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Research paper

Life strategies in intra-annual dynamics of wood formation: example of three conifer species in a temperate forest in north-east France

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We investigated whether timing and rate of growth are related to the life strategies and fitness of three conifer species. Intra-annual dynamics of wood formation, shoot elongation and needle phenology were monitored over 3 years in five Norway spruces (*Picea abies* (L.) Karst.), five Scots pines (*Pinus sylvestris* L.) and five silver firs (*Abies alba* Mill.) grown intermixed. For the three species, the growing season (delimited by cambial activity onset and cessation) lasted about 4 months, while the whole process of wood formation lasted 5–6 months. Needle unfolding and shoot elongation followed the onset of cambial activity and lasted only one-third of the season. Pines exhibited an 'extensive strategy' of cambial activity, with long durations but low growth rates, while firs and spruces adopted an 'intensive strategy' with shorter durations but higher growth rates. We estimated that about 75% of the annual radial increment variability was attributable to the rate of cell production, and only 25% to its duration. Cambial activity rates culminated at the same time for the three species, whereas shoot elongation reached its maximal rate earlier in pines. Results show that species-specific life strategies are recognizable through functional traits of intra-annual growth dynamics. The opposition between Scots pine extensive strategy and silver fir and Norway spruce intensive strategy supports the theory that pioneer species are greater resource expenders and develop riskier life strategies to capture resources, while shade-tolerant species utilize resources more efficiently and develop safer life strategies. Despite different strategies, synchronicity of the maximal rates of cambial activity suggests a strong functional convergence between co-existing conifer species, resulting in head-on competition for resources.

Keywords: cambial activity, competition, functional trait, mixed stand, phenology, tree growth, wood formation.

Introduction

Living organisms develop different life strategies to access vital resources and cope with intra- and inter-species competition (Grime 1977). These strategies can be seen in functional traits, which are measurable features that determine individual and species performance (e.g., survival, growth and reproduction) and, ultimately, their fitness in a given environment (Ackerly 2003). For tree species, growth timing and intensity may provide critical functional traits portraying their life strategies, and the result of these strategies may be evaluated using

the annual growth increment recorded in tree rings, which is known to be closely related to individual tree fitness (Baraloto et al. 2005, Poorter et al. 2008, Martinez-Vilalta et al. 2010). Indeed, during an annual cycle, trees must adjust their period of activity to maximize resource exploitation and minimize exposure to harmful events. According to their life strategies, tree species may respond differently to this unavoidable trade-off between growth and survival. Early onset and late termination of growth may involve high risks because they increase exposure to disturbance, such as herbivore damage, and

harmful climatic events such as spring or autumn frosts (Lockhart 1983, Hanninen 1991). However, a tree that begins growth early in spring can benefit from favorable conditions and avoid competition with its later neighbors (Augspurger et al. 2005). In contrast, a tree that begins late and ends early but demonstrates a high rate of growth may surpass its neighbors in longer-period growth.

It has been theorized that early-successional species are more prone to take risks, while late-successional species are associated with safer life strategies (Korner 2006, Korner and Basler 2010). Pioneer species, for example, are often photoperiod insensitive in spring and become temperature sensitive once their chilling demand has been fulfilled, allowing them to grow as soon as the climate is favorable. Late-successional species, however, can be controlled by photoperiod in spring, with temperature only exerting a limited modulating effect once the critical day length has come, preventing them from beginning too early. Such intertwined influences of environmental factors and life strategies are not documented in boreal and temperate conifer species, for which temperature is known to control spring phenological events (Wielgolaski 1999, Deslauriers et al. 2008, Rossi et al. 2008a, Lebourgeois et al. 2010a). However, the differences observed between early- and late-successional species in cambial activity resumption (Rossi et al. 2006a, 2008a) and budburst (Lebourgeois et al. 2010a) seem consistent with the theory of riskier life strategies in pioneer species. The contrasting strategies adopted by early- and late-successional species are also visible in their long-term growth dynamics. At the seedling stage, in an open field, early-successional species generally reach higher growth rates than late-successional species because they have higher capacities to capture resources (Reich et al. 1998, Lusk 2004, Niinemets 2006). Late-successional species, however, are more efficient in their resource utilization, which ultimately allows them to grow at higher rates and surpass early-successional species during forest closure (Lusk 2004, Niinemets 2006, Boyden et al. 2009).

Over 3 years, from 2007 to 2009, we monitored needle phenology and intra-annual dynamics of shoot elongation, cambial activity and wood formation in five silver firs (*Abies alba* Mill.), five Norway spruces (*Picea abies* (L.) Karst.) and five Scots pines (*Pinus sylvestris* L.). We chose to base our study on silver fir, Norway spruce and Scots pine because they are the three main conifer species in Europe and can be found growing in the same site despite contrasting autecologies. Selecting species of the same plant functional type (i.e., evergreen conifer) allowed us to compare their life strategies according to pioneer or late-successional status, without confusion with the deciduous or evergreen character. Finally, conifers produce wood comprising 90% of tracheids, which facilitates monitoring and comparing their intra-annual wood formation dynamics.

The very shade-tolerant silver fir is more frost- and drought-sensitive than the intermediate shade-tolerant Norway spruce, while the light-demanding Scots pine is the most drought- and cold-resistant of the three species (Lebourgeois et al. 2010b). Silver fir is also more thermophilic and has higher nutrient requirements than Norway spruce or Scots pine (Pinto and Gegout 2005). The three species also differ in their long-term growth dynamics (Bouriaud and Popa 2009). Scots pine has optimal growth at younger ages, and its productivity then rapidly decreases to become the lowest of the three species. In contrast, silver fir presents a slow growth rate at young age, but its productivity gradually increases and remains high even for old trees. Norway spruce demonstrated an intermediate behavior: its optimum growth is observed later than for Scots pine, then its productivity decreases but remains the highest of the three species until intermediate age. All these characteristics make Scots pine a pioneer species, while Norway spruce is an intermediate- and silver fir is a late-successional species.

The main objective of this study was to understand how the intra-annual dynamics of wood formation is related to species life strategies. As a pioneer species, we expected Scots pine to present longer growth durations due to earlier onsets and later cessations. As intermediate- and late-successional species, we expected Norway spruce and silver fir to produce greater growth increments due to higher cell production rates. Considering the contrasting life strategies of the investigated species, we expected that the functional diversity of the stand, with respect to the intra-annual dynamics of wood formation, was sufficient to lead to a mutual beneficial sharing of the resources in time, resulting in a 'soft' competition between trees.

Materials and methods

Study site and tree selection

The studied stand (70 × 50 m, see Figure S1 available as Supplementary Data at *Tree Physiology* Online) is located in a mixed forest composed of silver firs, Norway spruces and Scots pines in the Vosges Mountains (48°29'N, 7°09'E, and 643 m ASL), in north-east France. It was selected in consultation with the French national long-term monitoring network of forest ecosystems in order to be representative of submontane temperate coniferous forests of north-east France and to present the three studied species well intermixed. The study region is characterized by a mild continental temperate climate, with mean annual precipitation and temperature of 1600 mm and 9.4 °C. The three studied years were close to these mean values, with 1606 mm and 10.3 °C for 2007, 1121 mm and 9.9 °C for 2008 and 1162 mm and 10.1 °C for 2009.

Based on a complete inventory of the stand, five dominant and healthy silver firs, Norway spruces and Scots pines were

selected. Their total height and crown area were measured, and two standard cores were taken at breast height to estimate their age. Manual band dendrometers were installed at breast height in March 2007 and read weekly to monitor stem circumference variations. The annual circumferential increment (ACI, see Table S1 available as Supplementary Data at *Tree Physiology* Online for the list of variables used along with their notations and units) was computed as the median of the measurements taken after cambial activity cessation.

Needle phenology and shoot elongation monitoring

Shoot elongation and needle phenology were monitored weekly in the upper tier of the crown of each studied tree using binoculars. The length of new shoots was visually assessed, and needle unfolding was described using three successive phenological stages occurring after winter bud dormancy: (i) budburst, (ii) maturing needles and (iii) mature needles. In the three species, dormant buds were totally enclosed by scales and recognizable by their small size (Figure S2a and d available as Supplementary Data at *Tree Physiology* Online). Budburst differed between species and was distinguished according to the criteria of the phenological observations protocol of the French national long-term monitoring network of forest ecosystems (Ulrich and Cecchini 2009). In firs and spruces, budburst date was recorded when buds were fully open, scales had fallen and developing needles were clearly apparent (Figure S2b available as Supplementary Data at *Tree Physiology* Online). In pines, budburst date was recorded when developing needles were clearly visible at the bottom of the new shoots, emerging from the scales, even if buds were still elongating and only partially open (Figure S2e available as Supplementary Data at *Tree Physiology* Online). Maturation of needles was indicated by their color, which progressively changed from light to dark green. Current-year needles, in comparison with those of the previous years, were considered mature when they demonstrated the same color.

For each tree, onset ($t_{i,N}$) and cessation ($t_{f,N}$) of needle unfolding were defined, respectively, as the date at which 50% of buds were broken and 50% of needles were mature; the duration of needle unfolding (Δ_N) was computed as the time between $t_{f,N}$ and $t_{i,N}$. Onset ($t_{i,S}$) and cessation ($t_{f,S}$) of shoot elongation were defined, respectively, as the date at which 50% of shoots began to lengthen and 50% of shoots reached their final length; duration of shoot elongation (Δ_S) was computed as the time between $t_{f,S}$ and $t_{i,S}$. Daily dates were estimated based on weekly observations using linear interpolations.

To assess shoot elongation dynamics, length of new shoots was fitted with a logistic curve (Karkach 2006) using the SSlogis function of the R statistical software (R Development Core Team 2011):

$$IS(t) = \frac{A}{1 + e^{-\frac{(t_{p,S}-t)}{c}}} \quad (1)$$

where $IS(t)$ is the length of shoots at time t ; A is the upper horizontal asymptote parameter representing the final shoot length; $t_{p,S}$ is the date of the inflection point; and c is a numeric scale parameter of the time axis. From this model, the maximal ($r_{x,S}$) and mean ($r_{m,S}$) shoot elongation rates were estimated.

Overall measure of fit was assessed by the modeling efficiency (r^2), a statistic close to the coefficient of determination that can be used for nonlinear models (Mayer and Butler 1993).

Tree sampling and sample preparation

Microcores (2 mm diameter, 15–20 mm length) were collected weekly from April to November at breast height on the stems of the selected trees using a Trephor® (Vitzani, Belluno, Italy) (Rossi et al. 2006b) and following an ascending spiral pattern (Deslauriers et al. 2003). Successive microcores were taken about 1 cm apart from each other to avoid wound reaction without considerably increasing the influence of stem circumferential variability on the final dataset.

The collected microcores were placed in Eppendorf microtubes with ethanol solution (50% in water) and stored at 5 °C. Each sample was oriented under a stereomicroscope at $\times 10$ – $\times 20$ magnification, and the transverse side was marked with a pencil. Microcores were successively cleaned, dehydrated and infiltrated by immersion in baths of ethanol, d-limonene and paraffin using an automatic tissue processor (STP121, MM France, Francheville, France) (Rossi et al. 2006b). Microcores were then embedded in paraffin blocks with an embedding station (EC 350, MM France), and 5–10- μ m-thick transverse sections were cut with a rotary microtome (HM 355S, MM France). Sections were stained with cresyl violet acetate (0.16% in water) and permanently mounted on glass slides using Histolaque LMR®.

Microscopic observations

Overall, 1450 anatomical sections were observed using an optical microscope (Orthoplan, Leitz, Germany) under visible and polarized light at $\times 125$ – $\times 400$ magnification to distinguish the different phases of cell development. For each sample, the radial number of cells in the cambial (n_C), enlargement (n_E), cell-wall thickening and lignification (n_L) and mature zones (n_M) was counted along three radial files according to the criteria described by Rossi et al. (2006c). Cambial cells were characterized by thin cell walls and small radial diameters. Cells in the radial enlargement phase were larger than cambial cells and had thin walls that were not birefringent under polarized light. Cells in the cell-wall thickening and lignification phase were birefringent under polarized light and demonstrated violet and

blue walls. Tracheids were considered mature when walls were completely blue.

Timing of wood formation

A set of five critical dates and three durations was computed from the cell-counting dataset using logistic regressions (Rathgeber et al. 2011a). The onset of enlarging ($t_{i,E}$), cell-wall thickening and lignification ($t_{i,L}$) and mature phases ($t_{i,M}$), and the cessation of enlarging ($t_{f,E}$) and cell-wall thickening and lignification phases ($t_{f,L}$) were defined as the dates at which 50% of the radial files were active (onset) or non-active (cessation). The durations of enlarging (Δ_E) and cell-wall thickening and lignification (Δ_L) phases were the time between the onset and cessation of these phases.

As recommended by Rathgeber et al. (2011a), our assessment of tracheid production timing was based on the xylem cell enlargement phase rather than the cambial cell dividing phase. Thus, $t_{i,E}$, $t_{f,E}$ and Δ_E were also used as proxies for the onset, cessation and duration of cambial activity, respectively. $t_{i,E}$ and $t_{f,L}$ were used as proxies for onset and cessation of xylogenesis ($\Delta_X = t_{i,L} - t_{i,E}$).

Standardization

The number of cells varied according to the height and the orientation of the sample along the stem and consequently among the different samples within and between the trees (Wodzicki and Zajaczkowski 1970). According to Rossi et al. (2003), the number of cells from the previous year was counted on three radial files per sample and used to standardize the raw number of the current year. A dedicated function of the R package CAVIAR (Rathgeber 2011) was used to apply this standardization to all the samples in our dataset.

Cell number computation

The total number of tracheids produced at time t during the season was computed as the sum of the standardized number of cells belonging to the xylem ($n_T(t) = n_E(t) + n_L(t) + n_M(t)$). The final ring cell number was computed as the median of the total number of cells ($n_T(t)$) of all the samples taken after $t_{f,E}$. The number of cambial cells before and after the growing season was also computed as the median number of cambial cells in all the samples taken before $t_{i,E}$ or after $t_{f,E}$, respectively.

Rate of tracheid production

To assess tracheid production dynamics, the number of cells over time was fitted using a Gompertz function (Rossi et al. 2003) defined as

$$n_T(t) = A e^{-e^{-\beta - \kappa t}} \quad (2)$$

where $n_T(t)$ is the standardized total number of tracheids at time t ; A is the upper horizontal asymptote parameter representing

the final number of tracheids; β is the x -axis placement parameter that reflects the choice of origin time; and κ is the growth rate parameter that determines the spread of the curve along the time axis. These parameters were estimated for each tree using a dedicated function of the R package CAVIAR (Rathgeber 2011). Overall measure of fit was assessed by the modeling efficiency (r^2). The date of the inflection point ($t_{p,T}$) and the corresponding maximal rate of tracheid production ($r_{x,T}$) were computed, as well as the mean rate ($r_{m,T}$), accounting for the period during which 90% of the tracheids were produced (Rathgeber et al. 2011b).

To test whether the rate of tracheid production depended more on the number of cambial cells or on the division frequency of each cambial cell, the mean cell cycle length (CCL_m) was computed by dividing the mean number of cambial cells observed through the growing season by $r_{m,T}$, and the minimal cell cycle length (CCL_n) was calculated by dividing the number of cambial cells at $t_{p,T}$ by $r_{x,T}$.

Statistical analyses

Linear mixed-effects models were used to test the effect of species on the selected potential functional traits. Mixed-model approach is recommended in the case of repeated measurements because it evaluates the effects of fixed factors (in our case, the species), taking into account the effects of random factors (trees and years), on the response variable (the potential functional traits) (Zuur et al. 2009). The standard deviation of the year random effect allowed us to estimate the year-to-year variability of each potential functional trait. Estimations of the model parameters were performed with the R package lme4 (Bates et al. 2011).

Simple physical model of annual radial growth

The ACI was expressed as a function of the maximal rate ($r_{x,T}$), and duration (Δ_E) of tracheid production:

$$ACI = f(r_{x,T} \times \Delta_E) \quad (3)$$

The effect of species on this product was also tested using the same linear mixed-effects modeling procedure to check model consistency. Moreover, a sensitivity analysis of the model was performed to compare the contribution of the rate and duration to the annual increment (Cariboni et al. 2007).

Results

Dendrometric characteristics of the monitored trees

The monitored trees presented similar stem diameters but the silver firs and Norway spruces were on average 5 m taller and 45 years younger than the Scots pines (Table 1). Moreover, the firs had larger crown areas.

The three species demonstrated significant differences concerning their annual production of shoots and wood. Firs exhibited radial increments (either evaluated by number of cells, tree-ring width, or stem circumference variation) more than twice those of spruces and pines ($P < 0.01$). These differences were also visible in the wintering cambium, with firs showing two more dormant cambial cells than spruces and pines ($P < 0.01$), as well as during the growing season, with the firs producing more cells (Figure 1). Finally, pines

produced shoots 1–2 cm longer than firs and spruces ($P < 0.01$).

Needle phenology and intra-annual dynamics of shoot elongation

For firs and spruces, budburst occurred between late April and mid-May and was followed by the onset of shoot elongation the following week (Figure 2). In contrast, in pines, shoot elongation began at the end of April or the beginning of May,

Table 1. Main characteristics and annual production (mean \pm SE) of the monitored trees from the three studied species (silver fir, Norway spruce and Scots pine) illustrated by the diameter at breast height (DBH), height (H), age, projected crown area (CA), final ring cell number (RCN), tree-ring width (TRW), annual circumferential increment (ACI), number of cambial cells before the onset (ICN) and after the termination (FCN) of the growing season and final shoot length (FSL).

	DBH (cm)	H (m)	Age	CA (m ²)	RCN	TRW (mm)	ACI (mm)	ICN	FCN	FSL (cm)
Pines	53 \pm 2	27 \pm 1	119 \pm 3	29 \pm 2	29.7 \pm 3.0	1.1 \pm 0.1	6.8 \pm 0.7	5.5 \pm 0.2	5.9 \pm 0.2	10.5 \pm 0.3
Firs	57 \pm 3	31 \pm 1	73 \pm 3	37 \pm 6	67.9 \pm 7.5	2.5 \pm 0.2	15.5 \pm 1.2	7.2 \pm 0.3	7.8 \pm 0.3	8.5 \pm 0.3
Spruces	55 \pm 4	33 \pm 1	74 \pm 4	30 \pm 7	33.9 \pm 4.4	1.3 \pm 0.1	7.8 \pm 0.8	5.5 \pm 0.2	5.7 \pm 0.1	8.7 \pm 0.2

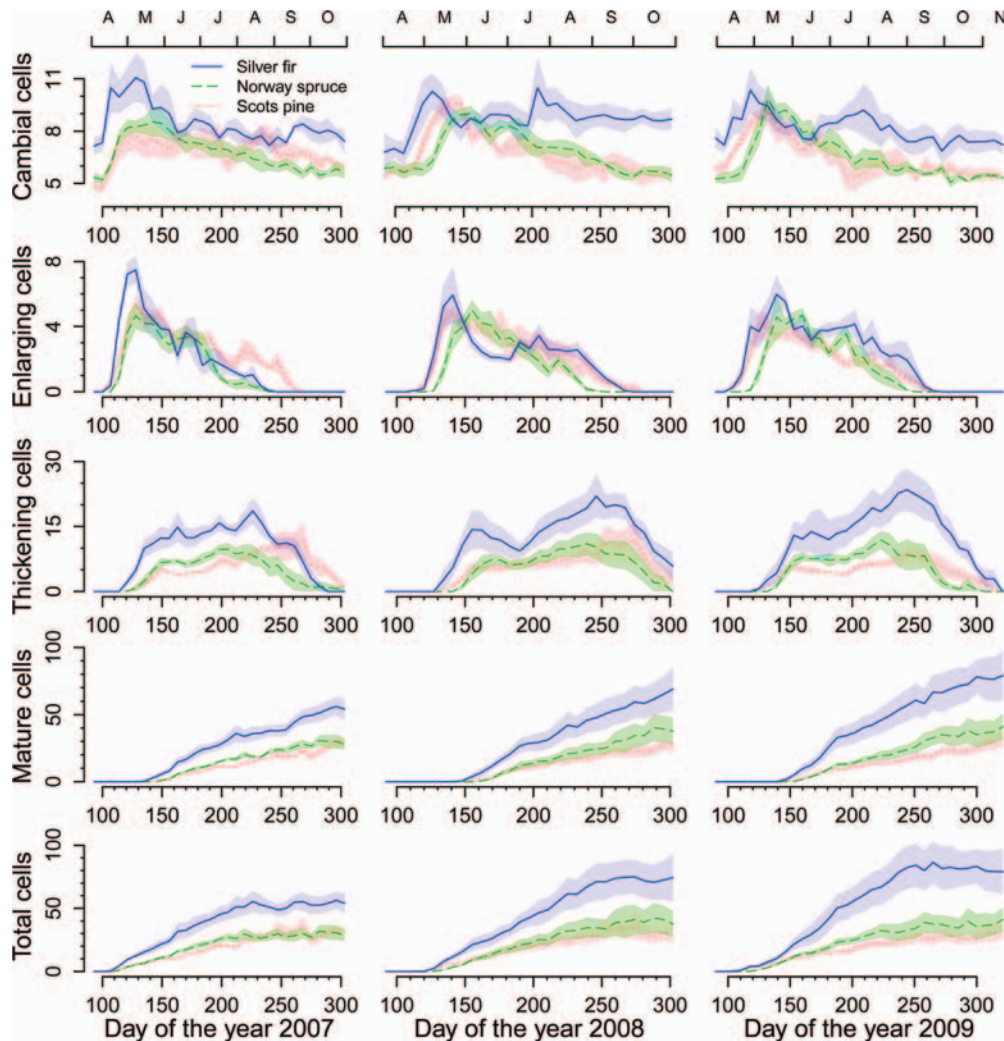


Figure 1. Intra-annual wood formation dynamics. For each species, the line represents the mean cell number of the five monitored trees, and the shadowed area delimits the 90% confidence intervals.

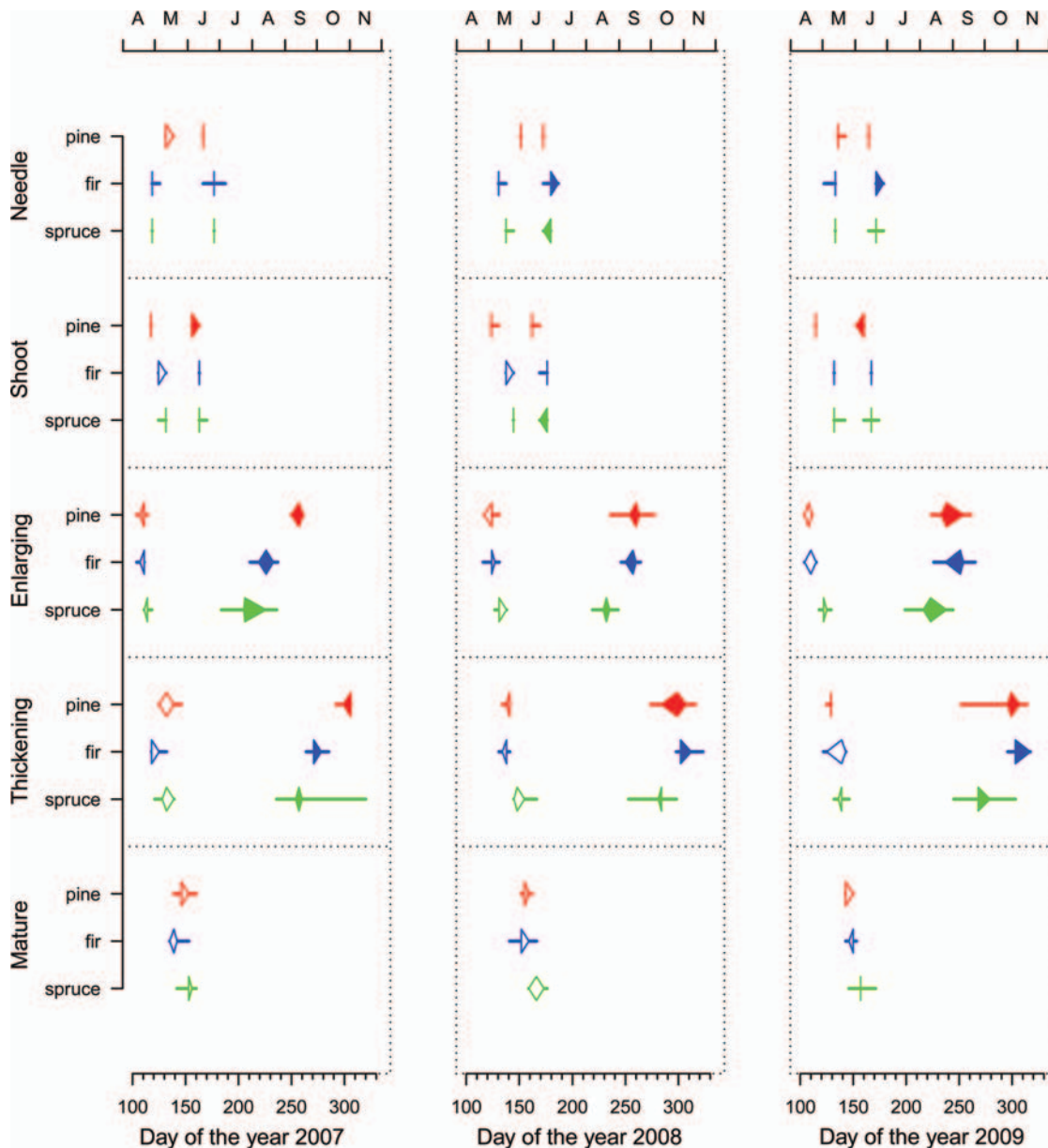


Figure 2. Needle unfolding, shoot elongation and wood formation calendar. Onset and cessation of needle unfolding and shoot elongation, along with critical dates of wood formation for pines, firs and spruces. Critical dates of wood formation are the onset and cessation of the enlarging and thickening phases along with the onset of the mature phase. Note: for each date, the five trees are represented by diamond-crossed-by-a-line marks; the left end of the line represents the first tree to begin or finish, the left end of the diamond the second tree, the middle of the diamond the third tree, the right end of the diamond the fourth tree and the right end of the line the fifth tree.

3 weeks before the onset of needle unfolding. Mixed-effect models estimated that budburst occurred 2 weeks earlier in firs and spruces when compared with pines ($P < 0.01$), but the onset of shoot elongation began 3 weeks earlier in pines ($P < 0.01$; Figure 3a). Overall, the onset of shoot elongation in pines was the first phenological event recorded during the year, occurring 1 week before budburst in firs and spruces ($P < 0.01$); thus, Scots pine was the first species to begin primary growth. A variability of 1 week was observed between years for the dates of budburst and shoot elongation onset.

On average, shoots reached their final length in June (Figure 2). Shoot elongation cessation varied by 4 days from year to year, but occurred 1 week earlier for pines than for firs and spruces ($P < 0.01$; Figure 3b). Shoots lengthened over 5–6 weeks, 1 week longer in pines than in firs or spruces ($P < 0.01$; Figure 3c), with an estimated year-to-year variability of only 1 day. For the three species, needles were mature 1 week after shoot elongation cessation, between mid-June and the very beginning of July (Figure 2). Needles were mature 1 week earlier for pines than for firs and spruces ($P < 0.01$; Figure 3d), with year-to-year variability estimated at 3 days.

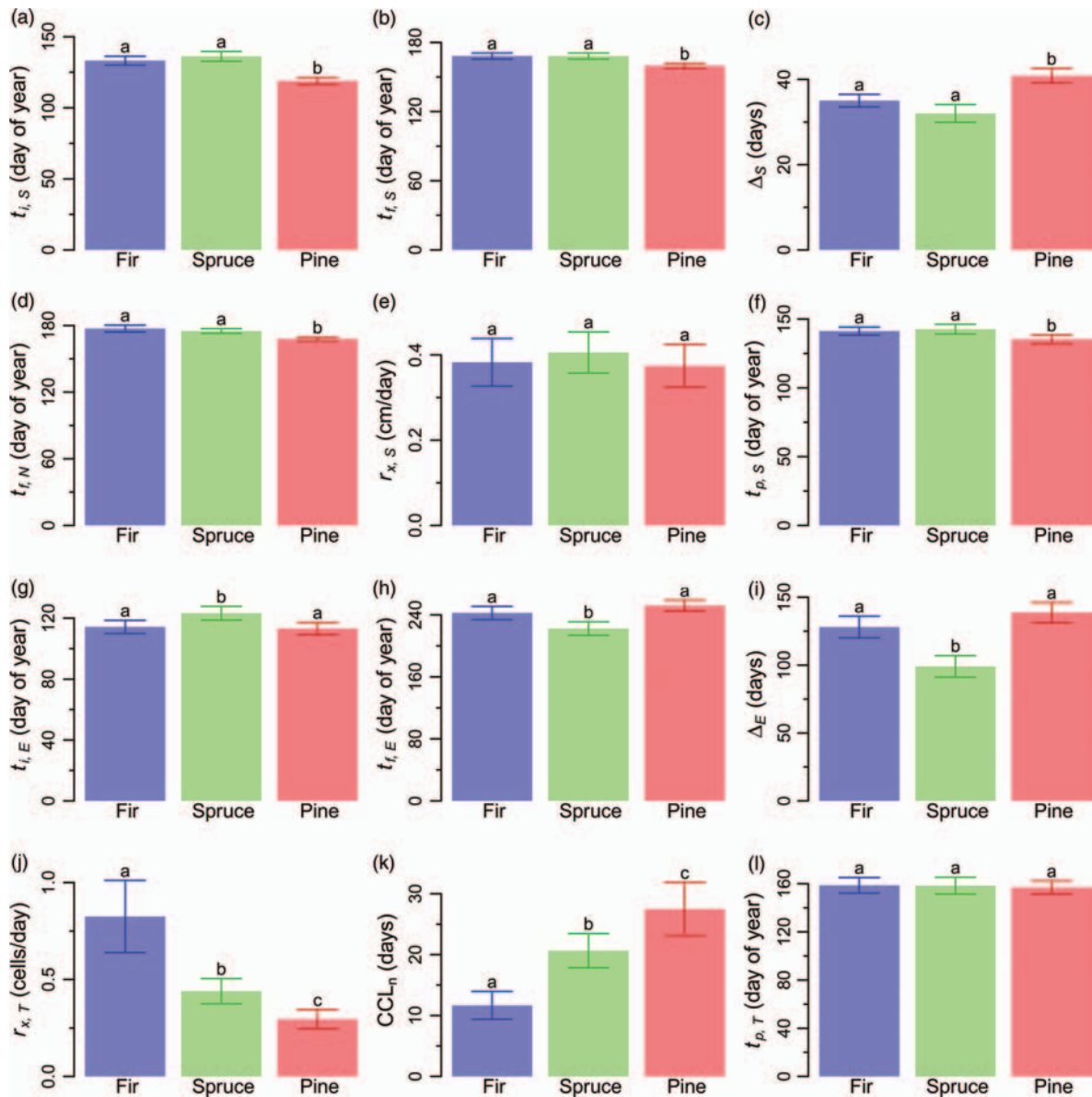


Figure 3. Comparison of the intra-annual dynamics of shoot elongation, needle unfolding and wood formation between the three studied species. Onset ($t_{i,s}$), cessation ($t_{f,s}$), duration (Δ_S), maximal rate ($r_{x,s}$), occurrence of the maximal rate ($t_{p,s}$) of shoot elongation and cessation ($t_{f,N}$) of needle unfolding. Onset ($t_{i,E}$), cessation ($t_{f,E}$), duration (Δ_E), maximal rate ($r_{x,\tau}$), minimal length of the cell cycle (CCL_n) and occurrence of the maximal rate ($t_{p,\tau}$) of tracheid production. Letters above bars indicate whether differences between species are significant ($P < 0.05$) based on linear mixed-effects models.

The logistic function yielded good results in fitting shoot elongation dynamics (Table S2, Figures S3, S4 and S5 available as Supplementary Data at *Tree Physiology* Online). The shoot elongation rates were similar between the three species, with a maximal rate of 0.39 ± 0.02 (mean \pm standard error) and a mean rate of 0.23 ± 0.01 cm/day (Figure 3e). Year-to-year variations of the maximal and mean rates were small (16 and 12%, respectively). Despite similar rate shapes and magnitudes across species, the earlier onset observed in pines was also visible in the pattern of shoot elongation rates through the growing season and resulted in a shift of the pine curve, while

spruce and fir curves tended to overlap (Figure 4). Thus, the maximal rate of shoot elongation occurred around the middle or end of May, 1 week earlier for pines than for firs and spruces ($P < 0.01$; Figure 3f), with year-to-year variability estimated at 6 days.

Intra-annual dynamics of xylem cell production and maturation

Regardless of species or year, the number of dividing, enlarging, thickening and mature cells followed a general pattern of variation through the vegetation season commonly described

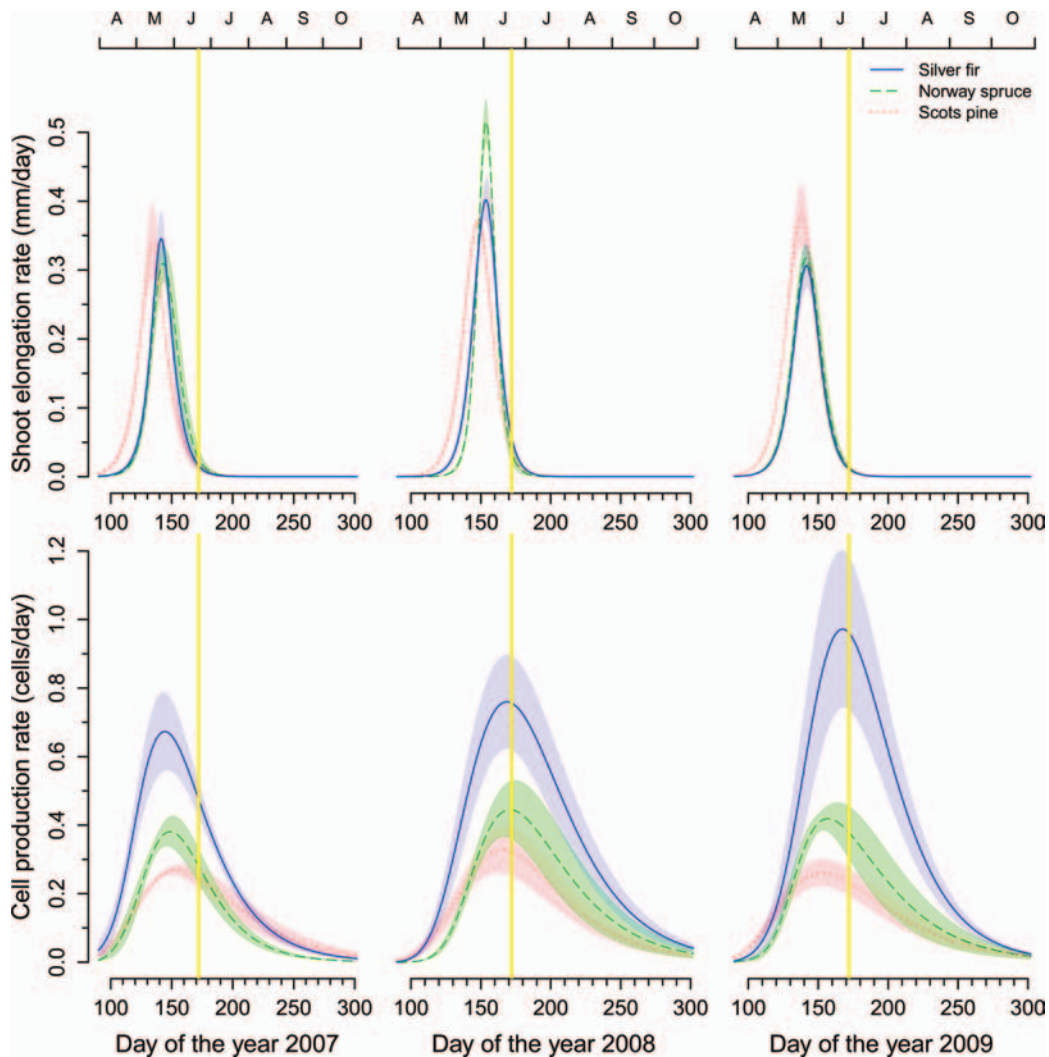


Figure 4. Rates of shoot elongation and tracheid production. For each species, the line represents the mean of the five monitored trees, and the shadowed area delimits the 90% confidence interval. The vertical yellow line represents summer solstice.

as three delayed bell curves followed by a sigmoid curve (Figure 1). However, the bell curves representing the number of dividing cells in the cambial zone were clearly skewed to the left, indicating a quick onset followed by a slow cessation of the process. Between mid-April and early May, the number of cambial cells increased, on average from 5.5 ± 0.2 to 9.0 ± 0.7 cells for the spruces and pines and from 7.2 ± 0.3 to 10.6 ± 1.4 cells for the firs. Then, it gradually decreased through the growing season and finally returned approximately to its initial value, corresponding to the number of cambial cells at rest.

Following the increase in the number of cambial cells by only a few days, xylem cells began to enlarge between mid-April and mid-May, depending on the year and the species (Figure 2). Pines and firs entered the enlarging phase 1 week earlier than spruces ($P < 0.01$; Figure 3g), even though a year-to-year variation of 1 week was estimated. As for the dividing cells, the number of enlarging cells through the growing period exhibited a bell curve clearly skewed to the left (Figure 1). It

increased quickly in spring to culminate at 4.8 ± 0.6 cells for the spruces and pines and 6.5 ± 1.2 cells for the firs, and then decreased progressively. Xylem cell enlargement ceased between the second part of July and mid-September, depending on species and year, with a greater variability among trees, both within and between species, than at its onset (Figure 2). Despite this greater variability, spruces ceased significantly (by 3 weeks) earlier than firs and pines ($P < 0.01$; Figure 3h), while a mean variation of 1 week was estimated between years. Due to their earlier onsets and later cessations, firs and pines had enlarging phase durations significantly longer than spruces (on average 4.5 and 3.5 months, respectively, $P < 0.01$; Figure 3i). These durations did not vary from year to year because the variations observed at the onset of the enlarging phase were counterbalanced by the variations observed at its cessation.

Secondary cell-wall formation began in late April or May, depending on year and species, about 2 weeks after enlarging phase onset (Figure 2). Pines and firs began 1 week earlier

than spruces ($P < 0.01$), even though the year-to-year variability was estimated at 1 week. In contrast to the dynamics observed for the dividing and enlarging cells, the number of thickening cells through the growing season demonstrated a bell curve slightly skewed to the right, indicating a slow onset and rapid cessation (Figure 1). The firs accumulated twice as many thickening cells (21.3 ± 4.3) than the pines and spruces (10.9 ± 2.8). Secondary cell-wall formation ended between September and November, depending on the year and the species, about 2 months after enlarging phase cessation, with a greater variability among trees (within as well as between species) than at its onset (Figure 2). Nevertheless, spruces ended 3 weeks earlier than firs and pines ($P < 0.01$), with an estimated year-to-year variation of 1 week. The duration of the thickening phase was 1 month longer for pines and firs than for spruces (on average 5.5 and 4.5 months, respectively, $P < 0.01$). The total duration of xylem formation also lasted 1 month longer for pines and firs than for spruces (on average 6 and 5 months, respectively, $P < 0.01$).

The first mature cells appeared at the end of May or the beginning of June, 2 weeks after the onset of the thickening phase and 1 month after the onset of the enlarging phase (Figure 2). A mean year-to-year variation of 1 week was estimated, but pines and firs began 1 week earlier than spruces ($P < 0.01$). Mature cells accumulated steadily through the season, making the expected sigmoid shape difficult to observe on the mean curves (Figure 1). The sigmoid shape, however, was clearly visible on the curves for the total number of xylem cells.

Regardless of species, Gompertz function yielded good results in fitting the production of xylem cells through the growing period (see Table S3, Figures S6, S7 and S8 available as Supplementary Data at *Tree Physiology* Online). However, the tracheid production rates derived from these fittings differed between the three species (Figure 3j). Pines presented the lowest rates, with maximal and mean rates around 0.29 ± 0.03 and 0.18 ± 0.02 cells/day, respectively. Spruces demonstrated higher rates than pines, with maximal and mean rates around 0.44 ± 0.03 and 0.26 ± 0.02 cells/day, respectively ($P < 0.01$). Firs exhibited maximal and mean rates two times greater (0.83 ± 0.10 and 0.50 ± 0.06 cells/day, respectively) than those of spruces and pines ($P < 0.01$). These rates were stable from year to year, with mean variations $< 5\%$. Significant linear relationships were found between the maximal rate and the number of cambial cells observed at its occurrence, as well as between the mean rate and the mean number of cambial cells through the growing season ($P < 0.01$).

The mean and minimal cell cycle lengths followed the same pattern of variation as the mean and maximal rates, with fir cell cycle lengths shorter than those of spruces, which were themselves shorter than those of pines ($P < 0.01$, Figure 3k). However, the differences between species were smaller when

considering the cell cycle length, with minimal and mean lengths of 12 ± 1 and 20 ± 2 days for firs, 21 ± 1 and 31 ± 2 days for spruces and 27 ± 2 and 43 ± 3 days for pines.

Although the cell production rates and cell cycle lengths differed between the three species, their evolutions through the growing season were similar (Figure 4). The most impressive feature was that the maximum rates of tracheid production (corresponding also to the minimal cell cycle length) occurred at the same time for the three species ($P > 0.5$), a couple of weeks before the summer solstice (Figure 3l). The date itself varied by about 9 days from year to year, but within a particular year trees, whatever their species, synchronized their maximal growth.

Simple physical model of annual radial growth

The onset ($t_{i, E}$), cessation ($t_{f, E}$) and duration (Δ_E) of cambial activity presented species-specific relationships with the ACI, with significant relationships for firs ($P < 0.01$) and spruces ($P < 0.01$) and no significant relationship for pines (Figure 5a). In contrast, a species-independent relationship was established between the maximal rate of cambial activity ($r_{x, T}$) and ACI ($P < 0.01$; Figure 5b). The relationship between $r_{x, T}$ and Δ_E was also species specific with significant and linear relationships for firs ($P < 0.01$) and spruces ($P < 0.1$) and no significant relationship for pines (Figure 5c). Three behaviors were identified through the manner in which species occupied the duration vs. rate space: (i) the spruces presented short durations and medium rates, (ii) the firs presented medium durations and high rates and (iii) the pines presented long durations and low rates.

The model $ACI = r_{x, T} \times \Delta_E$ yielded good results ($P < 0.01$; Figure 5d), with no significant effect of species on the rate-duration product. Sensitivity analysis revealed that when $r_{x, T}$ was kept constant to its mean value while Δ_E was made to vary around its mean within a range of twice its standard deviation, the resulting ACI varied from 7.1 to 12.3 mm (i.e., a range of variation of 5.2 mm). However, when Δ_E was kept constant to its mean value while $r_{x, T}$ was made to vary around its mean within a range of twice its standard deviation, the resulting ACI varied from 2.1 to 17.3 mm (i.e., a range of variation of 15.2 mm). Therefore, the simulated ACI was three times more sensitive to $r_{x, T}$ than to Δ_E , or ACI variability was attributable to $\sim 25\%$ of Δ_E and 75% of $r_{x, T}$.

Discussion

Intra-annual dynamics of wood formation in temperate and cold forest ecosystems

In our study site located at mid-altitude in a temperate coniferous forest, cambial activity began between late April and early May, a few days before the onset of needle unfolding and shoot

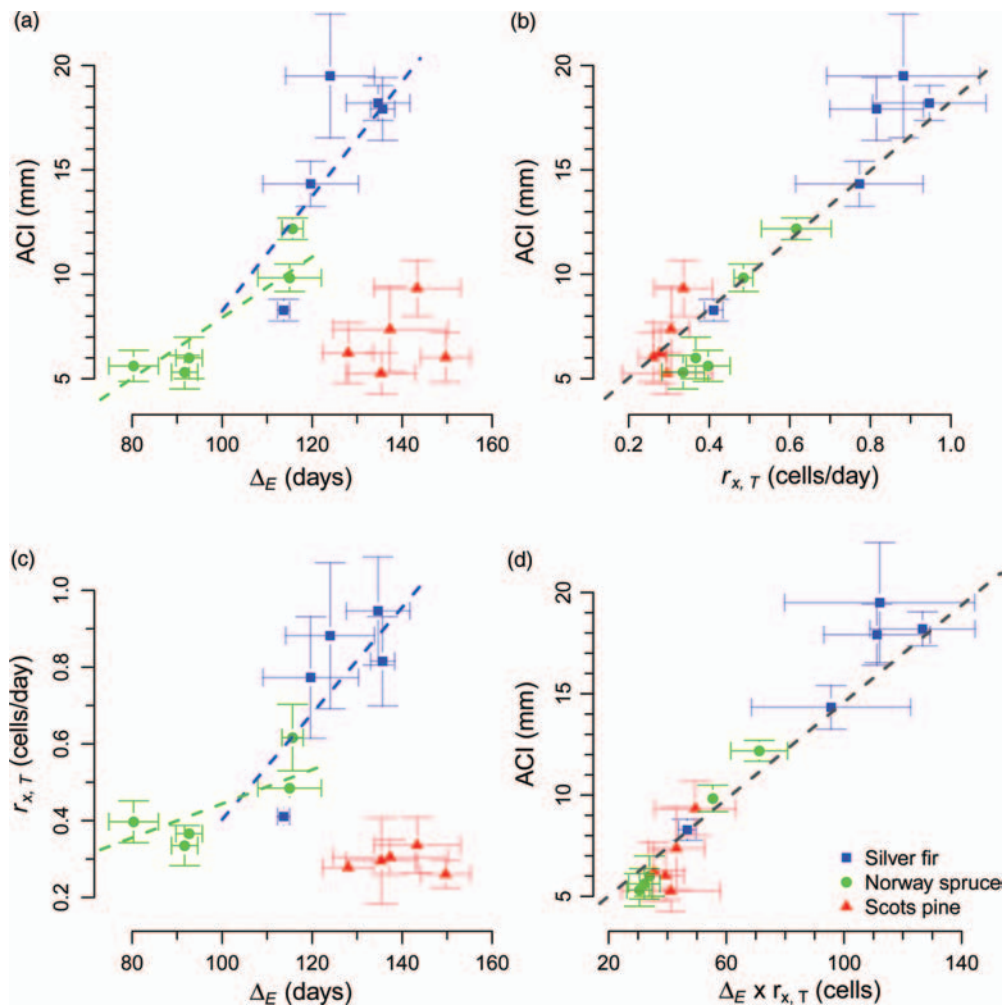


Figure 5. Relationships between the duration of the growing period (Δ_E), the rate of tracheid production ($r_{x,\tau}$) and the annual circumferential increment (ACI).

elongation. While needle unfolding and shoot elongation lasted only 1–1.5 months, tracheids accumulated over 3–4.5 months and matured over 5–6 months, so that the major part of wood formation occurred after the production of needles and shoots (Rossi et al. 2009). During the growth period, the cambium produced cells at an average rate of 0.31 ± 0.03 cells day⁻¹, for a final number of 44 ± 4 cells in the annual tree ring. Very similar timings and rates of tracheid production and differentiation have been described in temperate forests in central Europe, for Scots pine in Poland (Wodzicki 1971, 2001) and for Norway spruce and silver fir in Slovenia (Gricar 2007).

Conifers grown in subalpine and boreal forest ecosystems exhibit similar rates of cambial activity (Deslauriers and Morin 2005, Rossi et al. 2008b) but are associated with very different timing. In such cold environments, cambial activity begins 2–4 weeks later, finishes 2–4 weeks earlier, and thus lasts 1–2 months less than those in temperate forests, which results in lower annual production, generally <30 tracheids (Rossi et al. 2006c, 2007, 2011, Deslauriers et al. 2008, Lupi et al. 2010).

Influence of tree age and size on wood formation traits

Based on the theory that pioneer species adopt riskier life strategies than late-successional species (Korner 2006, Korner and Basler 2010), we expected an earlier onset of growth in pines. Primary growth actually began earlier in Scots pine, but surprisingly cambial activity began at the same time for pines and firs. The greater age and the lower height of pines (Table 1) may explain this unexpected result. Indeed, Rossi et al. (2008b) reported that cambial activity began later as trees aged, while Rathgeber et al. (2011b) found that, at the same age, smaller silver firs begin cambial activity later than taller ones. Therefore, because the selected pines were older and smaller than the firs and spruces, we probably overestimated their onset and underestimated their cessation and durations of wood formation. Moreover, by establishing the wood-formation calendar of similar-age (≈ 40 years old) trees grown in the same stand, Rathgeber et al. (2011a) found that cambial activity began 20 days earlier for Scots pine than for silver fir. This body of evidence supports the hypothesis that

early-successional species adopt riskier life strategies, as indicated in their wood formation phenology by early onsets and late cessations.

In our study, pines exhibited a lower cell production rate than firs and spruces. However, Rossi et al. (2008b) and Rathgeber et al. (2011b) found that cambial activity was less intense for older and smaller trees. Therefore, we calculated the rates of cell production in the dataset used by Rathgeber et al. (2011a) in order to compare trees of the same age and of comparable size, and we found that the maximum and mean rates of cambial activity were higher ($P < 0.01$) for silver firs (0.82 ± 0.18 and 0.49 ± 0.11 cells/day, respectively) than for Scots pines (0.50 ± 0.12 and 0.30 ± 0.09 cells/day, respectively).

The use of cell cycle length was also a method to minimize the effects of tree age and size by 'standardizing' the rate of cell production by the number of dividing cells, amplifying the intrinsic species behavior. The computation of this crucial functional trait slightly reduced the differences in cell production rate observed between species. However, the contrasting behaviors remained strong, with the minimal cell cycle duration being twice as long for Scots pine as for silver fir, Norway spruce being in between. Once again, this body of evidence supports the idea that early-successional species present a lower rate of production than late-successional species because they are less efficient at converting environmental resources into growth.

Life strategies in intra-annual wood formation dynamics

Our results demonstrated that the contrasting life strategies adopted by the three species were clearly apparent in the functional traits depicting the key features of their intra-annual wood formation dynamics. For example, to produce similar radial increment, cambial activity lasted 40% longer in pine than in spruce because its cell production rate was 30% slower. Firs were more difficult to compare with the two other species because they produced radial annual increments twice as large. However, the fir-specific relationships between radial increment and duration of cambial activity (see the slopes of the dashed lines in Figure 5a) and the non-specific relationship between radial increment and cell production rate (see Figure 5b) indicated that firs used less time than pines to produce similar radial increments because they exhibited higher rates. In summary, we described the Scots pine growth strategy as 'extensive' because it was characterized by long durations and low rates, illustrating the characteristic behavior of early-successional species, which are prone to explore space and time to capture resources but are not very efficient in their utilization. In contrast, we described the growth strategy adopted by fir and spruce as 'intensive' because it was characterized by higher rates and shorter durations, illustrating the behavior of late-successional

species, which are more cautious in their exploration of space and time but are more efficient in the utilization of the limited resources they have access to. The high rates of growth associated with the 'intensive' strategy suggest that at the dominant stage, silver fir, and to a lesser extent Norway spruce, are better competitors than Scots pine, while the long growing periods associated with the 'extensive' strategy suggest that Scots pine is closer to the stress top of Grime's C-S-R triangle (Grime 1977).

Firs reached higher rates of cambial activity than spruces, which themselves reached higher rates than pines because they have more dividing cambial cells during the growing season and, above all, because they have shorter cell cycles. The number of cambial cells is known to depend on tree species (Larson 1994) and vitality (Gricar et al. 2009), and its influence on the rate of cell production is already well documented (Vaganov et al. 2006). The strong influence of the cell cycle length is consistent with the results obtained by Deslauriers et al. (2009). As cell cycle length is closely linked to the quantity of sucrose available around dividing cells (Riou-Khamlich et al. 2000), the shorter cell cycles observed in firs and spruces suggest that they can produce more assimilates than pines. The results of Deslauriers et al. (2009), however, suppose differences in cell cycle length between two poplar genotypes that have similar photosynthetic capacities, indicating that genetic control can also be envisaged.

The suggested ability of fir and spruce to produce more assimilates may be related to their management of foliage turnover. Indeed, late-successional evergreen conifer species have slower foliage turnover than pioneer species. For example, leaf life span reaches 6–7 years in firs and spruces, compared with only 2–3 years in pines (Becker et al. 1995). A slow foliage turnover allows the accumulation of several leaf cohorts that finally develop larger leaf areas than those of shorter turnover species without spending more energy (Lusk 2004).

Life strategies, fitness and functional convergence of conifer species

When regrouping the data from the three species, we estimated that about 75% of the annual radial increment variability was attributable to the rate of cell production and only 25% to its duration. Rathgeber et al. (2011b) found the same values to explain the tree-ring width variability between trees belonging to different social status in a mature silver fir plantation. These results suggest that the rate of cell production is the main component of the growth process that influences the fitness of a particular tree compared with its neighbors, whether of the same or different species.

Mixed stands have been theorized to achieve greater overall productivity than monocultures because of the 'competitive production principle', assuming that the competition acting between trees of different species is inferior to that between

trees of the same species (Vandermeer 1989). This reduction of competition is a consequence of the functional diversity between species, which leads to a complementary resource use in two dimensions: first in space, e.g., by the complementarity of crown and root architectures; second in time, by the occupation of different time windows to carry out different vital processes (Richardson and O'Keefe 2009, Ishii and Asano 2010). Indeed, we observed that needle phenology and primary growth critical dates (onset, maximal rate occurrence and cessation) were spread over a few weeks depending on the species. In contrast, we found that the maximal rate of tracheid production culminated at the same time for the three species, indicating that most wood production occurs at the same time for all trees. For conifers, shoot production and wood formation mainly depend on current-year assimilates (Hansen and Beck 1990, Oribe et al. 2003). Shoots, however, represent <10% of the annual carbon allocation, whereas wood is the strongest carbon sink, representing around 45% of the annual allocation (Grote 1998). Therefore, even if the spreading observed in shoot production suggests complementary resource use in time, the low quantity of energy needed to produce new shoots indicates that its benefit is limited.

On the contrary, the synchronicity observed in the occurrence of the maximal rate of wood production shows that species allocate great quantities of carbon to their stem simultaneously. Similar results were found by Rathgeber et al. (2011b) for silver firs of different social status within a close monospecific stand. Thus, within a close conifer stand, the occurrence of the maximal rate of cell production is controlled by the same environmental factors regardless of tree species or size. Its convergence toward the summer solstice may suggest, as proposed by Rossi et al. (2006a), that the maximum day length is this common environmental factor triggering growth decrease, allowing trees to safely finish secondary wall lignification before winter. However, the inter-annual variations we observed concerning this trait are not fully consistent with this hypothesis and suggest that other climatic factors (soil humidity for example) may play a role. Whatever the environmental control behind this synchronization, it demonstrates a strong functional convergence between conifer species, leading to a 'hard' head-on competition for resources, with all the trees fully expressing their needs at the same time. This functional convergence may be heightened by the evergreen character of the studied species, which makes a real 'phenological escape' impossible and thus limits the benefit of a competition-avoidance strategy (Richardson and O'Keefe 2009). The fact that early-successional species are not able to avoid the head-on competition imposed by this synchronization with late-successional species may be a clue to explain their elimination during conifer forest maturation.

Conclusions

For the three studied conifer species, the growing season (as delimited by cambial activity onset and cessation) lasted about 4 months, while the whole process of wood formation lasted ~5–6 months. Needle unfolding and shoot elongation followed the onset of cambial activity and lasted about one-third of the season. Species-specific life strategies were recognizable through critical functional traits of intra-annual wood formation dynamics. Pines exhibited an 'extensive strategy' characterized by long duration and low rate of growth, while firs and spruces adopted an 'intensive strategy' with shorter durations and higher rates. The opposition between Scots pine extensive strategy and silver fir and Norway spruce intensive strategy is consistent with the theory that pioneer species are more disposed to take risks for exploring space and time in order to capture new resources, while shade-tolerant species safely favor maximal efficiency in the use of available resources.

Despite the fact that both the timing and rates of wood formation provided pertinent functional traits indicative of contrasting species life strategies, we estimated that about 75% of the annual radial increment variability could be attributed to the rate of cambial activity, and only 25% to its duration. Considering the strong link between secondary growth and fitness of forest trees, this result suggests that, whatever the life strategy, fitness relies mainly on one single crucial functional trait: the rate of cambial activity.

Moreover, this rate of cambial activity culminated at the same time for all the trees of the stand, whereas shoot elongation, for example, reached its maximal rate earlier in pines than in firs and spruces. These results suggest a strong functional convergence between co-existing conifer species concerning key traits that influence fitness. Furthermore, the synchronization of the rates of cambial activity indicates that all the trees express their maximal resource need at the same time, which must result in a hard head-on competition. The fact that early-successional species are not able to avoid this competition may be a clue to explain their elimination during conifer forest maturation.

Supplementary data

Supplementary data for this article are available at *Tree Physiology Online*.

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Conflict of interest

None declared.

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