INTRODUCTION GÉNÉRALE

Les espèces distribuées à grande échelle représentent de bons modèles d'adaptation (Leinonen et Hänninen 2002; Søgaard *et al.* 2007; Lu et Man 2011). En effet, l'environnement varie grandement en fonction de la taille de l'aire de répartition géographique ce qui peut amener à des adaptations spécifiques des individus. Ainsi, à l'intérieur d'une même espèce, les individus peuvent former des sous-groupes, représentant des populations génétiquement différenciées. De tels populations, adaptés à leur environnement spécifique, sont appelés écotypes. Ces écotypes sont à la fois issus des interactions entre les facteurs environnementaux et génétiques de même que des forces évolutives en présence (par exemple : la sélection naturelle). Ils sont par conséquence mieux adaptés (fitness optimale) aux conditions particulières d'un environnement (Eriksson 2005). L'adaptation locale est particulièrement importante pour les espèces de plantes pérennes, qui sont des organismes immobiles qui doivent donc « subir » leur environnement, sans possibilité de se déplacer.

La phénologie, consiste à relever des évènements périodiques (annuels très souvent) et récurrents (par exemple : la reproduction) qui varient en fonction des saisons et du climat. Ainsi la phénologie d'une plante, comme la feuillaison, reflète bien son adaptation à un environnement donné. Par exemple chez plusieurs espèces végétales, on observe au niveau intra-spécifique la présence de clines latitudinal ou altitudinal (Howe *et al.* 2003; Montesinos-Navarro *et al.* 2011). Chez les arbres, la phénologie s'intéresse beaucoup à la reprise d'activité photosynthétique au printemps et la fin de l'activité en automne, qui est balancée entre la croissance maximale et le risque minimum de gel (Chuine et Beaubien 2001). Jusqu'à présent on s'est surtout intéressé à la phénologie des bourgeons, qui implique le méristème primaire, responsable de la croissance en hauteur car il est facilement observable. Pour sa part la phénologie de la formation du bois, impliquant cette fois le méristème secondaire avec la croissance en largeur, a été très peu étudié. Pourtant chacune de ces composantes joue un rôle important dans la réponse des arbres aux variations climatiques (Delpierre *et al.* 2015).

L'épinette noire est l'espèce la plus représentée dans la forêt boréale nord-américaine (Lessard et Boulfroy 2010). Des études ont déjà montré la différentiation en écotype de cette espèce, qui présente des traits de croissance et de phénologie spécifiques à sa provenance d'origine (Beaulieu *et al.* 2004). Ainsi des tests de comparaison de provenances ont montré des différences au niveau du débourrement au printemps (Morgenstern 1978; Beaulieu *et al.* 2004). Les tests de provenances consistent en des plantations d'arbres provenant de différentes origines et ce, dans un même lieu, afin de soumettre les individus aux mêmes conditions environnementales (Mátyás 1996). Cela permet de mieux comprendre le déterminisme génétique de traits en contrôlant les effets environnementaux. La composante génétique correspond en partie à l'adaptation locale des populations et à la variabilité génétique présente au sein de l'espèce (King *et al.* 2013).

Ce mémoire, écrit sous forme d'article scientifique, a pour but de voir si les différences trouvées au niveau du débourrement peuvent aussi être observées au niveau du cambium (synchronisation des deux méristèmes) ainsi qu'à la fin de la saison de la croissance apicale, avec l'aoûtement. Nous posons donc l'hypothèse de travail suivante : les arbres des provenances prédéterminées comme hâtives et tardives (en terme de débourrement) seront hâtifs ou tardifs tout au long de la saison ce qui amènera a une même croissance annuelle. Seule la fenêtre de croissance sera déplacée au cours de la saison. Dans notre étude, les provenances ont été séparées en deux groupes, selon leur débourrement, et la phénologie des bourgeons et du cambium a été observée pendant 2 ans.

Avant-propos

J'aimerais d'abord remercier mon directeur, Sergio Rossi, et ma codirectrice, Nathalie Isabel. J'ai beaucoup appris d'eux et je pense qu'ils ont fait de moi une personne meilleure. J'avais tout le temps peur de mal faire, et ils ont réussi à me rentrer dans le crâne que "Magali, tout va bien, c'est correct ce que tu fais." Après 2 ans, je sais qu'ils ne sont pas juste des professeurs mais aussi des personnes sur qui je peux compter et avec qui je peux parler. Ensuite, beaucoup de personnes m'ont aidé avec mon projet de près ou de loin. Les gens de mon labo comme ceux du Centre de Foresterie des Laurentides (CFL) ont tous eu un impact, que se soit dans ma formation, pour du soutien moral, de l'aide, des conseils. Ainsi, je tiens a envoyer pleins de bisous à : Caroline Soucy, Valérie Néron, Annie Deslauriers, Marie-Claude Gros-Louis et toute l'équipe de foresterie du CFL. Vous le méritez tous. De plus, malgré mon amour pour les stats, j'ai quand même du poser quelques questions et je tiens à remercier Gonzalo Pérez de Liz Castro pour son aide précieuse sur R!

Ah, et pour les sous (parce que faire une maîtrise ça coûte cher en investissements personnels et en efforts de travail mais ça coûte aussi de l'argent), merci à Forêts, faunes et parcs avec leur Programme de financement de la recherche et développement en aménagement forestier et à Ressources naturelles Canada. Sans eux, je n'aurais pas fait cette expérience aussi paradoxale en sentiments qu'enrichissante. Parce que oui, j'ai voulu aussi bien laisser tout tomber dans le labo, que faire des câlins à tout le monde quand j'ai eu des résultats qui marchaient.

Table des matières

INTRODUCTION GÉNÉRALE ..... **II**

Avant-propos ..... **IV**

LISTE DES FIGURES ..... **VI**

LISTE DES TABLEAUX ..... **VII**

DEFINITIONS ..... **VIII**

CHAPITRE 1

Abstract **2**

Introduction **3**

Material and methods **5**

- **Site and provenance selection** **5**
- **Bud phenology** **8**
- **Phenology of wood formation** **8**
- **Statistical Analysis** **10**

Results **11**

- **Variance partition** **11**
- **Bud phenology and xylogenesis phenology: variability between classes and years** **13**

Discussion **19**

- **Bud and cambium phenology** **19**
- **Duration of the growing season** **20**
- **Factors influencing cambial activity** **21**
- **Factors influencing bud phenology:** **23**

Conclusion **26**

Acknowledgements **27**

References: **28**

CONCLUSION GÉNÉRALE **33**

LISTE DES FIGURES

Figure 1: Origin of the seven provenances selected. Black and white dots indicate early and late flushing class, respectively. The star represents the location of the provenance test..... 6

Figure 2: Example of cellular phases of tracheid formation: 1 is cambial cells, 2 is enlarging cells, 3 is cell-wall lignification cells and 4 is mature cells..... 9

Figure 3: Variance partitioning for the different phases of bud phenology (bud flush and bud set) and cambial phenology in black spruce provenances.....12

Figure 4: Proportion of trees reaching each phase of the bud flush process for early and late flushing classes of black spruce. 16

Figure 5: Proportion of trees reaching each phase of the bud set process for early and late flushing classes of black spruce. 17

Figure 6: Proportion of trees reaching each phase of the wood formation (xylogenesis) for early and late flushing classes of black spruce. A = beginning of the growing season, B = end of the growing season.....18

LISTE DES TABLEAUX

Table 1: Geographical and meteorological descriptions of studied provenances and trees 7

Table 2: Results for the logistic models. Z scores with the associated significance for each model and factor.....15

Table 3: Results of the two-way Anova comparing the annual tree-ring growth measured both in 2014 and 2015 in black spruce trees that represent early and late flushing classes.....15

DEFINITIONS

- Bud flush: The emergence of new leaves on plant at the beginning of each growing season
- Bud set: The bud formation for the next growing season after the growth cessation
- Cambium: Set of meristematic cells that produces the xylem and phloem and causes the radial plant growth.
- Cline: the graduation of changes in a trait or characteristic, within a species (or taxon) among different populations
- Cold hardiness: the ability of a plant to survive cold temperature during winter thanks to dormancy
- Determinate growth: The organism's growth stops when a particular genetically pre-determined phase has been reached.
- Phenology: derived from the Greek word *phaino* meaning to show or to appear, is the study of periodic biological events in the animal and plant world as influenced by the environment (T°, precipitations, etc.)
- Phenophase: an observable stage or phase in the annual life cycle of a plant or animal that can be defined by a start and end point. Phenophases generally have a duration of a few days or weeks

COMPARISON OF CAMBIUM AND BUD PHENOLOGIES AMONG BLACK SPRUCE PROVENANCES IN NORTH EASTERN CANADA

Abstract

Bud and cambial phenology represent the adaptation of a tree to its local environment. By maximizing its growth and taking the minimum risk of frost, a tree is adapted if its phenology is optimal for both primary and secondary growth. The phenology depends on genetic and environmental factors. The aim of this study was to assess cambial phenology in seven black spruce provenances, showing an early or a late bud flush development. We determined if there was a relationship between cambial growth and each of both timing of bud flush and timing of bud set and if they were synchronized. Microcores were sampled weekly from April to October in 2014 and 2015 in a provenance trial, located in Forêt Montmorency, Quebec, Canada. The samples were prepared and analysed with a microscope to identify the different cell stages. Bud phenology was monitored weekly from mid-May to the end of September. Data analyses were performed to assess the effects of environment and genetic background (provenances, family, individuals). The bud flushing trait, early or late, corresponded to differences in bud set and cambium phenologies: a tree early in bud flush was also early in all other growth processes. The results showed that the flushing class (genetic) and the environment (difference between years) were significant for the bud set and cambial phenology. Total growth did not differ significantly between trees from early- and late flushing classes. Growth lasted a similar period, but was shifted in time depending on the flushing class. Black spruce trees show local adaptation to their origin provenance for both bud and cambium phenology and shift their growth through the growing season.

Introduction

Phenology is one of the most important aspects of plant adaptation, which defines the seasonal timings of biological events, balancing between the optimal growth while minimizing risks of damage (Kaennel et Schweingruber 1995). In temperate and cold climates, the phenology represents the sequential phases occurring during the growth reactivation in spring and the beginning of dormancy in autumn (Chuine et Beaubien 2001). Investigations on bud phenology help to predict the ability of the species to adapt to various environmental conditions (Chmura et Rozkowski 2002). For instance, under climate change, leaf phenology is used to monitor climate change impacts (Bronson *et al.* 2009).

The timings and dynamics of apical (i.e. bud phenology) and lateral (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 has a strong impact on cold hardiness, which in turn plays a key role in species distribution (Bannister et Neuner 2001). Environmental impacts on the two meristems, buds and cambium, have already been investigated in forest trees, such as the sycamore maple, the European beech, the sessile oak and the Norway spruce in Switzerland and Slovenia (Basler et Korner 2014, 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 impacts of genetic variability and environment on the secondary growth still remain scarce (Deslauriers *et al.* 2015).

Previous studies in conifers using provenance trials have shown some variation among tree populations for bud phenology. For example, Parker *et al.* (1994) found between 11 and 17% of variation among 75 provenances of *P. mariana* for flushing date (dates of the needle flushing), but saw very little differences for shoot elongation. These provenances represented natural stands of black spruce across Ontario and were tested in three different environments. The

most favourable environment (greenhouse) showed the greatest differences among provenances for bud flushing dates and seedling heights. Søgaard *et al.* (2007) observed no difference among provenances in Norway spruce seedlings, but interactions between provenances and weather for bud burst. For cambium phenology, studies in Norway spruce showed a small effect of the provenance, but used a small number of tree (16 trees) (Dieset 2011; Kalliokoski *et al.* 2011). And so, a question remains whether differences in bud phenology could represent differences at the cambial level. To our knowledge, it is not clear if phenological traits observed in buds correspond to specific timings of cambial activity and wood formation. In particular, we raise the question whether provenances with a different bud phenology would exhibit similar pattern of xylem phenology.

In black spruce (*Picea mariana* (Mill.) B.S.P), differences among provenances for specific traits, for instance timing of bud flush and bud set, have indicated some genetic differentiation among provenances (Morgenstern 1978; Beaulieu *et al.* 2004). We used the provenance test described in Beaulieu et al. (2004) to assess phenology of wood formation and determine the relationships with bud phenology. As the phenology reflects the adaptation of plants to their environment, it is of great importance to understand how the provenances 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 flushing). We assessed the variation among and within provenance for bud and cambium phenology. We tested the hypothesis that provenances with an earlier bud flush exhibit an 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), which belongs to the balsam fir-white birch bioclimatic domain. The climate is cold and humid. The mean annual temperature over the last 30 years was 0.5°C, with a mean monthly temperature of -15.9°C and 14.6°C in January and July, respectively (climate station at 47.32° N and 71.15° O) (temperature conditions for 2014 and 2015 are presented in Figure 2). On average, there are 132 days with a minimum temperature >0°C. Annual precipitation is 1583 mm, of which 964 mm falling in form of rain. The snow is present from the end of October to mid-May.

The study was carried out in a provenance trial established in 1999, after harvesting the previous black spruce stand. Thirty black spruce provenances were planted in two main blocks, each including 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 (Figure 1), were chosen because they showed divergent bud flush, i.e. 4 early flushing class and 3 late flushing class, according to Beaulieu *et al.* (2004) which was confirmed by us in 2013. In average, 15 days of difference are observed between both classes through the bud flush. Three families per provenance and 2-3 trees per family were sampled for each of seven provenances, resulting in a total of 61 sampled trees (Table 1).

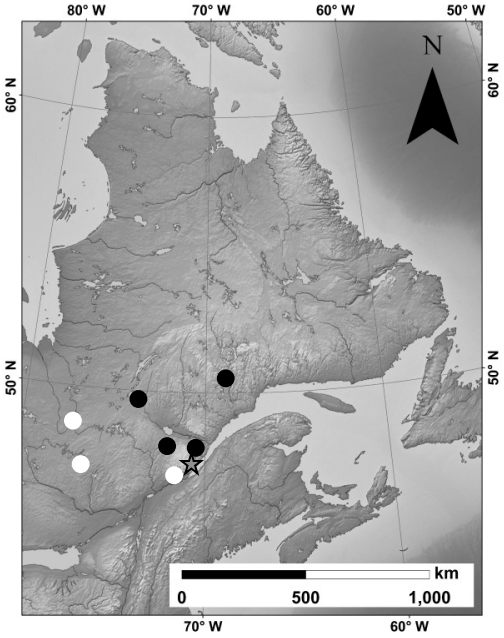

Figure 1: Origin of the seven provenances selected. Black and white dots indicate early and late flushing class, respectively. The star represents the location of the provenance test.

Table 1: Geographical and meteorological descriptions of studied provenances and trees

Provenance	Latitude (°N)	Longitude (°W)	Altitude (m a.s.l.)	Mean annual temperature (°C)	Total precipitation (mm)	Frost free days (days)	Growing season[1] (days)	Bud flushing class	Family	Number of trees
Nicabau Chibougamau	49.23	74.08	392	0.09	1026	89	114	Early	1	3
									2	3
									3	2
Parc des Laurentides	47.87	-71.2	861	-1.19	1340	89	113	Early	1	3
									2	3
									3	3
Manicouagan	50.67	-68.77	437	-0.59	1093	102	114	Early	1	3
									2	3
									3	3
Rivière Portneuf	48.5	-70.06	424	0.87	1207	90	162	Early	1	3
									2	3
									3	2
Parc de la Vérendrye	47.08	-76.55	394	1.81	1090	90	155	Late	1	3
									2	3
									3	3
Station Valcartier	46.54	-71.29	129	4.32	1284	103	183	Late	1	3
									2	3
									3	3
Senneterre	48.37	-76.95	363	0.35	1018	90	122	Late	1	3
									2	3
									3	3

[1]: Growing season is defined as the period between the last 3 consecutives days with frost (Tmin<0) in the spring and the first 3 consecutives days with frost (Tmin<0) in the fall.

Bud phenology

During 2014-2015, bud phenology was evaluated on the branches located at breast height, representing the lower-crown for most of the trees and mid-crown for the smallest ones, and weekly from April to October, according to Dhont *et al.* (2010). In spring, six phases of bud flush were defined: (BB1) open bud, with the scales starting to separate and a pale spot visible at the tip; (BB2) elongated bud, with lengthening scales; (BB3) swollen bud, with smooth and pale-colored scales but no visible needles; (BB4) translucent bud, with needles visible through the scales; (BB5) split bud, with open scales but needles still clustered; and (BB6) exposed shoot, with needles completely emerging from the surrounding scales and spreading outwards. In summer, five phases of bud set were defined: (BS1) white bud, initiation of the apical bud that is still hidden by the needles; (BS2) beige bud, with the bud increasing in size and showing beige scales; (BS3) brownish bud, with the bud increasing in volume and being enveloped by brown-turning scales; (BS4) brown bud, with a visible bud and needles starting to spread outwards; (BS5) spread needles, with an opaque brown, clearly visible bud, well developed, concave scales and needles spreading completely outwards.

Phenology of wood formation

Wood microcores were collected weekly from April to October on the stems of the trees, at breast height and all around the stems, with a Trephor (Rossi *et al.* 2006) The samples were stored in a mix of alcohol and water at 5°C. The microcores were dehydrated, embedded in paraffin, and cut in sections of 7 µm-in thickness 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.

Four different phases of cell development were identified: cambium, enlarging, wall-thickening, and mature cells. The important phases in cambium phenology were defined as following: (C1) first enlarging cell, (C2) first wall-thickening cell,

(C3) first mature cell, (C4) last enlarging cell and finally (C5) last wall-thickening cell. The enlarging cells showed a radial diameter at least twice larger than that of cambial cells. To discriminate wall-thickening cells, the polarized light was used. Indeed, because of the arrangement of cellulose microfibrils, the developing secondary walls shine when observed under polarized light. The colorant reacts with lignin, producing a color ranging from violet, for wall-thickening cells, to blue, for mature cells (Figure 3) (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).

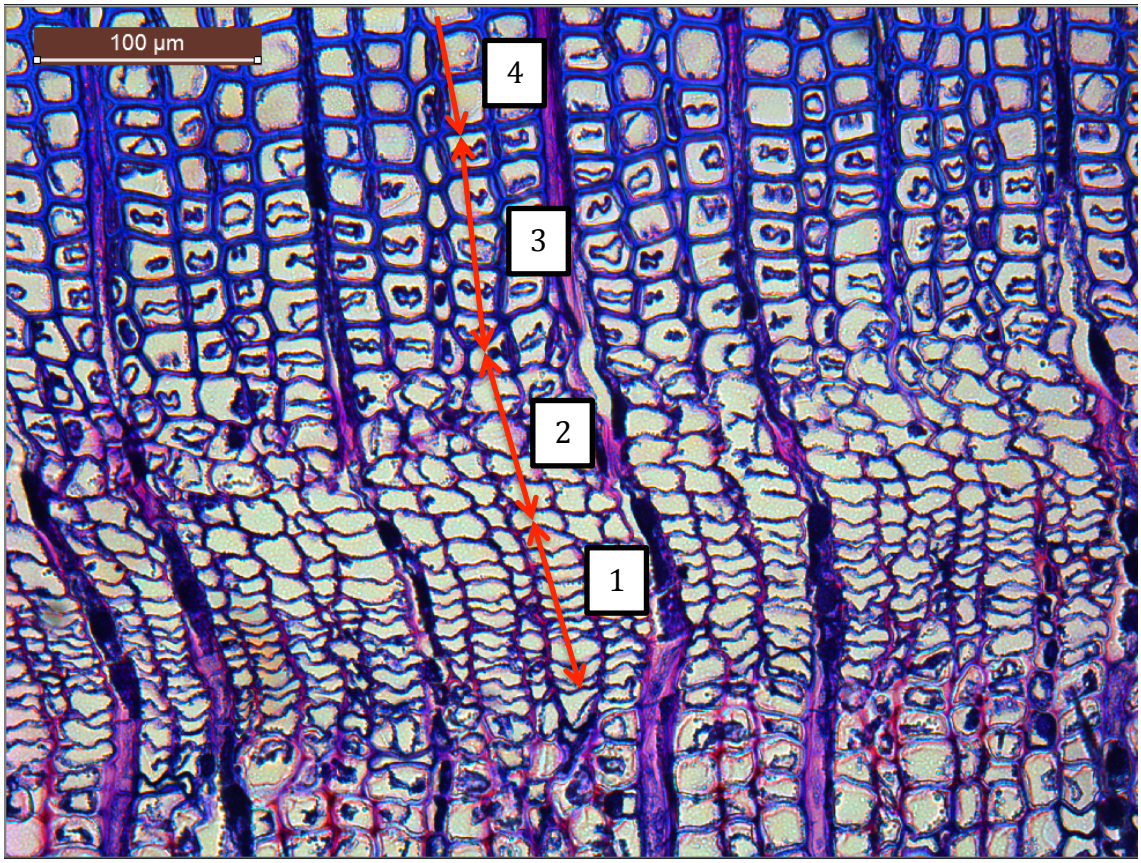

Figure 2: Example of cellular phases of tracheid formation: 1 is cambial cells, 2 is enlarging cells, 3 is cell-wall lignification cells and 4 is mature cells.

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) and year (y). The factor z was represented by either the Day of the Year (DOY) or the Heat Sum>5°C. We only studied the heat sum with a 5°C threshold because the literature was unclear about the best threshold (Man et Lu 2010) and it seems that results using 1°C, 5°C or 10°C are strongly correlated (personal communications, Julien Prunier). Only results based on DOY will be presented, as they showed similar trends as heat-sum results.

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*. A significant level of p=0.05 was used.

Results

Variance partition

The factor *tree* explained most of the estimated variance for the timing of bud flush, but its effect slightly decreased throughout the growing season from 45% to 30% (Fig. 3). The tree factor accounted for all the residuals, as it represents individual differences, others than year, provenance and family. This means that the tree effect takes in the phenotype, as it represents the individual genotype (G), part of the environmental variation (E) and their interactions (GxE). Forty-five percent of variation was explained by the differences among trees for open bud (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 year effect was noticed, from less than 5% (phases BB1 to BB5) up to 28% for exposed shoot (BB6) (Fig. 3)

The year effect explained most of the variance of the first two phases of bud set (BS1, 65% and BS2, 46%), and decreased to 11-1% for the successive phases (BS3-BS5). Conversely both family and provenance effects increased from 6% to 25%. A similar pattern was observed for the tree, which increased from 18% for white bud (BS1) to 60% at the end of bud set (BS5) (Fig. 3).

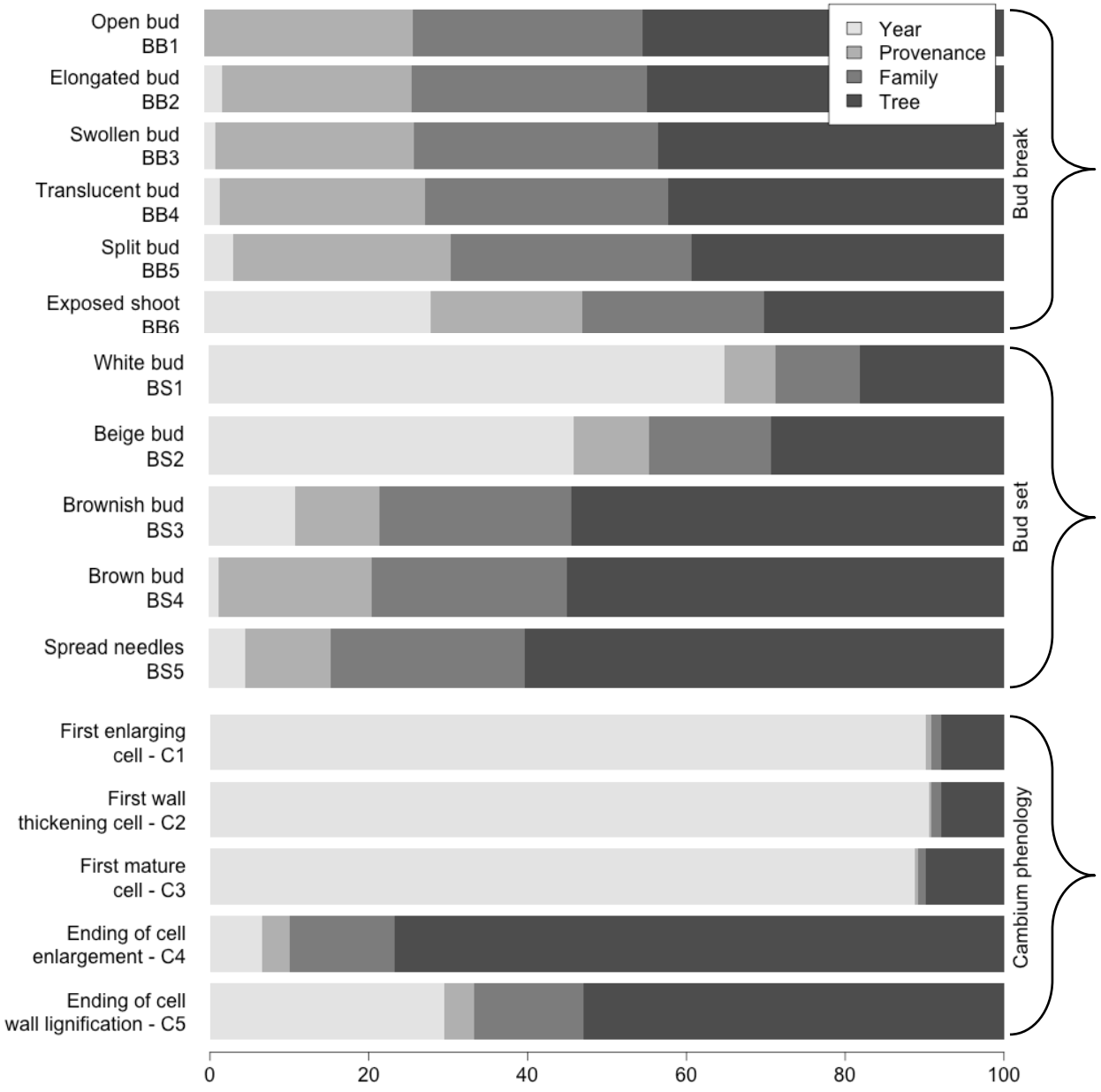

Figure 3: Variance partitioning for the different phases of bud phenology (bud flush and bud set) and cambial phenology in black spruce provenances

Xylem phenology showed a distinct pattern than that observed for bud flush and bud set. For phases C1 to C3 that occur during the spring, family and provenance had marginal effects (1-5%), while the year explained most of the variance (61-74%). The proportion of variance due to the tree ranged between 21% and 31%. For the last two phases C4 and C5 (ending of cell enlargement and cell wall lignification) the tree explained between 56 and 76% of the variance while the effect of the year was reduced to 20-4%. At the same time, family and provenance explained on average 18% and 2% of the variation of the same two phases (C4 and C5), respectively (Fig. 3).

Bud phenology and xylem phenology: variability between classes and years

The GLM produced significant models for all phases of bud flush, bud set, and wood formation. The factor DOY (Day Of the Year) was constantly highly significant ($p<0.001$) (Table 2). Indeed, we created the regressions in function of the DOY, so our variables (phases of each organ phenology) change according to it. As expected bud flush started earlier for all the early flushing provenances in both years. A small significant year effect was observed in bud flush, but only for open (BB1) and split bud (BB5) phases (Fig. 4). For the bud set, white bud (BS1), brownish bud (BS2) and brown bud (BS3) showed significant differences between years with 2015 being the earliest one (Table 2 and Figure 6). Such a result should be interpreted carefully for BS1 phase because some data were not recorded at the very beginning of this phase (missing data DOY 170-190) (Fig. 5). All phases of wood formation (C1 to C5) showed significant ($p<0.001$) differences between years for the logistic regression, confirming that 2015 was also the earliest year for cambium reactivation (Fig. 6a.), with about 5 days of differences between 2014 and 2015 for C1, C2 and C3. Very similar results were obtained with the heat sums models (results not shown), which means that the difference was not due to differences in temperature between years.

The bud flushing class (early vs late) impacted significantly all phases of bud set and all phases of xylogenesis, with the exception of the first wall-

thickening cell (C2) (Table 2). For bud flush, there was difference between both flushing classes in the starting dates (DOY of the beginning of each phase), ranging from 20.0 days for open bud (BB1) to 9.0 days for swollen bud (BB3) with an average of 12 days. (Fig. 4). The differences observed along the year between flushing classes was similar in both years. For the bud set, the difference in the DOY between both early and late flushing class was lower, 5.4 days on average, ranging between 0 for brownish bud (BS3) in 2014 and 10.0 days in 2015 for the white bud (BS1) (Fig. 5). For xylogenesis, the difference in days between the both flushing classes was 4.0 days on average, ranging from 1.0 days for the first wall-thickening cell (C2) to 5.0 days for the first enlarging cell (Fig. 5).

No significant difference in tree-ring growth was observed between early and late provenances (Table 3) although significant differences were observed between 2014 and 2015 (Table 3). The ANOVA showed no difference between flushing classes, and no interaction class×year. On average early provenances had a tree ring of 2169.8 μm in width, while late provenances had a tree ring of 2092.8 μm in width.

Table 2: Results for the logistic models. Z scores with the associated significance for each model and factor.

	Phase	DOY	Year	Flushing class
Bud break	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 phenology	C1 - First enlarging cell	15.19***	4.67***	6.03***
	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: Results of the two-way Anova comparing the annual tree-ring growth measured both in 2014 and 2015 in black spruce trees that represent early and late flushing classes.

	Df	F-value
Class	1	0.600
Year	1	54.134***
Class×Year	1	0.673
Residuals	23	
	6	

Three asterisks corresponds to p< 0.001

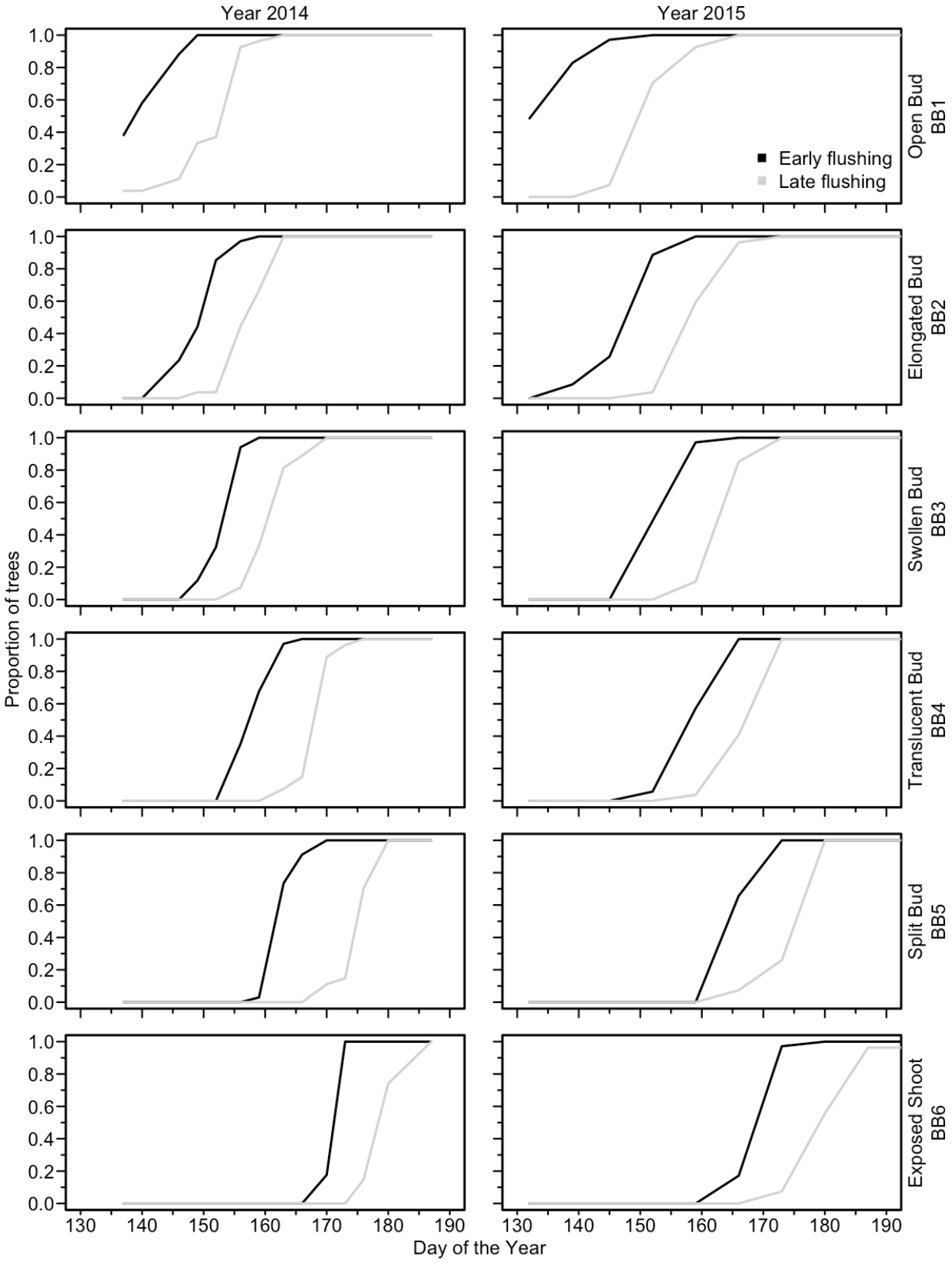

Figure 4: Proportion of trees reaching each phase of the bud flush process for early and late flushing classes of black spruce.

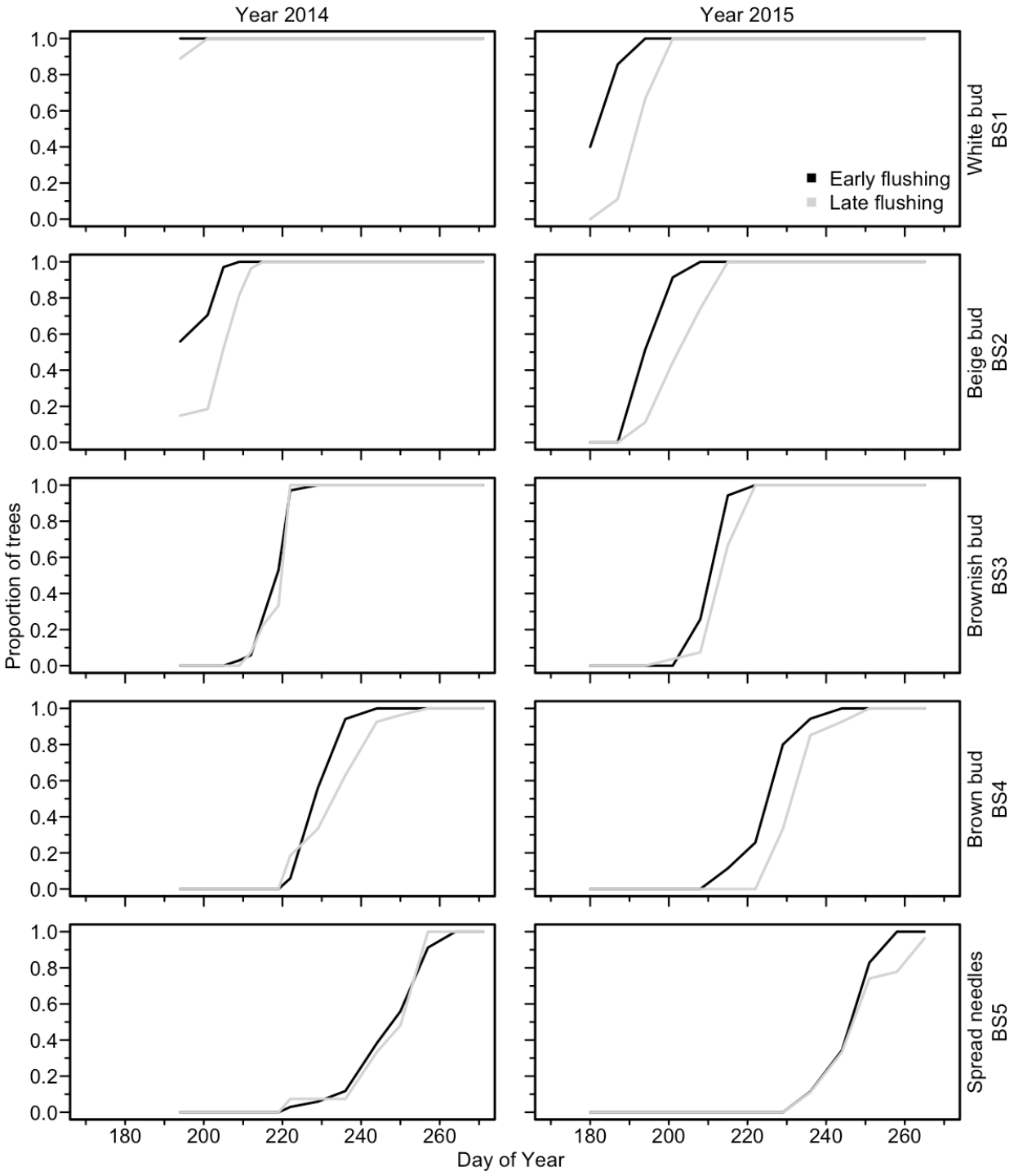

Figure 5: Proportion of trees reaching each phase of the bud set process for early and late flushing classes of black spruce.

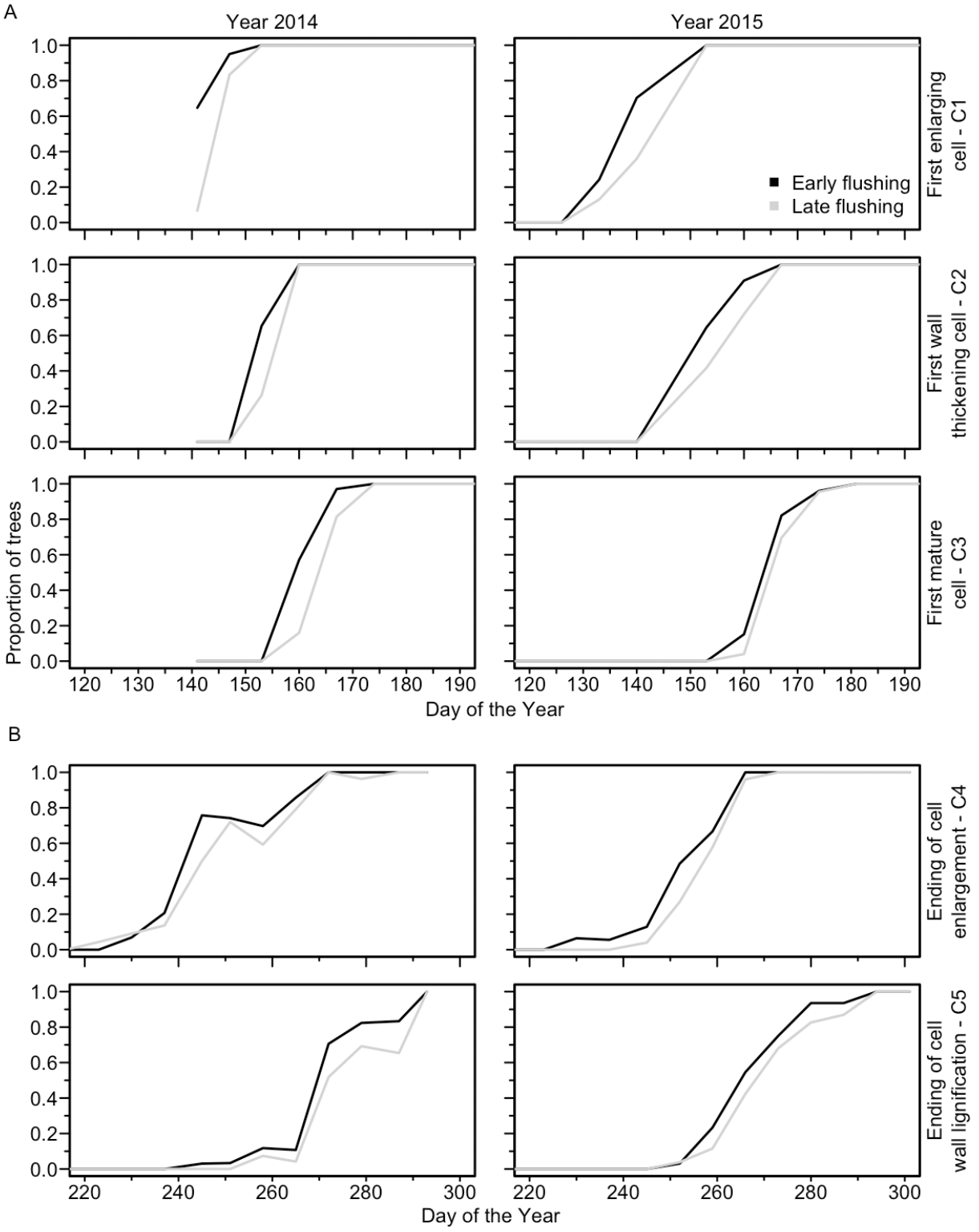

Figure 6: Proportion of trees reaching each phase of the wood formation (xylogenesis) for early and late flushing classes of black spruce. A = beginning of the growing season, B = end of the growing season.

Discussion

Bud and cambium phenology

In this study, we measured and compared the phenology of bud and cambium in seven black spruce provenances representing two flushing classes. We verify whether differences in bud flush timing (early vs late) corresponded to differences in the phenology of wood formation. The pre-assignment of provenances (either early or late flushing class) was based on observations conducted at the seedling stage (Beaulieu et al. 2004) and was confirmed for bud flush in our study. This means that the flushing classes remained the same across the beginning of the tree life (seedlings and young trees). This is in agreement with previous study in conifers that showed a high age-age correlations for bud flush phenophase (Aitken et Hannerz 2001).

The flushing traits corresponded well to differences in cambium phenology. We confirmed the hypothesis that provenances with an early bud flush have an early reactivation of cambial activity, and therefore start xylem cell differentiation earlier (7 days between flushing classes). A similar trend was also observed at the end of the growing season, (6 days of differences between the flushing classes). Concerning the bud set, differences between early and late flushing class were found, but to a lesser extent: the end of bud set showed little to no difference (0 to 1 days)

As early flushing provenances started and completed their growth earlier than the late flushing provenances, the bud flushing classes corresponded well to timings of cambial and apical growth. In a precedent study in natural stand of black spruce, Antonucci *et al.* (2015) showed a high correlation between both phenology

of cambium and bud flush. However in the abovementioned study, these results were observed for the phenophases occurring at the beginning of the growing season. Our observations from a provenance trial confirmed these previous findings while adding new information on the dynamics of xylem formation and bud set at the end of the growing season. A high correlation between both meristems was found throughout the whole 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 tree age, as we measured younger trees than Kalliokoski et al. (13 vs 80 year-old trees). It has already been shown that older trees are more adapted to their planting site and therefore show less differences among provenances (Beuker 1994). Moreover, young trees have a different lateral growth pattern from older trees, with ring areas, ring density and ring latewood proportion changing over the lifetime of an individual (Koubaa *et al.* 2004).

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 of conifers, for example in red and white spruce as shown in Blum (1988) and in black spruce (Johnsen et Seiler 1996a). Accordingly, the provenances classified as early in spring were also early to complete their growth, 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.

In the studied provenances, radial growth was similar, with an activity being shifted in time according to the flushing class. It has been suggested that an earlier growth resumption would lengthen the growing season in conifers of the northern hemisphere, leading to more cells to be produced by the cambium (Kalliokoski *et al.* 2011; Rossi *et al.* 2015). Our results did not confirm such 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, composed by genetically different provenances submitted to the same growing conditions. In addition, we characterized provenances only 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 the Northern ones (Morgenstern 1978; Girard *et al.* 2015). This raises new questions on the variability in xylem phenology within species and within populations. Our two bud flushing classes represented geographically distinct provenances of boreal forest region with different temperature conditions (average temperature for early = -0,21° and late = 2,16°, Table 1) showing different phenotypes, despite their similar growth. These populations have likely experienced significant selective pressures as suggested by Prunier et al., (2011) who detected significant allelic variations on genes involved in climate adaptation (Prunier *et al.* 2011).

Factors influencing cambial activity

Our study demonstrated that cambium phenology is controlled by both endogenous (genetic) and environmental drivers. These factors that influence xylem differentiation change along the growing season. Indeed, the year was the main factor for the beginning of cambium phenology (phases of first enlarging,

wall-thickening and mature cells). Differences accounted for years correspond with the meteorological conditions that vary between the years. Therefore, the reactivation of xylem growth seems to be more dependent on environmental conditions, as previously shown by Dufour et Morin (2010). However, the differences in cambial phenology found between the early and late flushing classes in the regressions indicated that adaptive component likely plays a significant role, although 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 could be affected by different 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 weather conditions occurring at their origin site (Rossi et Isabel 2016).

The end of xylem growth (i.e. the timing of the last enlarging and cell-wall thickening cell) is potentially under a higher 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 logical continuation of previous tree phenophases in the canopy, also controlled by genetics. Moreover, 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. For instance, several studies involving the mechanisms inducing dormancy in Norway spruce showed that growth cessation was mainly under endogenous control, and that meteorological stimuli had a minor effect (Cooke *et al.* 2012).

Factors influencing bud phenology:

In black spruce, temperature and photoperiod are the two main environmental drivers (natural selection) that affect tree growth responses (Morgenstern 1978). In our study, we have assumed that the year reflects the environmental variation. For bud flush, only two phases (open bud and exposed shoot) were impacted significantly by year. However in the case of the open bud, the year explained only 0.1% of the variation. On average 2014 and 2015 showed only 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). We can hypothesize that it is probably the case for black spruce, since the beginning of bud flush is only partly influenced by environment.

The effects of provenance, family and tree decrease according to the phases: the beginning of the bud flush (the first two phases) seems to be under stronger genetic control than the subsequent phases. These results are new, and can be explained by the phase classification used, which varies across literature (Beuker 1994; Dhuli *et al.* 2014). Indeed, the use of 6 phases is rare. In the majority of studies, the bud is observed as closed vs open and the bud flush is considered happening at the equivalent of the exposed shoot (Søgaard *et al.* 2007). Moreover, the difficulty to reach some sites at the very beginning of the spring (due to snow cover) might have restricted such observations in precedent studies.

The differences among provenances in bud flush were also found in studies for the red and white spruce (Blum 1988) and sessile oak in an altitudinal gradient (Alberto *et al.* 2011) but are opposed at what was found by Vitasse *et al.* (2009) in temperate deciduous trees, where no differences were observed. Indeed, Vitasse *et al.* (2009) studied only two populations with a reduced latitudinal gradient:

Pyrenean mountains and Paris. The local adaptations in bud flush to climate might need a greater range of temperature. Indeed, in black spruce seedlings, boreal provenances were found to initiate their growth (timing of bud flush) and bud formation earlier than southern provenances (Morgenstern 1978; Johnsen et Seiler 1996b). Some studies observing young trees (23 years-old) found similar results than seedlings (Johnsen et Seiler 1996a). With our trees being 16 and 17 years old when the observations were conducted, our results fall within the same range that those already found (Johnsen et Seiler 1996a; Johnsen et Seiler 1996b).

For the bud set, there were also two main tendencies, with white and beige bud (beginning of the bud set) under different controlling factors than the other phases (end of bud set). Thus, the beginning of the bud set was majorly under environmental control. Indeed, environmental stimuli such as temperature and photoperiod are essential to start bud formation and subsequent dormancy processes (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 the growing conditions of 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, the family and the provenance, which means that the end of bud formation and growth cessation is mainly under genetic control. Indeed, we know that there is determinate growth in conifers: the growth cessation is mainly genetically pre-determined and effects of the environment on the phenology are only minor (Junttila 1976; Cooke *et al.* 2012).

Bud and cambium phenology is affected by different factors: the beginning of bud phenology in spring (bud flush) is majorly controlled by genetic whereas the beginning of xylem formation in spring (first enlarging, first wall-thickening and first mature cells) are under environmental control. On the other hand, the end of the growing season shows an inverse tendency: the bud set is first largely

influenced by environmental components followed by an increase of the degree of genetic control, and the cambium phenology is controlled by genetics.

Conclusion

In this work, we compared timings of bud phenology and cambium phenology among black spruce provenances assigned to early and late bud flushing class. The aim was to verify if a different timing of bud flush in spring would influence the bud set phenology as well as the cambial phenology. Our results showed that trees from different locations but growing under the same environmental conditions can have different phenologies, with timings of events shifted throughout the growing season. This means that early flushing trees and late flushing trees experience a different timing in growing season. Bud and xylem development are complex growth processes, regulated by several interacting factors. The environment and genetics both influence these processes in different proportion, depending on the phase studied and with interactions: an effect can have a small impact and still be significant. Our study raise the need to identify the environmental factors involved in growth resumption and their interaction with the endogenous factors, for a better understanding and a better preparation for the climate change.

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CONCLUSION GÉNÉRALE

La croissance d'un arbre dépend de la phénologie des méristèmes primaire et secondaires, dont les liens sont encore flous et peu étudiés. Cette étude a permis de voir que le type de débourrement influence l'aoûtement ainsi que la phénologie du cambium. En effet, les individus hâtifs dans le débourrement le sont aussi au niveau de l'aoûtement et du cambium, quelle que soit la phase phénologique. L'hypothèse principale d'une conservation de l'écotype hâtif ou tardif le long de la saison de croissance ainsi que dans les différents types de méristèmes a été confirmée. Ces résultats sont différents des précédentes études réalisées sur l'épinette de Norvège, ou aucune différence entre provenances n'avait été prouvée (Kalliokoski *et al.* 2011). En revanche, des relations entre le débourrement et la phénologie du cambium avait déjà été étudié pour le début de la saison de croissance par Antonucci *et al.* (2015). Nos résultats confirment et prolongent ces recherches à la fin de la saison de croissance, durant l'aoûtement et la mise en dormance du cambium.

Le décalage entre arbres hâtifs et arbres tardifs entraine que les saisons de croissance sont de la même longueur, peu importe le type de débourrement. Les résultats de l'étude de Rossi *et al.* (2014) montrent que la longueur de la saison de croissance plus important que le taux pour la productivité chez l'épinette noire. Avec une même longueur, la production totale de bois des arbres hâtifs ou tardifs est donc la même pour tous.

Au niveau des facteurs contrôlant les processus, nous avons pu observer l'impact de la génétique ainsi que de l'environnement. Le débourrement dépend surtout de la génétique alors que l'aoûtement est majoritairement sous control de l'environnement. Ces résultats confirment les précédentes recherches (Pelgas *et al.* 2011; Delpierre *et al.* 2015). Certaines études ont montré des différences avec nos résultats (Basler et Korner 2014) mais cela est probablement du à l'utilisation de 6

stades différents pour décrire chaque chronologie, ce qui n'est pas utilisé dans les articles précédents.

Pour ce qui est du cambium, le début de la saison de croissance est majoritairement influencé par l'environnement. La réactivation du cambium est connue pour être sous contrôle de signaux externes comme la température ou les précipitations, en accord avec d'autres résultats précédents (Ren *et al.* 2015; Vieira *et al.* 2015). En revanche, la génétique est quand même importante puisque les différences entre provenances se sont montrées significatives.

La fin de la croissance secondaire est surtout contrôlée par la génétique. Des études ont montré que la dormance dépendait de la température pendant l'embryogenèse (Kvaalen et Johnsen 2008). Le lieu de provenance de la mère, et donc l'origine de l'arbre sont donc des plus importants, puisqu'il va affecter l'embryogenèse et plus tard la phénologie.

De façon générale, nous avons confirmé notre hypothèse de départ, qui était que le type de débourrement influencerait la phénologie du bourgeon à l'automne et du cambium tout au long de la saison. De plus, nous avons pu observé que le type d'arbre, hâtif ou tardif, n'impacte pas la production totale de l'arbre. Et enfin l'étude des facteurs contrôlant les différents stades de la phénologie nous a permis de voir les relations complexes entre l'environnement et la génétique pour l'adaptation locale de l'épinette noire. Cela sera utile pour permettre de prévoir l'impact des changements climatiques globaux et l'effet des provenances lors de ces changements. Cette étude pourrait être continuée dans le temps pour voir l'effet de l'environnement, changeant à chaque année, de façon plus systématique. De plus, cela permettrait de voir si les arbres finissent par converger à une adaptation à leur nouvel environnement et confirmer ainsi les propos de Beuker (1994) mais au niveau de *P. mariana*.