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

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ABSTRACT

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

INTRODUCTION

Phenology is one of the most important aspects of plant adaptation that defines the seasonal timings of biological events, balancing between optimal growth and minimizing risks of damage (Kaennel and Schweingruber 1995). In temperate and cold climates, phenology represents the sequential phases occurring from growth reactivation in spring to the beginning of dormancy in autumn (Chuine and Beaubien 2001). Investigations on bud phenology help to assess the ability of species to adapt to local environmental conditions (Chmura and Rozkowski 2002) and predict changes in the timings of growing season under current global warming (Bronson et al. 2009).

The timings and dynamics of apical (i.e. bud phenology) and radial (i.e. phenology of wood formation) growth are quantitative traits influenced by both environmental conditions and endogenous factors (Klug et al. 2006). The dates of bud flush and bud set have a strong impact on cold hardiness, which in turn plays a key role in tree distribution (Bannister and Neuner 2001). Environmental impacts on the two meristems, buds and cambium, have recently been investigated in forest trees (Basler and Korner 2014; Bosio et al. 2016; Gricar et al. 2014). Results showed that external factors such as temperature, photoperiod and precipitations are key drivers of these processes. However, studies looking at the different impact of genetic variability and environment on secondary growth are still scarce (Deslauriers et al. 2015).

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

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

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

MATERIAL AND METHODS

Site and provenance selection

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

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

Bud phenology

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

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

Phenology of wood formation

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

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

Statistical Analysis

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

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

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

by solving the regression

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

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

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

RESULTS

Variance partitioning

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

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

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

Phenological variability between classes and years

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

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

On average, early provenances had tree rings of 2169.8 μm in thickness, while in late provenances they were 2092.8 μm. The difference in growth between early and late 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 ($p>0.05$).

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DISCUSSION

Bud and cambium phenology

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

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

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

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

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

Duration of the growing season

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

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

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

Factors influencing cambial activity

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

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

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

Factors influencing bud phenology

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

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

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

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

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

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

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

CONCLUSIONS

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

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511

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

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

TABLE 2

	Phase	DOY	Year	Flushing class
Bud flush	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***
Bud set	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***
Cambium	C1 - First enlarging cell	15.19***	4.67***	6.03***
phenology	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

	Df	F-value
Class	1	0.600
Year	1	54.134***
Class×Year	1	0.673
Residuals	236	

Three asterisks corresponds to p< 0.001

FIGURE 1

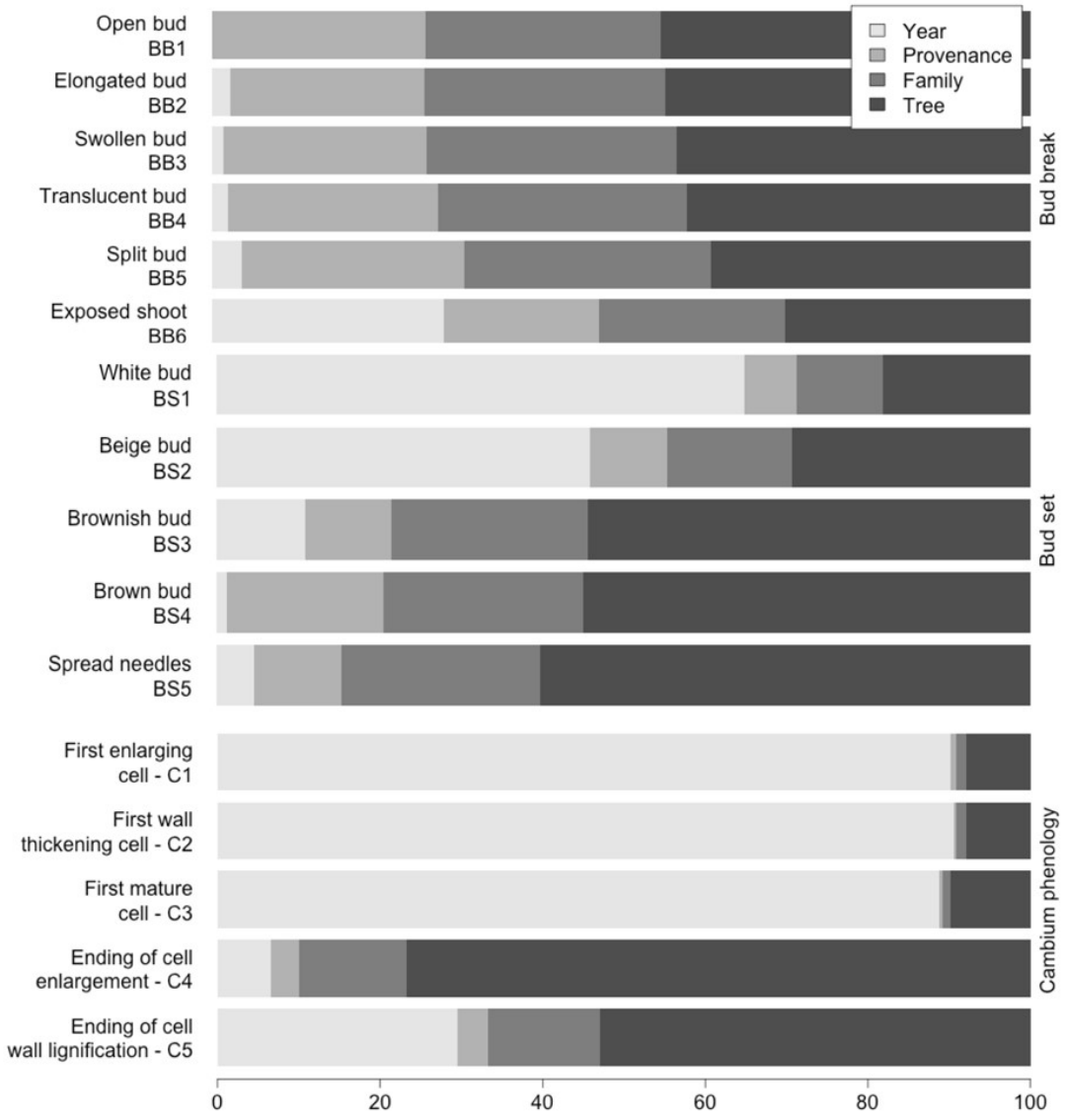

Open bud
BB1
Elongated bud
BB2
Swollen bud
BB3
Translucent bud
BB4
Split bud
BB5
Exposed shoot
BB6
White bud
BS1
Beige bud
BS2
Brownish bud
BS3
Brown bud
BS4
Spread needles
BS5
First enlarging
cell - C1
First wall
thickening cell - C2
First mature
cell - C3
Ending of cell
enlargement - C4
Ending of cell
wall lignification - C5
Year
Provenance
Family
Tree
Bud break
Bud set
Cambium phenology
0
20
40
60
80
100

FIGURE 2

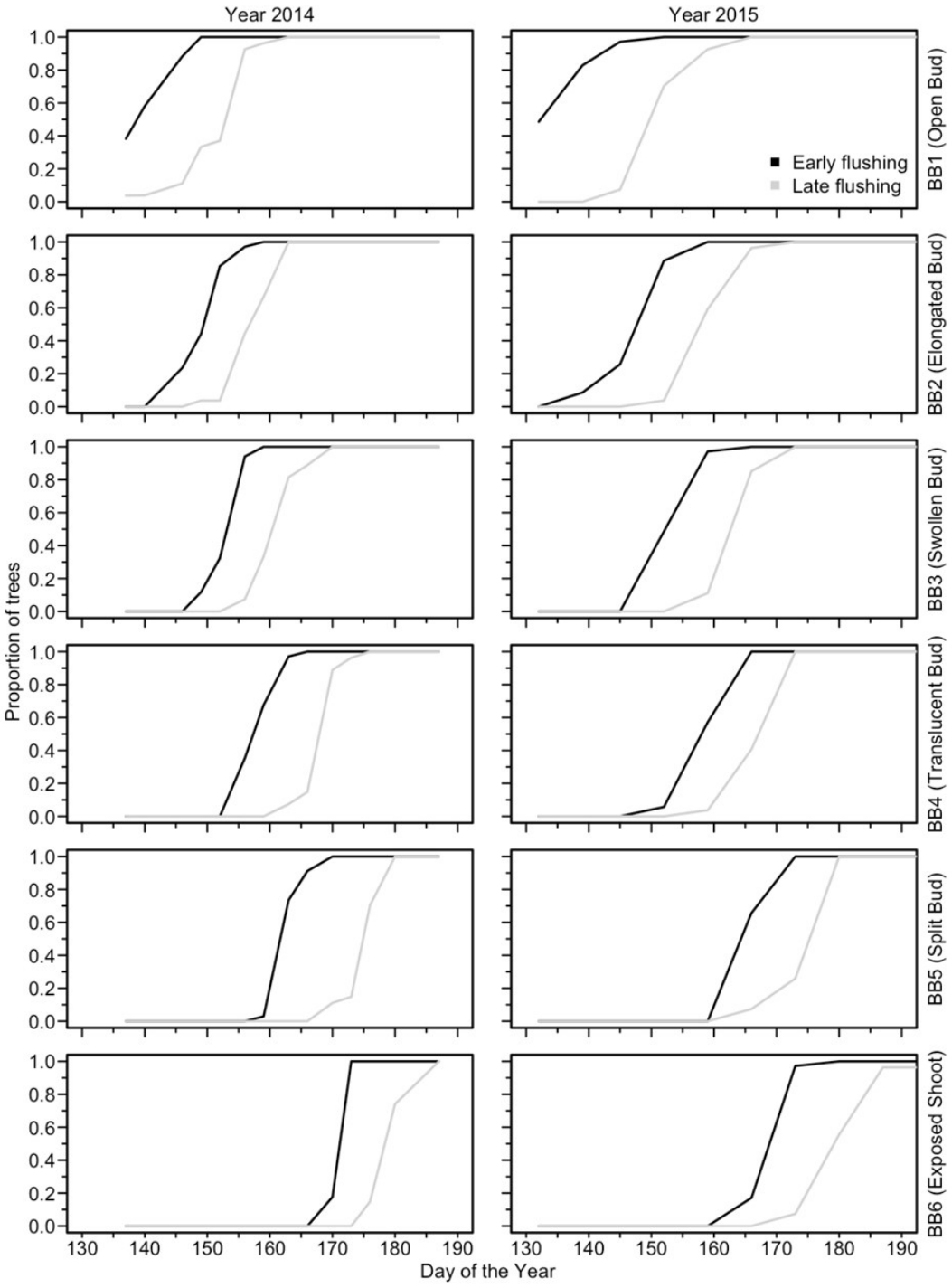

Year 2014
Year 2015
Early flushing
Late flushing
BB1 (Open Bud)
BB2 (Elongated Bud)
BB3 (Swollen Bud)
BB4 (Translucent Bud)
BB5 (Split Bud)
BB6 (Exposed Shoot)
Proportion of trees
Day of the Year
0.0
0.2
0.4
0.6
0.8
1.0
130
140
150
160
170
180
190

FIGURE 3

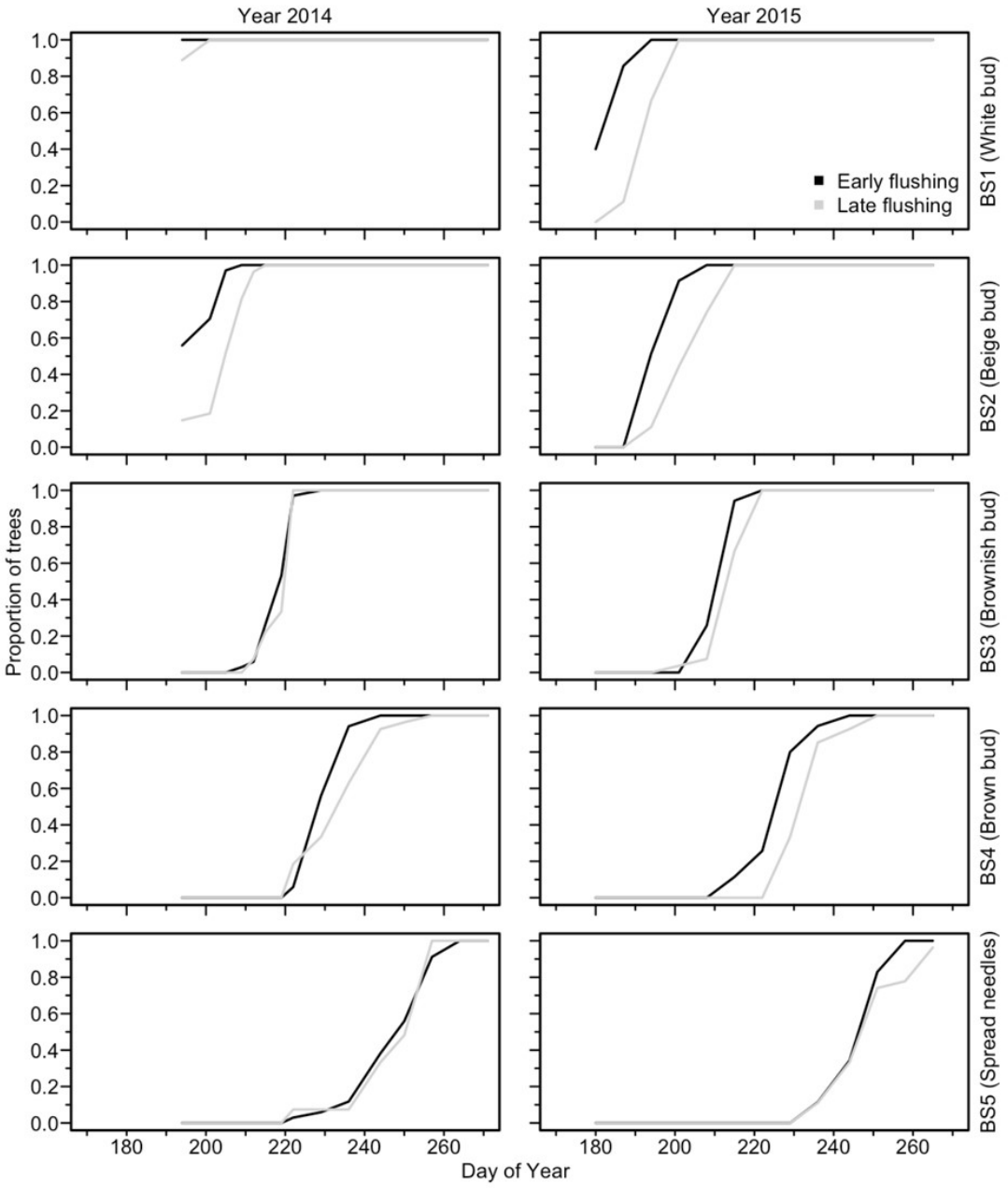

Year 2014
Year 2015
Early flushing
Late flushing
BS1 (White bud)
BS2 (Beige bud)
BS3 (Brownish bud)
BS4 (Brown bud)
BS5 (Spread needles)
Proportion of trees
Day of Year
0.0
0.2
0.4
0.6
0.8
1.0
180
200
220
240
260

FIGURE 4

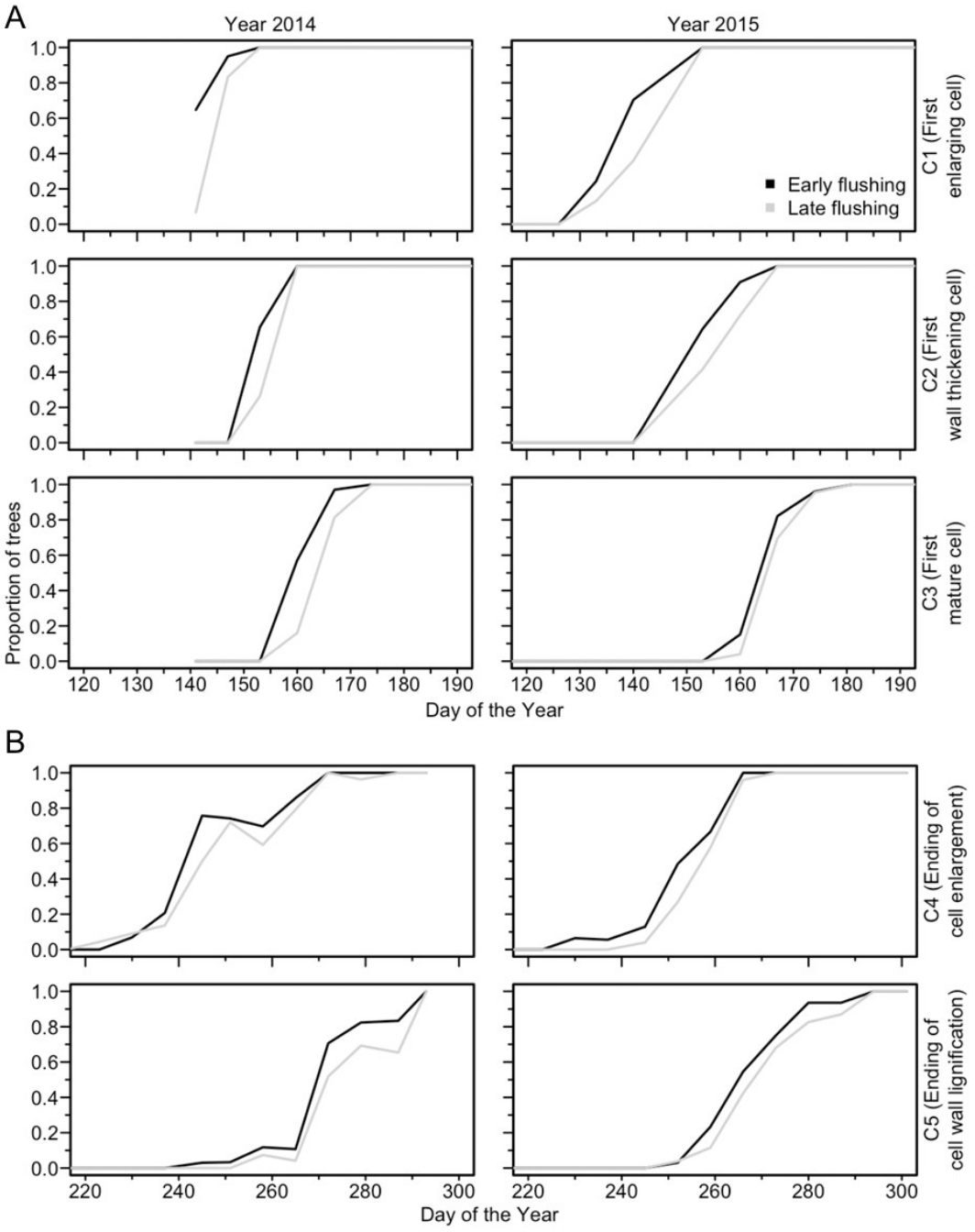
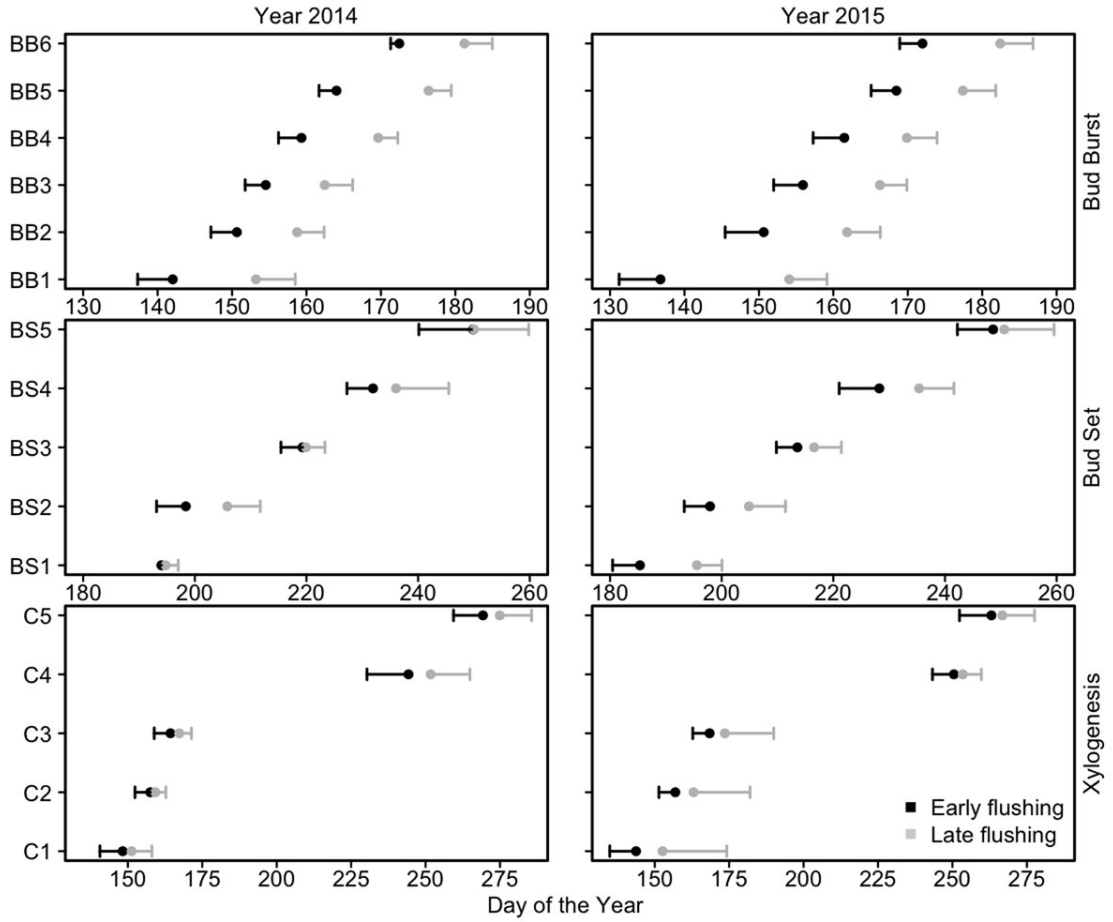

A
Year 2014
Year 2015
Early flushing
Late flushing
C1 (First enlarging cell)
C2 (First wall thickening cell)
C3 (First mature cell)
Proportion of trees
Day of the Year
B
C4 (Ending of cell enlargement)
C5 (Ending of cell wall lignification)
Day of the Year

FIGURE 5


Year 2014
Year 2015
BB6
BB5
BB4
BB3
BB2
BB1
BS5
BS4
BS3
BS2
BS1
C5
C4
C3
C2
C1
130 140 150 160 170 180 190
180 200 220 240 260
150 175 200 225 250 275
Bud Burst
Bud Set
Xylogenesis
Early flushing
Late flushing
Day of the Year