SYNCHRONISMS AND CORRELATIONS OF SPRING PHENOLOGY BETWEEN APICAL AND LATERAL MERISTEMS IN TWO BOREAL CONIFERS

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Abstract

Phenological synchronisms between apical and lateral meristems could clarify some aspects related to the physiological relationships among the different organs of trees. This study correlated the phenological phases of bud development and xylem differentiation during spring 2010-2014 in balsam fir (*Abies balsamea* Mill.) and black spruce [(*Picea mariana* Mill. (BSP)] of the Monts-Valin National Park (QC, Canada) by testing the hypothesis that bud development occurs after the reactivation of xylem growth. From May to September, we conducted weekly monitoring of xylem differentiation using microcores and bud development with direct observations on terminal branches. Synchronism between the beginning of bud development and xylem differentiation was found in both species with significant correlations between the phases of bud and xylem phenology. Degree-day sum was more appropriate in assessing the date of bud growth resumption, while thermal thresholds were more suitable for cambium phenology. Our results provide new knowledge on the dynamics of spring phenology and novel information on the synchronisms between two meristems in coniferous trees. The study demonstrates the importance of precisely defining the phases of bud development in order to correctly analyse the relationships with xylem phenology.

Introduction

Phenology is one of the most responsive plant traits driven by climate (Badeck et al. 2004), making trees reliable indicators of climate change (Donnelly et al. 2004). Phenological observations record earlier events in terms of bud break, shooting, leafing and flowering, which may be related to the recent spring warming and extended growing seasons (Chuine and Beaubien 2001, Menzel et al. 2006). In spring, bud development is mainly regulated by temperature (Sarvas 1972), although other interacting factors such as chilling and photoperiod can play an important role (Heide 1993, Häkkinen et al. 1998, Partanen et al. 2001, Rossi et al. 2007). Air temperature is a good predictor of bud development and shoot elongation, once ontogenetic development begins (Schwalm and Ek 2001).

Bud swelling is a phenological event of growth resumption composed of sequential events during which the embryonic shoots and leaves rapidly proliferate and emerge from the bud scales. Generally, studies on apical meristem have identified bud swelling with the phase of leaf unfolding (Takahashi and Koike 2014, Cuny et al. 2012, Moser et al., 2009), while few studies have taken into account all bud development phases (Dhont et al. 2010, Huang et al. 2014, Rossi and Bousquet 2014).

As with bud development, stem growth also occurs through different phases. Secondary xylem production is a complex process that derives from cell periclinal and cataclinal division in the vascular cambium. The differentiation of annual rings in conifers involves the formation of tracheids that pass through several phases before reaching their final form (Deslauriers et al. 2003). Several studies have revealed an early onset of cambium activity and xylem cell production as well as a longer duration of xylem formation and this has

61 often been related to the influences of the on-going climate change (Huang et al. 2011,
62 Boulouf-Lugo et al. 2012). The activity of meristems involves a number of biochemical
63 processes resulting in a sequence of phases of development and maturation. These phases
64 are identified according to the morphological or anatomical changes and can last from a
65 few days, in primary meristems, to several weeks, in the case of secondary meristems
66 (Rossi et al. 2013, Rossi 2015).
67 Aloni (2015) showed how leaf development (related to the primary meristems) and biomass
68 accumulation (related to the secondary, or lateral, meristems) are regulated by a number of
69 environmental factors. These factors influence the wood production and its biochemical
70 traits, but also the amount and differentiation of vascular cells. Moreover, physiological
71 factors, such as carbohydrate availability and hormone distribution, could determine the
72 dynamics of wood formation (Uggla 1998, 2001, Rossi et al. 2008). Studies on bud and
73 cambium phenology have revealed different patterns and links between primary and
74 secondary meristems. In conifers, the last phase of bud development, often identified as bud
75 break, has been found to occur either before or after the onset of xylem cell production
76 (Ladefoged 1952, O' Reilly and Owens 1989, Rensing and Owens 1994, Rossi et al. 2009,
77 Cuny et al. 2012), while in diffuse porous species, cambial reactivation takes place
78 immediately after bud break (Schmitt et al. 2000, Čufar et al. 2008). Thus, the relationships
79 between apical and lateral meristems are still unclear, at least in conifer species.
80 Understanding these relationships is crucial for identifying the timings of C-uptake and
81 seasonal exchanges of water, nutrients and gases between the land surface and atmosphere
82 (Chen and Xu 2012). In order to elucidate the role of meristems as indicators of the effects
83 of climate change, in particular warming temperature, the relationships between primary

and secondary growth need to be defined for a mechanistic understanding of the growth dynamics in all tree organs (Huang et al. 2014).

There are still few ecophysiological studies aiming to explain the relationships between bud and cambial phenology and their synchronism over the growing season (Deslauriers et al. 2009, Huang et al. 2014). Characterisation of the relationships between environmental factors, particularly temperature, and meristem activity is crucial to understand the fate and differentiation of meristem cells and their coordination under environmental stresses. Heat accumulation above a threshold temperature, commonly expressed in degree-days, has often been used to predict or verify the effect of temperature on biological processes (Baskerville and Emin 1969). In this study, having monitored and compared the timings of bud and xylem development in two conifer species of the boreal forest, we identified the spring temperature thresholds and cumulated degree-days required for triggering the first phases of bud and cambium development. The study was conducted during five growing seasons, investigating in detail all the phenological phases for growth resumption of bud and cambium and their climatic drivers, to improve understanding of seasonal patterns and responses to inter-annual and long-term variation in climate. The hypothesis that the beginning of bud development occurs after the reactivation of xylem growth was tested.

Materials and Methods

Study site and tree selection

This study was conducted in the Monts-Valin National Park (QC, Canada). Two sites at different altitudes were selected in the black spruce-feather moss bioclimatic domain, Gaspard (abbreviated as GP) and Lagacé (LA), located at 227 and 900 m a.s.l., respectively (Table 1). The climate in the region is dominated by dry and moderately polar air masses in winter and moist maritime air masses in summer (Sheridan 2002). Absolute minimum temperature reaches -41 °C, and mean air temperature ranges between -1.4 and 2.5 °C, with annual precipitation higher than 1000 mm. The sites experience between 204 and 256 frost days $year^{-1}$.

Primary and secondary growth was monitored weekly from May to September, during the 2010-2014 growing seasons, in balsam fir [*Abies balsamea* (L.) Mill.] and black spruce [*Picea mariana* (Mill.) BPS]. Three trees per species were randomly selected in each site, choosing healthy dominant or co-dominant individuals.

Bud phenology

Two north-facing and two south-facing branches per tree were selected in the bottom part of the canopy. On each branch, the phases of bud development were recorded on terminal buds according to Rossi and Bousquet (2014): (1) open bud, with the scales starting to separate and a pale spot visible at the tip; (2) elongated bud, with lengthening scales; (3) swollen bud, with smooth and pale coloured scales but no visible needle; (4) translucent bud, with needles visible through the scales; (5) split bud, with open scales but needles still clustered; and (6) exposed shoot, with needles completely emerged from the surrounding

125 scales and spreading outwards. All data were computed in days of the year (DOY), by
126 averaging the data observed in the field.
127
128 **Cambial phenology**
129 Wood microcores (2 mm in diameter) were extracted from the stem at breast height, using a
130 Trephor tool (Rossi et al. 2006*a*). The samples contained the preceding 4-5 tree rings and
131 the developing annual layer with the cambial zone and adjacent phloematic tissue. The
132 microcores were placed in Eppendorf microtubes with an ethanol solution (50% water) and
133 stored at 5 °C to avoid tissue deterioration. Microcores were dehydrated with successive
134 immersions in ethanol and D-limonene and then embedded in paraffin according to Rossi et
135 al. (2006*a*). In order to obtain thin slices, the samples were cut (7 µm in thickness) using a
136 rotary microtome. The sections were stained with cresyl violet acetate (0.16% in water) and
137 examined within 10-15 min under visible and polarized light at 400-500× magnification to
138 differentiate the developing and mature xylem cells.
139 The radial number of cells was counted along three radial rows, according to the criteria
140 described in Rossi et al. (2006*b*). Observations under polarized light discriminated the
141 zones of enlarging and cell wall thickening of tracheids. The progress of cell wall
142 lignification was detected with cresyl violet acetate reacting with lignin (Antonova and
143 Shebeko 1981). Lignification was shown by a colour change from violet to blue. The colour
144 change over the whole cell wall revealed the end of lignification and the tracheid reaching
145 maturity (Gričar et al. 2005). The dormant cambium consisted of 3-4 cells. In spring, when
146 the diameter of the new xylem cells was at least twice that of a cambial cell, the onset of
147 cell division was considered to occur. Xylogenesis was considered to have started when at

least one radial file of enlarging cells was observed (Rossi et al. 2008) (Fig.1). In late summer, when no further cell was observed in wall thickening and lignification, xylem formation was considered complete.

Weather stations

In both sites, a standard weather station was installed in a forest gap to measure temperature and precipitation. Data were collected every 15 min during the five years of monitoring and recorded as hourly averages by means of CR10X data-loggers (Campbell Scientific Corporation, Logan, UT, USA). Daily mean values were calculated with the time series obtained from the 24 measurements per day.

Data analysis

Timings of bud phenology were compared between species, DOY and years with analysis of covariance (ANCOVA). Phases of bud and xylem phenology were compared using analysis of variance (ANOVA), while the Tukey test was applied for multiple comparisons. The correlations between phenological phases were tested through Pearson correlations. Degree-days sum (DDS) at the onset of bud and cambium phenology were calculated as a sum of the positive differences between mean daily air temperature and the threshold value 0 °C (Man and Lu 2010). This threshold was used for calculating DDS according to Man and Lu (2010), who suggested 0 °C as an optimal base temperature for black spruce. Thermal thresholds (THR) for bud and xylem phenology were assessed using logistic regressions to calculate the probability of active meristem growth at a given measured daily temperature. Binary responses were coded as non-active (value 0) or active (value 1)

171 growth, and temperature thresholds were calculated when the probability of active
172 meristem growth was 0.5 (Rossi et al. 2008). Coefficient of variation was used for
173 comparing DDS and THR, given that these measurements take non-negative values. All
174 statistical analyses were conducted using JMP (Version 11, SAS Institute Inc., Cary, NC,
175 USA).
176

Results

Bud phenology

The phases of bud phenology occurred earlier in fir than in spruce at both altitudes, though showing different trends in the investigated years. The period of bud development was shorter in fir than in spruce, and longer in 2010 and 2013 than in other years. In 2014, the trend of the two species was similar at lower elevation, and almost identical at higher elevation. Occasionally, some intermediate phases were not recorded, especially at low elevation, because they occurred within the same sampling week. The first bud development phase occurred in the second half of May. The two species showed a week of difference for phase 1, occurring on average on DOY 138 and 145 in fir and spruce, respectively. Phase 6 occurred approximately 4 and 5 weeks after phase 1 in fir and spruce, respectively (Figure 2). In LA (900 m a.s.l.), bud phenology started 2 weeks later than in GP (227 m a.s.l.). ANCOVA model was highly significant ($p<0.001$) for both altitudes, with F=435.77 and 431.51 for 227 m and 900 m a.s.l., respectively (Table 2). As expected, the bud development phases occurred on different DOY. The two species showed different phenological development behaviour and the years considered differed from each other. All the factors considered were statistically significant with $p<0.001$.

Xylem phenology

In the analysed cross section, cambial cells were characterized by thin cell walls and small radial diameters. At the onset of cambial activity, the cambial zone began to widen rapidly as the number of cells increased, revealing that cell division had started. During cell

enlargement, the tracheids were composed of protoplast still enclosed in the thin primary wall, but with radial diameter at least twice that of a cambial cell.

The start of xylem cells production was similar and within the same week in the two species (Figure 3). As expected, xylem growth and differentiation started first at lower elevation, at the beginning of May. At higher elevation, cambial resumption occurred between the end of May and beginning of June. The first mature cells were recorded 22 days and 24 days after the start of enlargement in fir and spruce, respectively. The difference in days of mature cell production was greater in LA than in GP. In 2010 and 2013, too few micro-cores were available to determine the date of cell maturation with precision.

Comparing xylem and bud phenology

The correlations between the date of bud and xylem phenology were highly significant (Table 3). A strong association was detected between the open bud (phase one) and date of the first enlargement for both fir (r=0.91) and spruce (r=0.90) (p<0.001). The first mature xylem cell and the stage of exposed shoot showed a significant correlation in fir (r=0.98, p<0.01), but not in spruce (r=0.57, p>0.05), meaning that the variation between both stages is not always synchronous for spruce.

The multiple comparison of phenological events, performed with ANOVA (p<0.001), showed that the dates (DOY) of first cell enlargement and open bud phases were not statistically different in fir. In addition, DOY of the first mature cell and exposed shoot phases did not show statistical difference. In spruce, only the first phases of xylem differentiation and bud development at higher elevation showed no statistical differences.

222 On the contrary, differences were observed at lower elevation between all the analysed
223 phases (Figure 4).
224
225 **Degree-days sum and thermal threshold**
226 Open bud occurred in fir and spruce when 230±44 (mean ± standard deviation) and 284
227 ±33 degree-days had accumulated, respectively. The occurrence of the first enlargement
228 cell was observed at lower DDS than the open bud phase, with 217±43 degree-days in
229 spruce and 201 ±37 degree-days in fir. DDS in the exposed shoot phase were higher than
230 DDS in the first mature cell phase (Figure 5). The mean THR were estimated at 7.9±1.2 °C
231 for the first enlarging cell phase in spruce, and 7.4±1.5 °C in fir. The open bud phase had a
232 THR of about 9.1±1.2 °C in spruce, and 7.7±1.8 °C in fir (Figure 5). The standard
233 deviations of DDS and THR were similar between the first phases of xylem differentiation
234 and the first phases of bud phenology, in both species. For spruce, the coefficient of
235 variation showed higher values for the first enlarging cell phase than for the other phases,
236 though the exposed shoot phase showed higher values than the open bud and first mature
237 cell phases. The same trend was observed for the coefficient of variation of THR. In fir, the
238 highest coefficient of variation was found in the open bud phase, which occurred for both
239 DDS and THR. The coefficient of variation was smaller for DDS than for THR for the open
240 bud phase, in both species. Finally, the first enlarging cell phase showed higher coefficient
241 of variation for DDS than THR (Table 4).
242

Discussion

Synchronisms and correlations between meristems

In this study, we monitored all the phenological phases of bud development and xylem differentiation in black spruce and balsam fir during five growing seasons, investigating the possible relationships between bud development and xylem phenology. This is the first study, to our knowledge, that examines long-term chronologies of the two meristems in these boreal species. The hypothesis that the beginning of bud development in conifers occurs after xylem reactivation was only partially accepted. This result was related to the criteria defined a priori in this study. Generally, bud break has been represented by the last phases of bud development, leaf unfolding (Rossi et al. 2009, Moser et al. 2010, Cuny et al. 2012, Takahashi and Koike 2014). However, the bud growth resumption begins one month before or even earlier, when the scales start to separate following bud swelling. Although the phases of bud phenology are well known and documented (Dhont et al. 2010, Rossi and Bousquet 2014, Sutinen et al. 2012), we are not aware of any studies that considered their relationships with xylem phenology in detail. Indeed, according to the general definition of bud swelling, apical phenology can occur before or after cambial resumption. However, from a biological point of view, the phase of bud swelling, occurring when the scales start to separate and a pale spot is visible at the tip (Rossi and Bousquet 2014), and not that of leaf unfolding, seems to be the most appropriate to represent the break of bud dormancy, as also suggested by Sutinen et al. (2012). As a consequence, we are confident that the beginning of xylem differentiation was synchronous with the beginning of bud development in both the studied species, although no causal relationship between the two meristems can be deduced by this monitoring. Temperature is known to control the

266 phenological events in spring, thus the synchronisms observed between meristems could be
267 the results of a response of the meristems to a common driving factor (e.g., high
268 temperature and available liquid water in early spring may promote photosynthetic activity
269 and carbohydrate storage).
270 Recent studies have demonstrated that localized heating anticipated cambium reactivation
271 and earlywood vessel formation in seedlings of a broadleaved deciduous species, in
272 comparison with non-heated seedlings, and occurred before bud break (Kudo et al. 2014).
273 Through artificially heated and cooled stems, several other authors were able to induce an
274 earlier break of cambial dormancy and higher rates of cambial cell production (during
275 heating) and a delay in cambial reactivation and lower rates of cambial cell production
276 (during cooling) (Oribe and Kubo 1997, Oribe et al. 2001, 2003, Gričar et al. 2006, Begum
277 et al. 2007, 2010, 2012, 2013). Nevertheless, our results showed a strong coupling between
278 phases of bud development and those of xylem phenology in spring, under natural growing
279 conditions in the boreal forest. This means that both phenological patterns varied in a
280 similar manner along the time axis, i.e., when the phase of open bud occurred earlier, the
281 first enlarging cell was also observed earlier. The synchronization between the last two
282 phases, exposed shoot in bud phenology and mature cell in xylem differentiation was
283 unclear, because significant correlations between the last phases of the two meristems were
284 observed only in fir. Variability in xylem and phloem phenology among years and between
285 species might be determined by exogenous factors (contrasting temperatures prevailing at
286 the start of the growing season, water availability) and intrinsic factors (gene expression,
287 hormonal signals) (Swidrak et al. 2014). Correlation between primary and secondary
288 growth processes over the growing season would indicate optimal mechanisms to

289 simultaneously allocate photosynthetic products and stored non-structural carbon for the
290 growth of the different organs in trees (Huang et al. 2014).
291
292 **Synchronisms between species**
293 Phenological differences were observed between the two species. Bud phenology began
294 between mid-May and late-June. Fir began earlier than spruce, which might be attributed to
295 a greater sensitivity to spring temperatures, fir being a microthermal species in comparison
296 with spruce. This behaviour could also be explained by genetic differences related to the
297 species-specific capacity to perceive exogenous signals through hormones (Aloni 2001).
298 Jones and Cregg (2006) studied the timings of bud break in different types of North
299 American firs, showing that the date varied with locations, species and years. As expected,
300 in all the years considered, open bud occurred first in trees at lower elevation. This was
301 attributed to earlier spring warming at lower elevation (Moser et al. 2010). Several studies
302 suggest that the accumulation of lipids in the cortex area of the buds would trigger a series
303 of events connected to the onset of bud development (Sutinen et al. 2012, Alaudinova and
304 Mironov 2010). However, studies on xylem phenology showed similar timing between
305 species. Thibeault-Martel et al. (2008) showed no differences in onset of cell enlargement
306 for *A. balsamea* and *P. mariana,* whereas the same cambium activity date was observed for
307 fir and pine trees in temperate environments (Cuny et al. 2012, Gričar et al. 2014).
308 By comparing bud phenology, it was possible to disentangle the differential responses to
309 climate patterns in these co-occurring species. While conservative responses (low
310 sensitivity to warming temperature) may opportunistically minimize the risk of late (spring)
311 or early (autumn) frost at little cost (Guy 2014), with greater climate warming,

phenologically flexible species may advantageously occupy new early or late temporal niches within a short time (Wolkovich et al. 2013). Black spruce has a current competitive advantage over balsam fir in coniferous forests of the boreal shield due to a greater tolerance to cooler temperatures and soil waterlogging (Messaoud et al. 2014). However, warmer temperatures will potentially cause asynchrony in biological activities, disrupting ecological interactions (e.g., between these conifers and spruce budworm and/or fire). A longer period for primary and secondary tree growth with warming temperature could advance black spruce phenology, making this species a more suitable host for spruce budworm.

Temperature of growth

Accumulated temperature in spring is important for bud development and the onset of radial growth (Tadaki et al. 1994). DDS increased in the order of first cell enlargement, open bud, first mature cell and exposed shoot phases, as did the thermal threshold. In our study, we used the coefficient of variation for comparing the methods generally applied in agriculture and forestry to evaluate the influence of temperature on phenological phases. The coefficient of variation for the open bud phase was lower in DDS than in THR. The opposite occurred for the first cell enlargement phase. This coefficient indicated that DDS better explained the first phase of bud phenology, whereas thermal thresholds were more suitable for the first phase of xylem differentiation. The degree-days, therefore, appeared to be the more appropriate monitoring technique for apical meristems, while the thermal threshold was more advisable for cambium phenology. Rossi et al. (2008) determined common critical temperature thresholds for xylogenesis in conifers at different latitudes and

altitudes in Europe and Canada, with average daily temperature values of 8–9 °C. Analyses at a wider geographical scale comparing several conifer species have confirmed convergence of the thermal threshold around specific critical values, demonstrating the existence of a precise thermal limit in radial growth and tree ring formation (Deslauriers et al. 2008, Rossi et al. 2008). Prislan et al. (2013) observed that the onset of cambial cell division in *Fagus sylvatica* L. was associated with an extended period of preconditioning that varied with site, rather than with a threshold temperature. Patterns of cambial activity and climate thresholds have been found to vary among sites in *Picea abies* (Gričar et al. 2014), suggesting that local adaptation may play a role in coupling processes of wood formation and foliage renewal within populations. Gričar et al. (2014) showed site-specific amounts of accumulated heat units at the onset of cambial cell production. In contrast, Schmitt et al. (2004) reported that growing degree-days for the onset of cambial activity in *Pinus sylvestris* were similar along a latitudinal gradient in the boreal forests of Finland. Instead, DDS have been used to predict the growth stages of major crops and trees, particularly in tracking bud development (Wang 1960, Førland et al. 2004, Jones and Cregg 2006). It should be pointed out that longer and warmer summers at higher elevations and northern latitudes might result in disproportional resource requirements for these species, namely water.

Conclusions

To our knowledge, this is the first study that examines the synchronisms between the phases of growth resumption in buds and xylem, using chronologies of 5 years of data collected from two species and two sites. Detailed phases of bud break and xylem cell

differentiation showed a significant synchronism in the timings of activation between the two meristems. Considering the bud swelling as break of dormancy and not exposed shoot, we found that the beginning of bud development occurred in the same period as xylem differentiation in both species. This demonstrates the importance of considering all phases of bud break in order to correctly evaluate the relationships with xylem phenology. Data from the two meristems were strongly correlated to one another, although fir dormancy release was earlier than spruce. This study allowed the dynamics of spring phenology to be explored in detail, providing novel information about the potential physiological correlation between meristems and filling an important gap in the knowledge on the growth synchronisms in conifer species. A straightforward quantification of the dynamics of growth and response of phenology to climatic drivers is essential to link plant-level events of life cycles with the environmental factors occurring at short time scale. The presence of synchronism in leaf and cambial activity in these conifers may be conveniently used to relate field (leaf phenology and wood production) and satellite (vegetation indices) data and explain seasonal variability in boreal forest productivity.

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Conflict of interest

None declared

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543

CAPTION LIST

Table 1. Mean and standard deviation of the characteristics of the trees in the two study sites.

Table 2. *F*-value and resulting probability of the ANCOVA model for the phases of bud phenology.

Table 3. Correlation matrix between dates of bud and cambium phenology during 2010-2014 in spruce (white background) and fir (grey background). One, two and three asterisks correspond to a probability lower than 0.05, 0.01, and 0.001 respectively.

Table 4. Mean, standard deviations and coefficient of variation (expressed as a percentage in brackets) for degree-day sum and thermal threshold for bud and cambium phenology in spruce and fir.

Figure 1. Beginning of xylem differentiation with dividing cambium and cells in enlargement phases (DOY 160, Site LA). Ph, phloem; Cz, cambial zone; Ec, enlarging cells.

Figure 2. Average of days of occurrence of the phases of bud development in spruce and fir observed during 2010-2014 at two altitudes (1, open bud; 2, elongated bud; 3, swollen bud; 4 translucent bud; 5, split bud; 6, exposed shoot). Some phases are missing because they were not observed during the weekly monitoring.

Figure 3. Days of occurrence of the phases of xylem differentiation in spruce and fir observed during 2010-2014 (FE, first enlarging cell; FWT, first wall-thickening cell; FM, first mature cell). Error bars indicate standard deviation between trees.

Figure 4. Bud and cambium phenology observed during 2010-2014 for spruce and fir (error bars indicate standard deviation between years). Bars with the same letters are not statistically different ($p>0.05$).

567 **Figure 5.** Degree-days sum and thermal threshold assessed for bud and cambium phenology

568 (error bars indicate standard deviation between years).

569

570 **TABLE 1**

Species	Site	Stand age (yr)	Height (m)	Diameter at breast height (cm)
Spruce	LA	72.7 (20.3)	4.6 (1.04)	14.0 (0.03)
	GP	39.7 (3.7)	14.2 (1.6)	24.7 (1.4)
Fir	LA	25.3 (2.5)	7.6 (0.7)	16.8 (4.0)
	GP	49.7 (3.7)	17.6 (2.6)	32.2 (9.1)

571

572 **TABLE 2**

Altitude	Model		Factor	Statistics	
	F-value	R^2		*F*-value	*P*
900 m a.s.l.	431.51	0.67	Year	53.25	<.0001
			DOY	1281.49	<.0001
			Species	93.64	<.0001
227 m a.s.l	435.77	0.66	Year	49.44	<.0001
			DOY	1266.52	<.0001
			Species	156.93	<.0001

573

574 **TABLE 3**

	Open Bud	Exposed shoot	First enlarging xylem cell	First mature xylem cell
Open bud	-	0.82**	0.90***	0.81*
Exposed shoot	0.82**	-	0.77*	0.57
First enlarging xylem cell	0.91***	0.78**	-	0.88*
First mature xylem cell	0.93*	0.98**	0.89*	-

575

576 **TABLE 4**

		DDS	THR
Spruce	First enlarging cell	217±43 (19.9)	7.9±1.2 (14.9)
	Open bud	230±44 (11.7)	9.1±1.2 (12.9)
	First mature cell	468±50 (10.8)	11.8±1.1 (9.3)
	Exposed shoot	795±103 (13.0)	13±1.7 (13.1)
Fir	First enlarging cell	201±37 (18.4)	7.4±1.5 (18.7)
	Open bud	284±33 (19.2)	7.7±1.8 (22.8)
	First mature cell	420±68 (16.3)	11.3±1.3 (11.2)
	Exposed shoot	585±98 (16.8)	11.3±1.1 (9.4)

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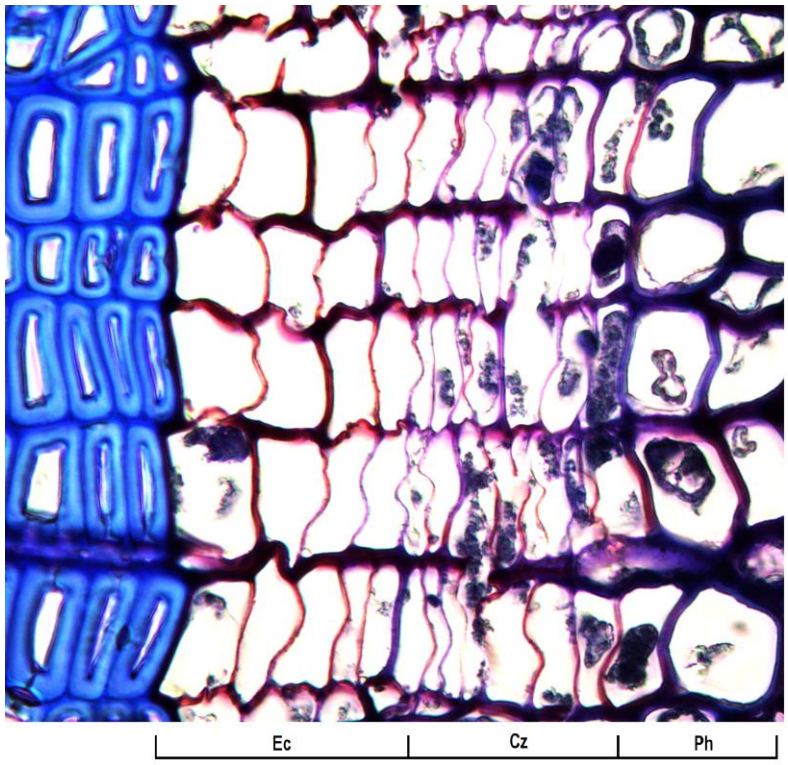
579

580 **FIGURE 1**



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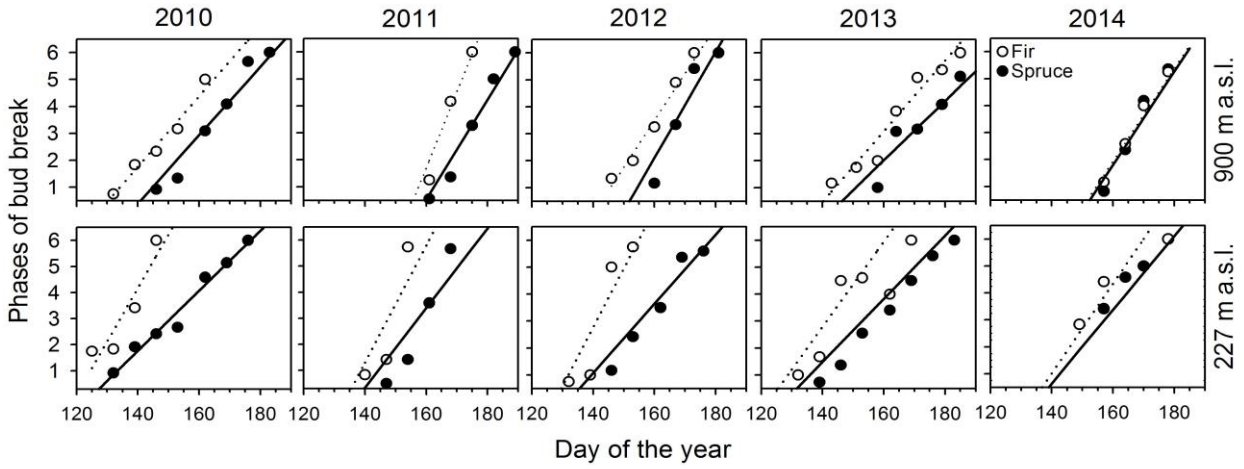
582

583 **FIGURE 2**



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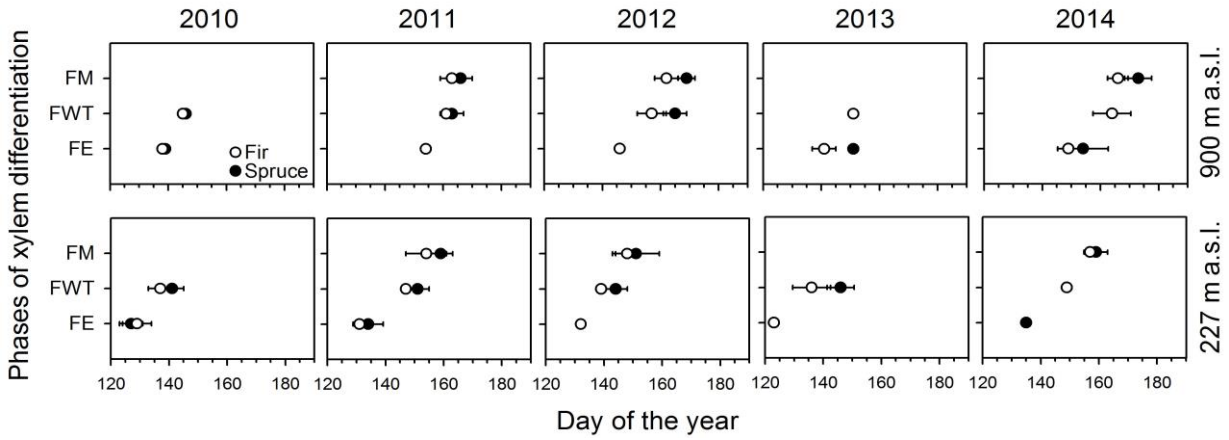
585 **FIGURE 3**



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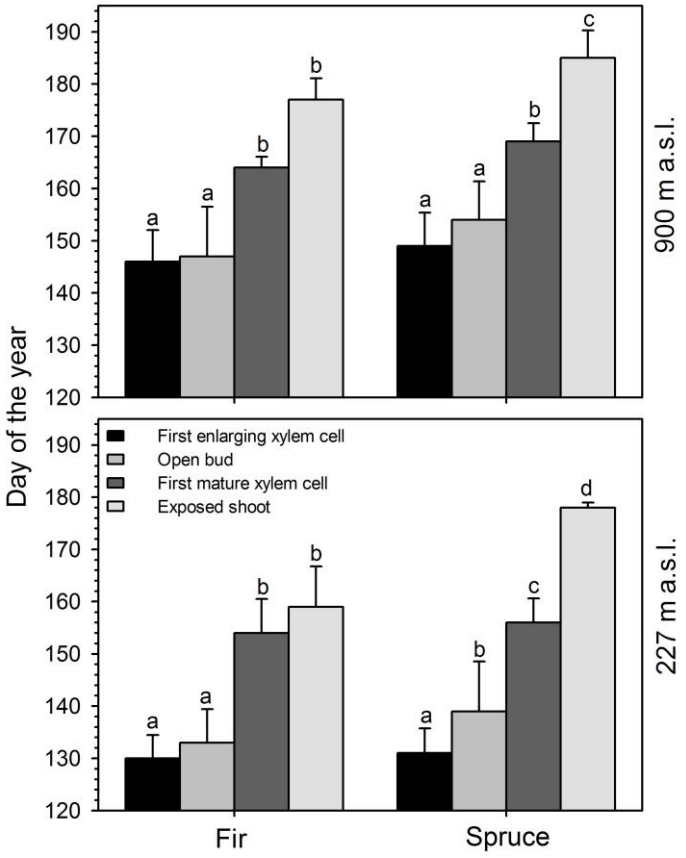
587 **FIGURE 4**



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589 **FIGURE 5**

590