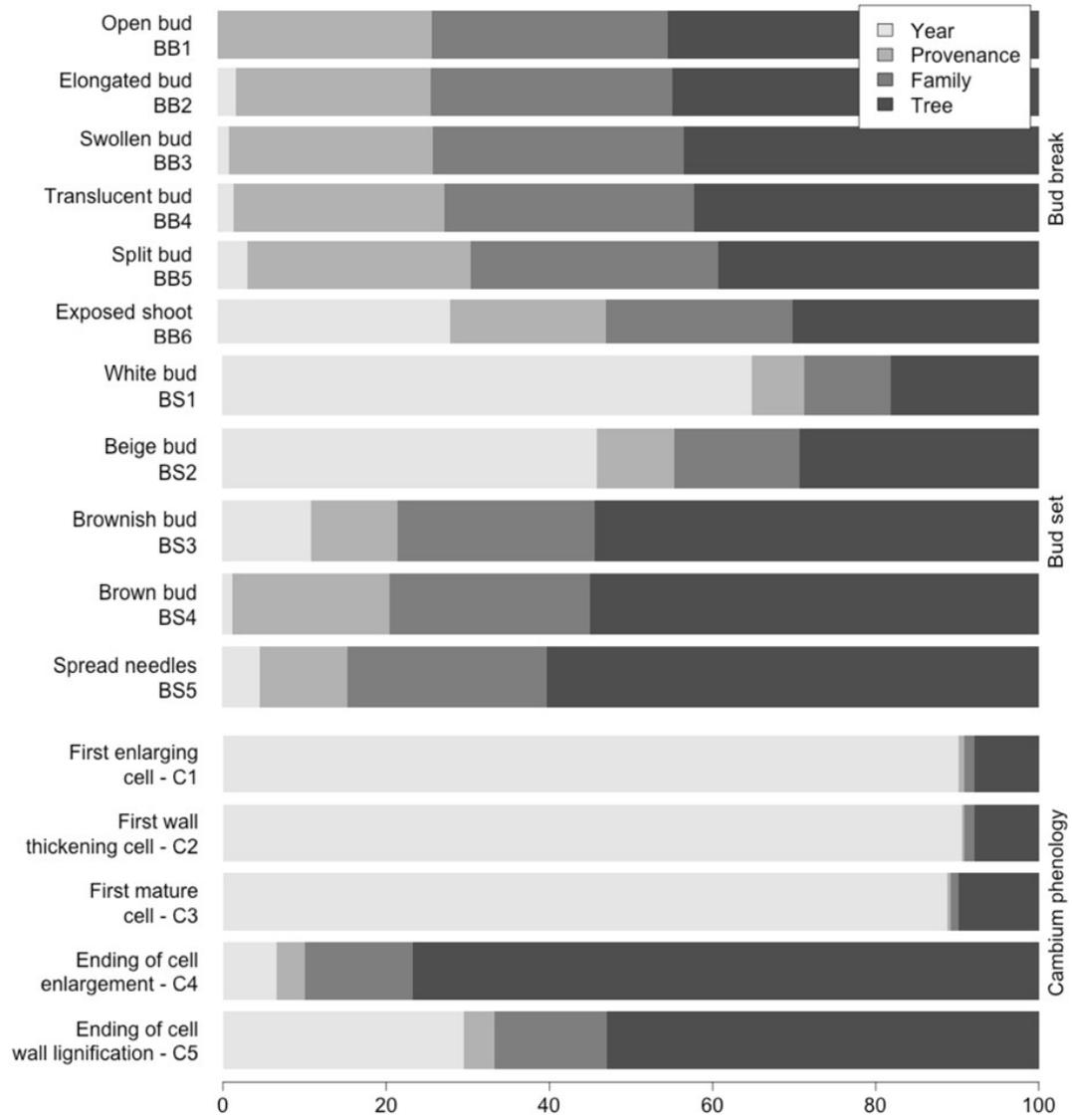


FIGURE 2

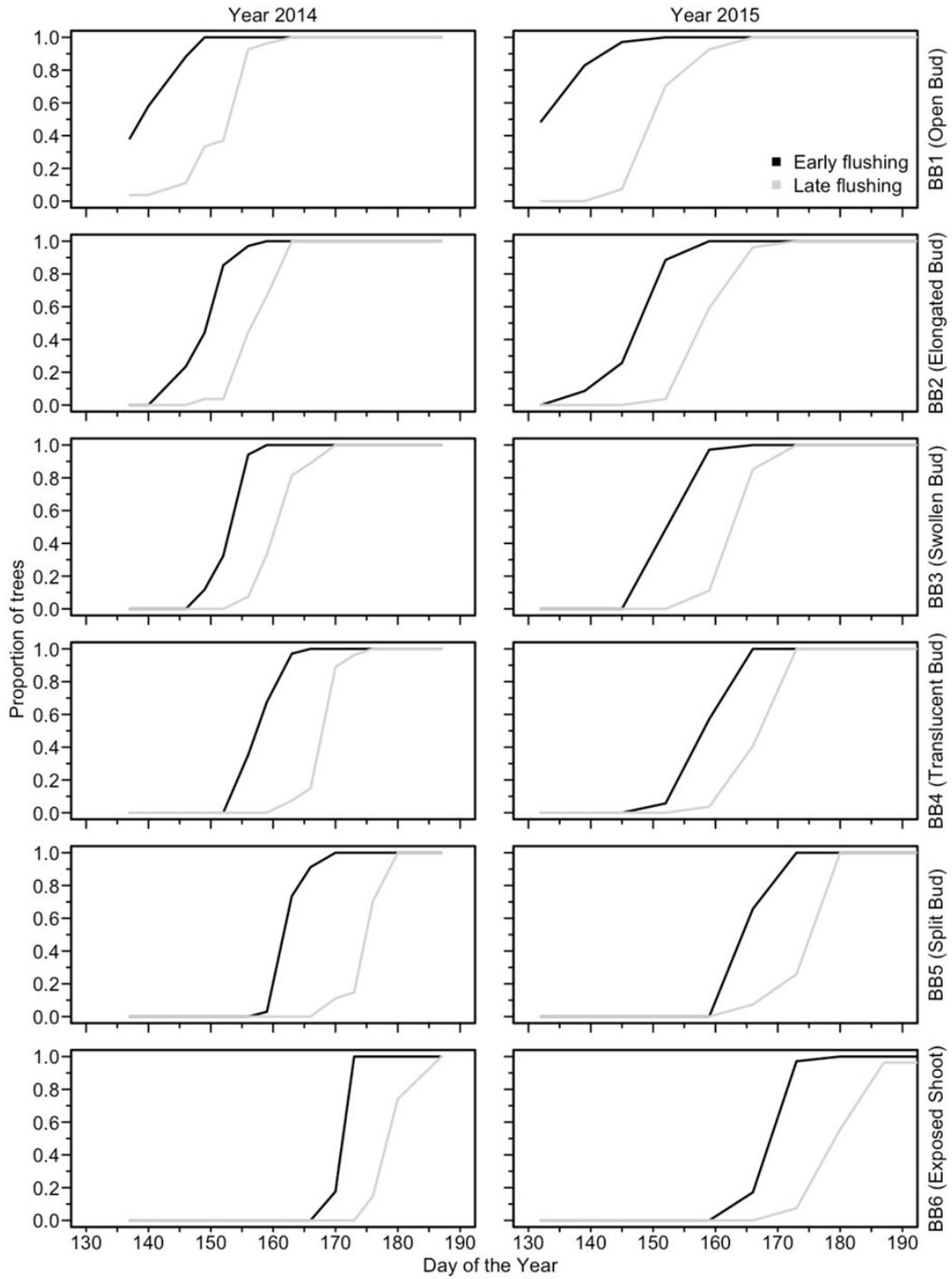


FIGURE 3

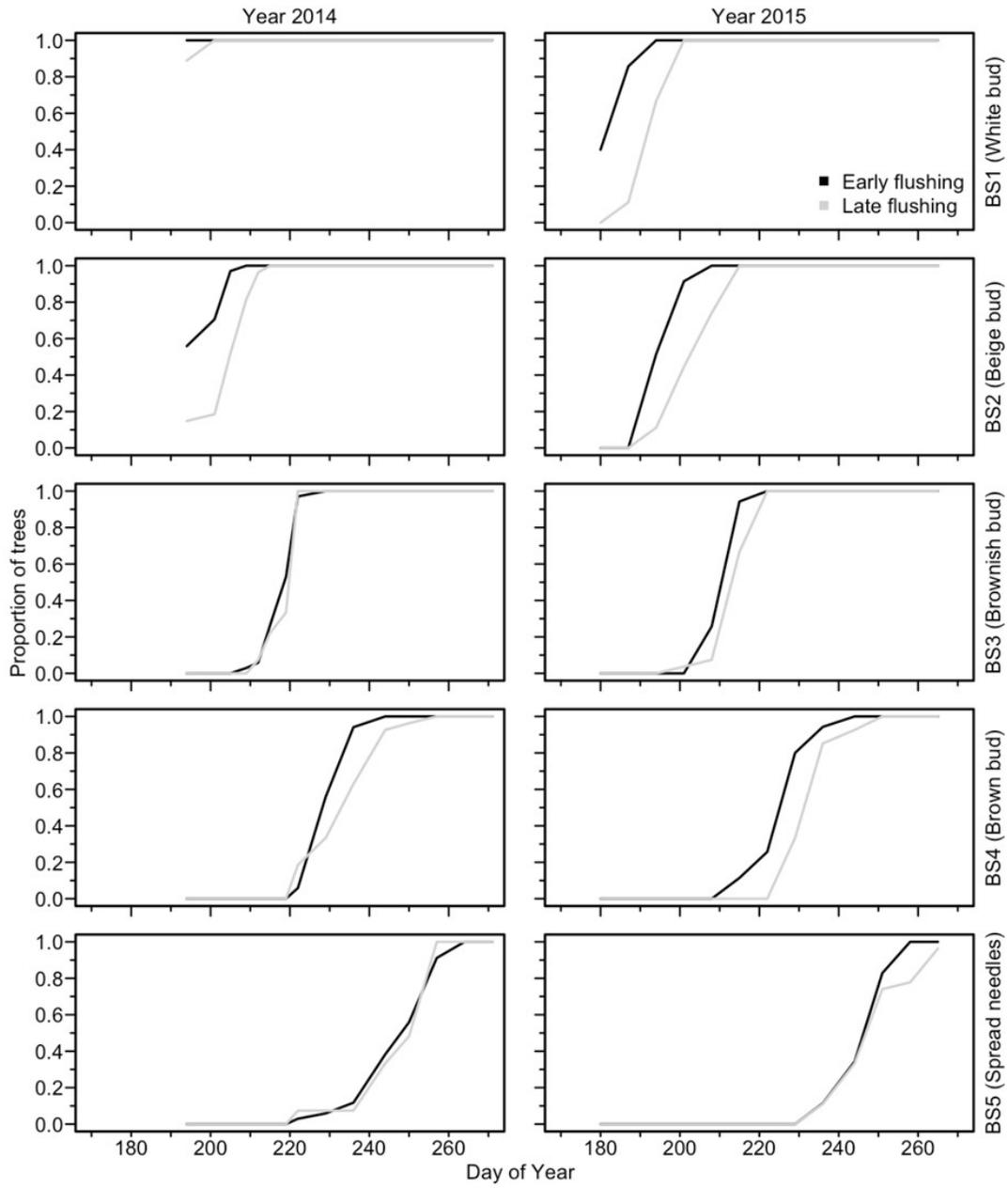


FIGURE 4

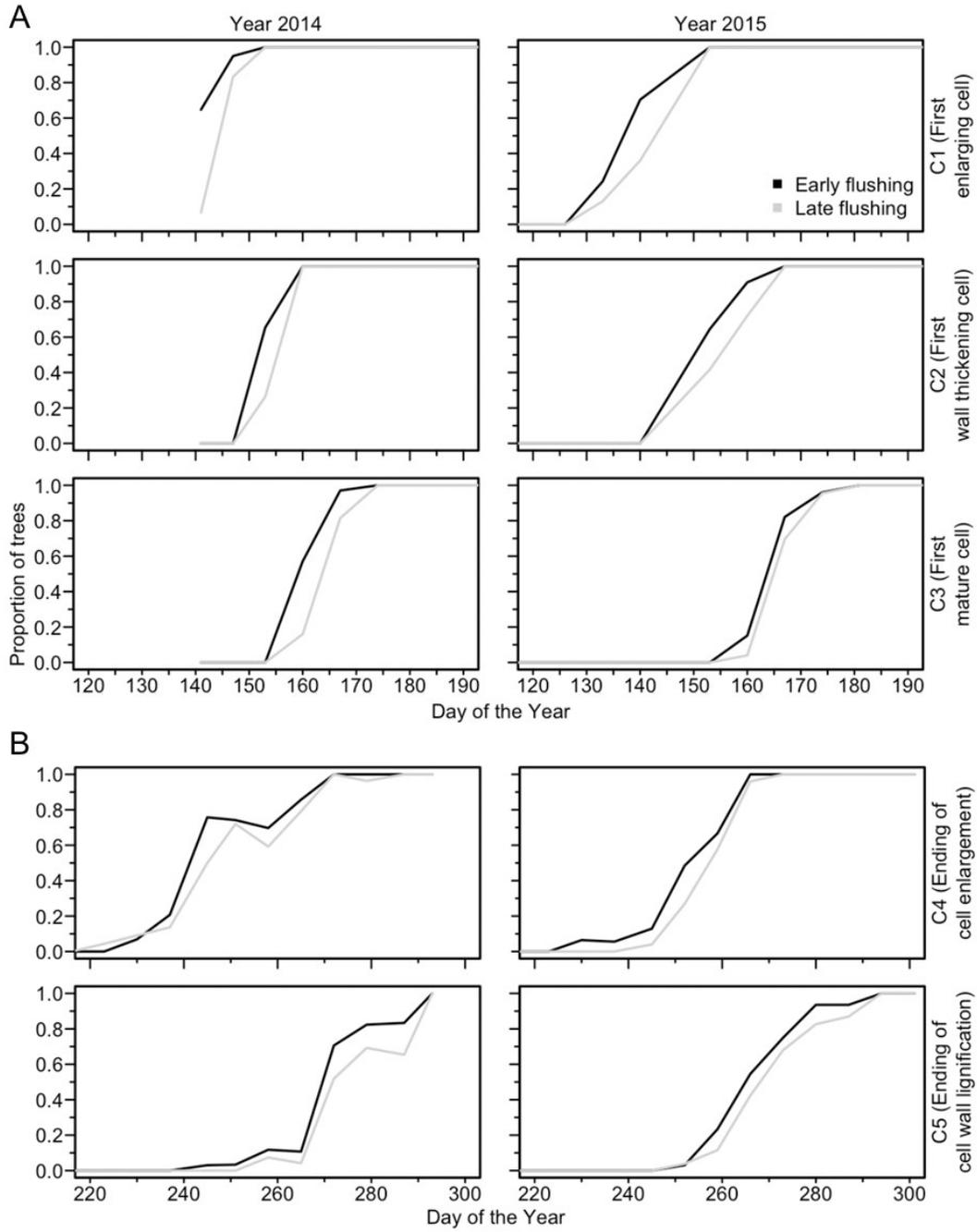


FIGURE 5

