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A meta-analysis of cambium phenology and growth: linear and non-linear patterns in conifers of the northern hemisphere

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• *Background and Aims* Ongoing global warming has been implicated in shifting phenological patterns such as the timing and duration of the growing season across a wide variety of ecosystems. Linear models are routinely used to extrapolate these observed shifts in phenology into the future and to estimate changes in associated ecosystem properties such as net primary productivity. Yet, in nature, linear relationships may be special cases. Biological processes frequently follow more complex, non-linear patterns according to limiting factors that generate shifts and discontinuities, or contain thresholds beyond which responses change abruptly. This study investigates to what extent cambium phenology is associated with xylem growth and differentiation across conifer species of the northern hemisphere.
• *Methods* Xylem cell production is compared with the periods of cambial activity and cell differentiation assessed on a weekly time scale on histological sections of cambium and wood tissue collected from the stems of nine species in Canada and Europe over 1–9 years per site from 1998 to 2011.
• *Key Results* The dynamics of xylogenesis were surprisingly homogeneous among conifer species, although dispersions from the average were obviously observed. Within the range analysed, the relationships between the phenological timings were linear, with several slopes showing values close to or not statistically different from 1. The relationships between the phenological timings and cell production were distinctly non-linear, and involved an exponential pattern
• *Conclusions* The trees adjust their phenological timings according to linear patterns. Thus, shifts of one phenological phase are associated with synchronous and comparable shifts of the successive phases. However, small increases in the duration of xylogenesis could correspond to a substantial increase in cell production. The findings suggest that the length of the growing season and the resulting amount of growth could respond differently to changes in environmental conditions.

Key words: Cambium, cell differentiation, cell production, climate change, conifers, growth, meristem, phenology, productivity, secondary wall formation, xylogenesis.

INTRODUCTION

The timing of seasonal activities, or phenology, is a fundamental aspect of both plant and animal functioning at organization levels ranging from the individual to the ecosystem (Forrest *et al.*, 2010). Phenology is an integrated response to the environmental conditions accomplishing growth and reproduction of species. For this reason, archives of phenological observations have assumed particular importance in the last decades because shifts in phenological events have pointed out changes in the environment (Menzel *et al.*, 2006; Anderson *et al.*, 2013) or a mismatch among species along food chains (Johnson *et al.*, 2010). In plants, the phenology of growth represents a trade-off between environmental constraints and resource availability, and identifies the period when resources are acquired and used (Nord and Lynch, 2009). The growing season in temperature-limited

environments, for example, is an optimization between frost damage avoidance and maximization of annual carbon assimilation (Chuine, 2010).

The cambium is the secondary meristem of plants that produces layers of phloem and xylem cells that envelope the wood cylinders of stem, branches and roots, and results in seasonal radial growth. In temperate, boreal and some tropical ecosystems, the cambium undergoes winter dormancy, producing annual tree rings. During development, the xylem undergoes several different biochemical processes throughout its sequential stages of maturation (Prislan *et al.*, 2009). Thus, secondary growth represents an intriguing model of complex phenological processes of single cells that gradually and successively proliferate during a growing season. In contrast to the primary meristems (leaf and flower buds), cambial activity (e.g. cell division and differentiation) occurs within the plants and cannot be directly observed during the growing season.

Species native to colder climates are associated with a short growing season and low growth and productivity. At higher latitudes and altitudes, growth has to be completed within a limited time period and in less favourable conditions than in temperate climates (Chuine, 2010). Species adjust their phenology with shifted or compressed growth and reproduction phases, according to specific regional environmental drivers, local adaptations and individual plasticity to climate (Nord and Lynch, 2009; Diez *et al.*, 2012). However, it is unclear if and how this pattern applies to the cambium and its phenology across taxonomic groups and locations. In addition, changes of environmental conditions could have several effects by modifying the phenology of native species or, in the long run, driving migration towards higher latitudes and altitudes. Accordingly, the timings of phenological events are expected, and have already been in part observed, to change with the ongoing global warming (Menzel *et al.*, 2006). The challenge is to determine to what extent these modifications in phenology are associated with variations in forest productivity, in particular carbon uptake by trees (Forrest *et al.*, 2010).

Biological systems are composed of numerous interacting components that can be characterized by multivariate relationships such as covariation, causality and feedback, that may be directly proportional (i.e. linear) or not. Linear models are the most widely known and applied methods to quantify proportional changes, and have been demonstrated to be a valid representation of several processes, or at least of parts of them (Sparks *et al.*, 2009). However, a linear pattern can be considered as a special case: generally, nature follows more complex relationships, revealing non-linear or even non-monotonic behaviour (Burkett *et al.*, 2005; Porter and Semenov, 2005). Biological relationships may be, or appear to be, approximately linear within a central, bounded interval of variation, but with marked deviations from linearity at the extreme margins of the range (Sparks *et al.*, 2000). Living organisms experience limiting factors that generate shifts and discontinuities, or contain thresholds beyond which responses change abruptly (Stenseth and Mysterud, 2002; Körner, 2012). Coexistence or interaction of factors can have cumulative effects and produce disproportional responses of biological systems (Burkett *et al.*, 2005; Körner and Basler, 2010). The detection and understanding of non-linear relationships of ecological processes benefit from analyses that span a wide enough response range – be it spatial, temporal or system response states – that extend beyond linear regimes.

The questions that this paper aims to answer are: do the phenological phases of xylem production and differentiation occur independently of each other? If any relationships exist among the cambial phenological timings, what is their form (e.g. linear or not) and what does this imply about cambial and growth processes? Answers to these questions could contribute to a more complete understanding of the growth dynamics of forest ecosystems and their possible large-scale responses to climate change.

MATERIALS AND METHODS

Study species and tree selection

Data were collected from nine conifer species at numerous sites distributed in six countries (Table 1). The sites consisted of permanent plots located at different altitudes and latitudes in Europe and Canada and represented temperate-cold to cold environments. In each site, adult trees with upright and injury-free stems were selected for the study. Trees with polycormic stems, partially dead crowns, reaction wood or evident damage due to parasites were preferentially avoided. Between one and 20 trees were sampled per site and year.

Xylem sampling and preparation

Xylem formation was studied at all sites for one or more years during the 1998–2011 period. The different research teams followed the same technique of sample and data collection according to a common protocol. Wood microcores were collected weekly, or occasionally fortnightly, on the stem from 30 cm below to 30 cm above breast height (1·3 m) using surgical bone sampling needles or a Trephor (Rossi *et al.*, 2006a). Samples usually contained several previous xylem rings including the developing annual increment with the cambial zone and the adjacent phloem. The microcores were stored in solutions of propionic or acetic acid mixed with formaldehyde and ethanol at 5 °C to avoid tissue deterioration.

Microcores were dehydrated in ethanol and D-limonene, and embedded in paraffin (Rossi *et al.*, 2006a) or glycol methacrylate (Gruber *et al.*, 2010). The microcores collected in Switzerland were not embedded (Moser *et al.*, 2010). Transverse sections of 10–30 μm thickness were cut from the samples with rotary or sledge microtomes, stained with cresyl violet acetate (Rossi *et al.*, 2006b) or safranin and astrablue (Moser *et al.*, 2010), and examined under bright-field and polarized light to differentiate the developing and mature xylem cells.

Microscopic observations

In each sample, the radial number of cells in the cambial zone, as well as cells in the radial enlargement, secondary cell wall thickening and mature phases were counted along three radial rows. In cross-section, cambial cells were characterized by thin cell walls and small radial diameters (Rossi *et al.*, 2006b). Enlarging cells were composed of a protoplast still enclosed in the thin primary wall but with radial diameter at least twice that of a cambial cell. Observations under polarized light discriminated between enlarging and wall-thickening tracheids. The developing secondary walls shine when observed under polarized light, whereas no glistening is observed in enlarging cells (Abe *et al.*, 1997). Lignification was identified by a colour change from violet to blue with cresyl violet acetate or from blue to red when using safranin and astrablue. Completed colour change over the whole cell wall and lumina free of cell contents marked the end of lignification and tracheid maturity (Gričar *et al.*, 2005).

The number of cells in each developmental phase for every section was averaged over the three rows and used to assess onset and ending of each phase of cell differentiation. In spring, when at least one tangential row of differentiating or mature cells was observed, the differentiation or maturation phase was considered to have begun, respectively. In late summer, when no differentiating cell was observed, xylem formation was considered complete. Cambium phenology was represented by the dates corresponding to (1) the first enlarging cell; (2) the first wall-thickening cell; (3) the first mature cell; (4) the last enlarging cell; and (5) the last wall-thickening cell.

TABLE 1. *Studied species, location of the sites, years of monitoring and number of sampled trees*

Species	Site	Country	Latitude	Longitude	Altitude (m a.s.l.)	Study years	Number of trees
Abies alba	Amance	France	48°44′N	06°19′E	270	2006–2007	15–20
Abies alba	Grandfontaine	France	48°29′N	07°09′E	643	2007–2009	5
Abies balsamea	Arvida	Canada	48°26′N	71°09′W	80	1999–2000	18
Abies balsamea	Liberal 23	Canada	49°58′N	72°30′W	380	1998–2000	10
Abies balsamea	Liberal 24	Canada	49°58′N	72°30′W	430	1998–2001	5–10
Abies balsamea	Simoncouche	Canada	48°13′N	71°15′W	338	2005–2008	10
Larix decidua	Cinque Torri 1	Italy	46°27′N	12°08′E	2085	2001–2005	5
Larix decidua	Cinque Torri 2	Italy	46°27′N	12°08′E	2156	2002–2005	5
Larix decidua	Cinque Torri 3	Italy	46°27′N	12°08′E	2085	2004–2005	5
Larix decidua	Lötschental N08	Switzerland	46°23′N	07°45′E	800	2008	4
Larix decidua	Lötschental N13	Switzerland	46°23′N	07°45′E	1350	2007–2008	4
Larix decidua	Lötschental N16	Switzerland	46°23′N	07°45′E	1600	2007–2008	4
Larix decidua	Lötschental N19	Switzerland	46°23′N	07°45′E	1900	2007–2008	4
Larix decidua	Lötschental N22	Switzerland	46°23′N	07°45′E	2150	2007–2008	4
Larix decidua	Lötschental S16	Switzerland	46°23′N	07°45′E	1600	2007–2008	4
Larix decidua	Lötschental S19	Switzerland	46°23′N	07°45′E	1900	2007–2008	4
Larix decidua	Lötschental S22	Switzerland	46°23′N	07°45′E	2150	2007–2008	4
Larix decidua	Val di Susa	Italy	45°03′N	06°40′E	2030	2003–2004	5
Picea abies	Cinque Torri 1	Italy	46°27′N	12°08′E	2085	2001–2005	5
Picea abies	Cinque Torri 2	Italy	46°27′N	12°08′E	2156	2002–2005	1
Picea abies	Cinque Torri 3	Italy	46°27′N	12°08′E	2085	2004–2005	5
Picea abies	Grandfontaine	France	48°29′N	07°09′E	643	2007–2009	5
Picea abies	Lötschental N08	Switzerland	46°23′N	07°45′E	800	2008	4
Picea abies	Lötschental N13	Switzerland	46°23′N	07°45′E	1350	2007–2008	4
Picea abies	Lötschental N16	Switzerland	46°23′N	07°45′E	1600	2007–2008	4
Picea abies	Lötschental N19	Switzerland	46°23′N	07°45′E	1900	2007–2008	4
Picea abies	Lötschental S16	Switzerland	46°23′N	07°45′E	1600	2007–2008	4
Picea abies	Lötschental S19	Switzerland	46°23′N	07°45′E	1900	2007–2008	4
Picea abies	Menina Planina	Slovenia	46°16′N	14°48′E	1200	2009–2011	6–8
Picea abies	Panska reka	Slovenia	46°00′N	14°40′E	400	2009–2011	6
Picea abies	San Vito di Cadore	Italy	46°26′N	12°13′E	1000	2003	1
Picea mariana	Bernatchez	Canada	48°51′N	70°20′W	611	2002–2010	5–10
Picea mariana	Camp Daniel	Canada	50°41′N	72°11′W	487	2002–2010	5–10
Picea mariana	Mistassibi	Canada	49°43′N	71°56′W	342	2002–2010	5–10
Picea mariana	Simoncouche	Canada	48°13′N	71°15′W	338	2002–2010	5–10
Pinus cembra	Cinque Torri 1	Italy	46°27′N	12°08′E	2085	2001–2005	5
Pinus cembra	Cinque Torri 2	Italy	46°27′N	12°08′E	2156	2002–2005	5
Pinus cembra	Cinque Torri 3	Italy	46°27′N	12°08′E	2085	2004–2005	5
Pinus cembra	Patscherkofel krummholz	Austria	47°12′N	11°27′E	2180	2007	5
Pinus cembra	Patscherkofel timberline	Austria	47°12′N	11°27′E	1950	2007	6
Pinus cembra	Patscherkofel treeline	Austria	47°12′N	11°27′E	2110	2007	6
Pinus cembra	Val di Susa	Italy	45°03′N	06°40′E	2030	2003–2004	5
Pinus leucodermis	Pollino	Italy	39°54′N	16°12′E	2053	2003–2004	10
Pinus sylvestris	Grandfontaine	France	48°29′N	07°09′E	643	2007–2009	5
Pinus sylvestris	Tschirgant dry-mesic	Austria	47°14′N	10°50′E	750	2007–2010	5–8
Pinus sylvestris	Tschirgant xeric	Austria	47°14′N	10°50′E	750	2007–2010	6–8
Pinus uncinata	Val di Susa	Italy	45°03′N	06°40′E	2030	2003–2004	5

The timings of cambium phenology, computed in day of the year (DOY), as well as the number of radial cells produced annually by the cambium were calculated for each tree, site and year. The number of xylem cells varies around the stem and among samples, even if collected on the same day. Thus, cell production was quantified for each tree by fitting a sigmoid curve to the total number of cells produced during the year and estimating the upper asymptote of the function (Deslauriers *et al.*, 2008). The trees showing low goodness of fit or no representative asymptote were excluded from the analysis (29 of 927, representing 3·1 % of all tree–year combinations).

Statistical analyses

Relationships between phenological timings, durations of cell differentiation and cell production were performed using correlations and regressions. Before statistical analyses, variable distributions were inspected for normality. The distribution of the total cell production diverged significantly from normality and data were transformed using the common logarithm. The dates of the first enlarging cell showed a partially asymmetric distribution, but no transformation was applied due to the relatively low skewness.

Bootstrapped Spearman correlations (r_S) were calculated by resampling the original data set 10 000 times (Chernick, 2008). Bootstrap calculated robust statistics irrespective of the huge size of the sample, allowing only the relationships really correlated to emerge as significant. The resulting distribution of the coefficient r_S was skewed, clearly not normal, and with an upper limit at 1. Thus, a Fisher's *z*-transformation using the inverse hyperbolic tangent of r_S was applied to obtain statistics following a normal distribution. Correlations were considered

significant when both 95 % confidence intervals of the distributions were either higher or lower than zero (Chernick, 2008).

Reduced major axis (RMA) regressions were performed when correlations were significant. RMA minimizes the sum of the product of both vertical and horizontal deviations, which is equivalent to the area of triangles formed by the deviation of a point from the line in the vertical and horizontal directions (Smith, 2009). RMA was more appropriate than the conventional ordinary least squares (OLS) method because both dependent and independent variables were measured with error. The bootstrap procedure with 10 000 replications was adopted for computing 95 % confidence intervals of the intercept and slope. For the identification of the dependent and independent variables of the regressions, the temporal rule in which effects, if any, might propagate only from preceding to subsequent phenological phases was assumed. The last cell of the tree ring had to be produced before completing enlargement; accordingly, the radial number of cells within the tree ring was considered as an independent variable in respect to the ending of cell enlargement and wall thickening. However, the assignment of the variables was only a formal procedure, because RMA regression symmetrically defines the bivariate relationship, regardless of which variable is dependent and which is independent (Smith, 2009).

RESULTS

Phenological timings and cell production

The study concerned an analysis of cambium phenology based on >800 tree–year combinations in Europe and Canada. Overall, the first enlarging cells, corresponding to the onset of xylem differentiation, were observed between mid-April (DOY 100) and the end of June (DOY 180), although very occasionally enlargement was observed earlier in *Abies alba*, *Pinus sylvestris*

and *Picea abies* (Fig. 1). On average, the first wall-thickening cells were detected 15 d after the onset of the enlargement phase, between the beginning of May and mid-July, with 75 % of observations occurring in June (DOY 150–180). The first mature cells appeared between mid-May and the end of July, on average 17 d after the onset of cell wall thickening (Fig. 1). The earliest onsets of each phenological phase were observed at the lower altitudes, while cell differentiation began later in the sites at the higher altitudes.

On average, 35 xylem cells were produced by the cambium along a radial row of a tree ring, within a range of 5–144, with most observations (84 %) being between ten and 50 (Fig. 1). The number of cells showed the highest dispersion among the analysed variables, with a coefficient of variation of 62 %, at least five times larger than the phenological timings. The distribution of these data was right-skewed to exponential, with only 5 % of individuals producing >80 cells. In general, the highest cell production was observed in *P. abies* and *A. alba*, while fewer cells were produced by *Picea mariana* and *P. sylvestris*.

The ending of cell enlargement generally occurred between the beginning of July and mid-September (on average in mid-August), although in 1·6 % of cases cessation of the enlargement phase was observed in June and October (Fig. 1). The last cells in the phase of lignification, which corresponded to the ending of both cell wall thickening and lignification and xylem differentiation, were observed in September, on average 40 d after the conclusion of the enlargement phase. Most trees completed cell maturation in the period between September (DOY 250) and the beginning of October (DOY 280).

Duration of xylem differentiation

Durations of the phases of cell differentiation were extremely variable, as evidenced by the wide range of the distributions
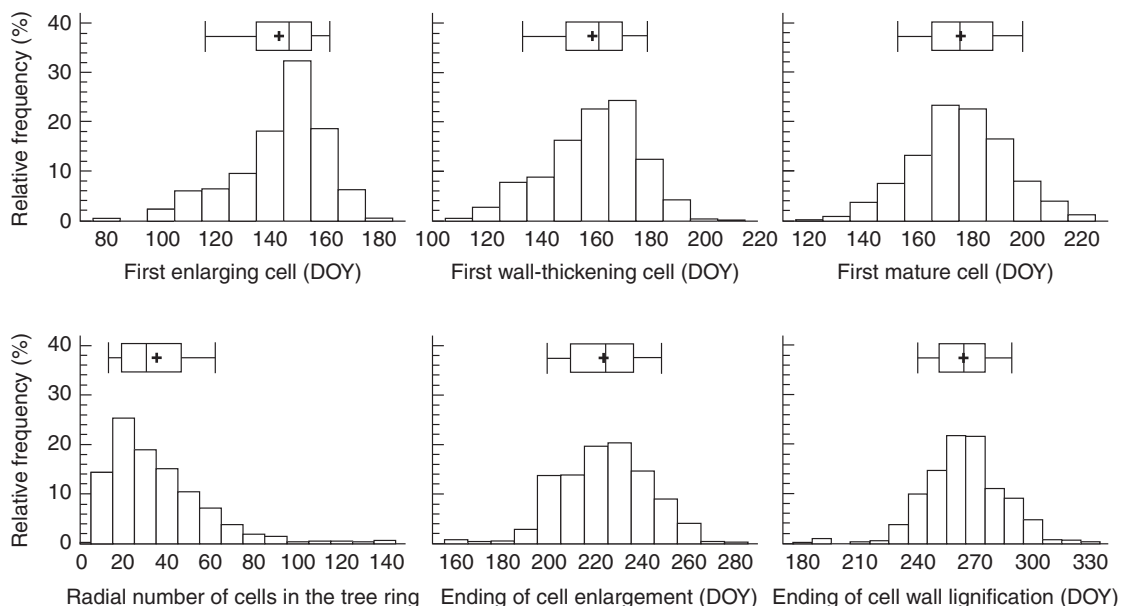

FIG. 1. Frequency distributions of the dates of cambium phenology (first enlarging, wall-thickening and mature cell, ending of cell enlargement and cell wall lignification) and the radial number of cells produced annually by the cambium of nine conifer species of Europe and Canada. Horizontal boxes represent the upper and lower quartiles, whiskers achieve the 10th and 90th percentiles, and the mean and median are drawn as plus signs and vertical solid lines, respectively. DOY, day of year.
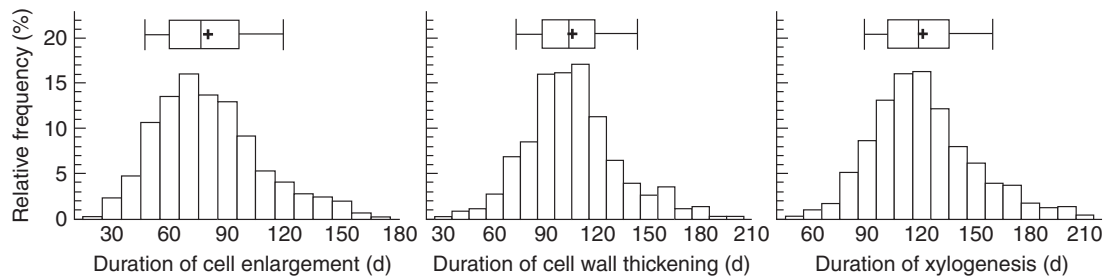
FIG. 2. Frequency distributions of the durations of cell enlargement, cell wall thickening and xylogenesis observed in tree rings of nine conifer species of Europe and Canada. Horizontal boxes represent upper and lower quartiles, whiskers achieve the 10th and 90th percentiles, and the mean and median are drawn as plus signs and vertical solid lines, respectively.

(Fig. 2). Cell enlargement lasted 80 d on average, with 81 % of observations between 50 and 110 d. Only 2·4 and 2·5 % of observations showed enlargement phases shorter than 30 d and longer than 150 d, respectively. *Picea mariana* tended to have the shortest period of cell enlargement. Secondary wall differentiation and the overall duration of xylogenesis lasted on average 105 and 120 d, respectively (Fig. 2). Longer periods of wall thickening and lignification and xylogenesis were observed in *A. alba* and *P. sylvestris* in France.

Relationship between phenological timings and xylem cell production

The dates of emergence of the first enlarging, wall-thickening and mature cells were strongly correlated with positive and significant r_S (Fig. 3). Thus, late onset of cell enlargement was associated with delayed onset of secondary cell wall formation and late emergence of mature cells. Similarly, the ending of cell enlargement was significantly and positively correlated with the ending of cell wall lignification. The models estimated by RMA regressions between these variables showed slopes varying between 0·99 and 1·08 (Table 2). In contrast, no correlation was observed between the phases of onset and ending of differentiation (Fig. 3). Except for the date of the first enlarging cells, all phenological timings were significantly correlated with the logarithm of the number of xylem cells in a radial row, even if with different slopes. The logarithm of cell production was positively correlated with the emergence of the first wall-thickening and mature cells, and negatively correlated with the ending of cell enlargement and lignification. Accordingly, later dates of first observed wall-thickening and mature cells corresponded to lower annual xylem cell production, and greater numbers of cells were observed when the phases of cell enlargement and cell wall lignification were completed later in autumn (Fig. 3).

Relationship between duration of cell differentiation and cell production

All variables reported in Fig. 4 showed significant correlations with positive r_S. Consequently, higher annual xylem cell production (in the form of common logarithm) was associated with longer durations of cell enlargement, wall thickening and xylem formation (Table 3). Similarly, longer periods spent for cell enlargement were associated with longer periods spent for completing cell walls, and, as a consequence, a longer overall duration of xylogenesis. The slopes of the RMA regressions between the durations of differentiation and cell production ranged between 0·99 and 1·02, with 95 % confidence intervals always including 1 (Table 3).

DISCUSSION

Linear patterns of phenology

This study regarded a meta-analysis that correlated the phenological timings of xylem and cell production in order to verify the existence of common relationships across nine conifer species living at different latitudes and altitudes in Europe and Canada. The dynamics of xylogenesis were surprisingly homogeneous between the conifer species: despite the wide geographical range considered, the number of studies included and the variability among trees, the dates of successive phenological timings significantly converged towards positive correlations. This result was somehow partially intuitive: time is directional and asymmetric, with effects that can clearly propagate only from preceding towards subsequent events, and not vice versa (Forrest *et al.*, 2010). Thus, a certain phenological phase can take place only after the previous one has occurred. However, more interestingly, regressions showed a novel and unexpected aspect of the relationship between the phenological timings: the models were linear within the range analysed by our data, with several slopes showing values very close to 1. Indeed, it seems that, on average, shifts of one phenological phase lead to comparable shifts of the successive phases. Accordingly, it could be deduced that the average time required for differentiating the first cells of the tree ring is independent of the date of growth resumption, and is estimated as 15 and 17 d for cell enlargement and wall thickening, respectively. Similarly, the last latewood tracheids spend approx. 40 d to complete thickening and lignification of their cell walls. In addition, the durations of cell differentiation and the overall period of xylogenesis are linearly proportional according to the factor 1. These results considered the most general pattern between phenological timings, although dispersions from the average were obviously observed, mainly for the end of differentiation, which indicated the occurrence of other factors affecting the phenology within and between species.

Non-linear patterns of cell production

Correlations and regressions were performed with the variable cell production transformed into the common logarithm.
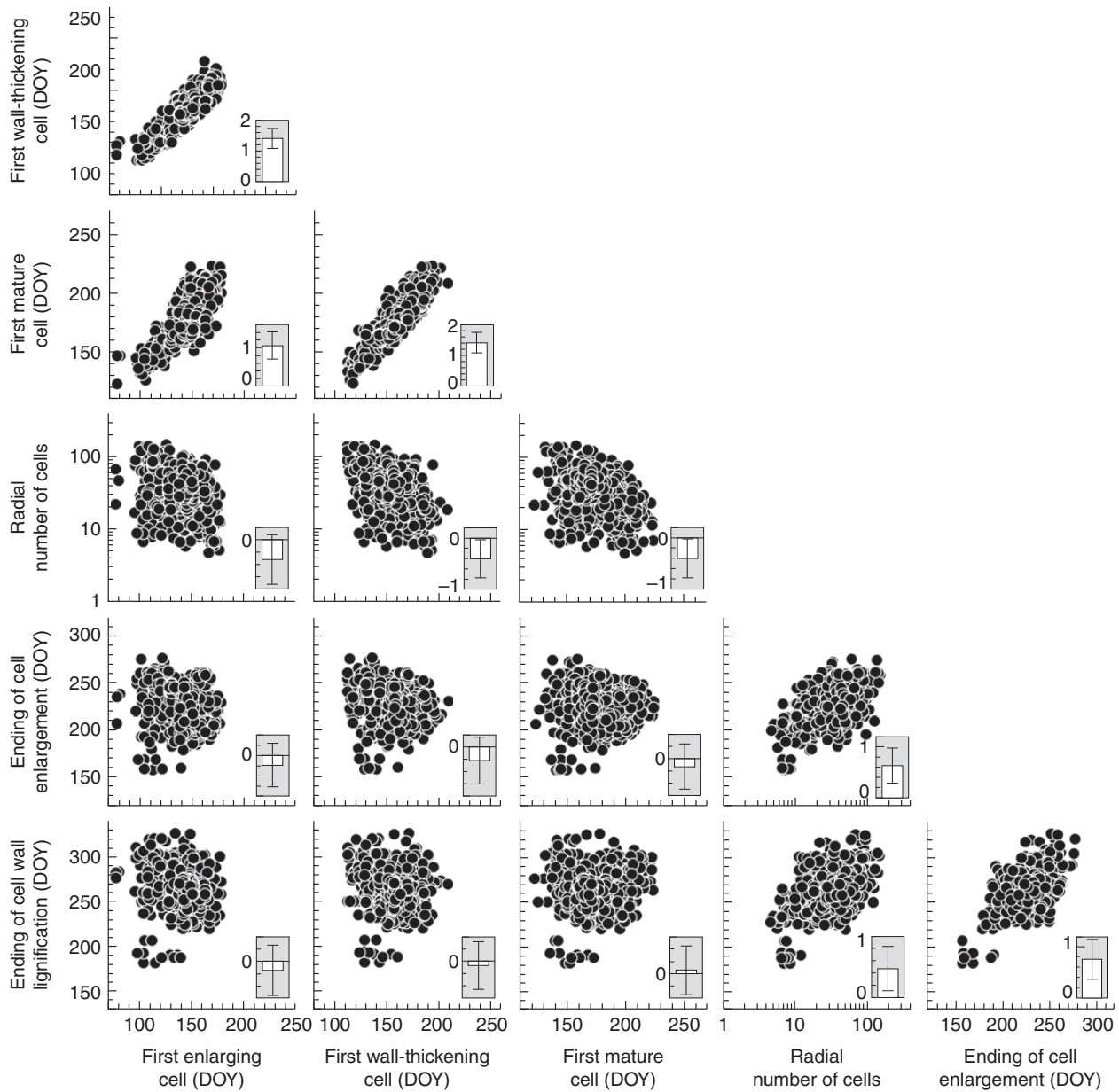
FIG. 3. Correlation matrix between the dates of cambium phenology (first enlarging, wall-thickening and mature cell, ending of cell enlargement and cell wall lignification) and the radial number of cells produced annually by the cambium of nine conifer species of Europe and Canada. The enclosed vertical bars represent the mean and 95 % confidence interval based on the Fisher's z-transformation of the bootstrapped Spearman correlation coefficient calculated by 10 000 replications. The correlation is considered significant when both confidence intervals are either higher or lower than zero. Note the $\log_{10}$ scale on the axis for the number of cells. DOY, day of year.

Consequently, the relationships between the phenological timings and cell production were distinctly non-linear, and involved an exponential pattern. Earlier spring phenology and later conclusion of cell differentiation were associated with more than proportional increases in the number of cells. As a result, small increases in the duration of xylogenesis could correspond to a substantial increase in cell production, both within and across species. Higher cell production implies more derivatives to be differentiated, and greater volumes of cell walls to be thickened, the process of wall thickening being longer and more demanding in resources than enlargement (Fig. 2). This necessarily results in higher forest productivity and involves greater carbon assimilation by

trees. Similarly, a wider xylem area results in a greater amount of water supplied for producing and maintaining leaves, with a consequent positive feedback of increased potential productivity of plants. These findings suggest that, in temperature-limited ecosystems, the environmental conditions leading to increases in the period of growth could generate disproportionate responses in terms of tree growth.

The divergent patterns observed between phenological timings and cell production could be related to the different mechanism regulating the physiological processes. At the latitudes where the study was carried out, a phenological phase such as cambial resumption is an event triggered by temperature,

TABLE 2. *Intercepts and slopes resulting from the RMA regressions comparing the dates of cambium phenology and the radial number of cells produced annually by the cambium of nine conifer species of Europe and Canada*

Dependent variable	Independent variable	Intercept	Slope	CI of intercept	CI of slope	R^2
First wall-thickening cell	First enlarging cell	15·48	0·99	−10·33 to 39·54	0·83 to 1·17	0·77
First mature cell	First enlarging cell	28·32	1·02	−4·88 to 58·87	0·81 to 1·25	0·60
First mature cell	First wall-thickening cell	12·44	1·02	−13·81 to 37·39	0·86 to 1·19	0·78
Log_{10} radial number of cells	First wall-thickening cell	3·98	−0·01	2·54 to 5·33	−0·02 to −0·006	0·14
Log_{10} radial number of cells	First mature cell	4·17	-0·01	2·54 to 5·68	−0·02 to −0·006	0·13
Ending of cell enlargement	Log_{10} radial number of cells	119·60	70·68	92·07 to 146·08	52·90 to 89·13	0·30
Ending of cell wall lignification	Log_{10} radial number of cells	151·82	76·34	106·56 to 199·42	44·36 to 106·76	0·19
Ending of cell wall lignification	Ending of cell enlargement	22·63	1·08	−35·13 to 80·47	0·81 to 1·34	0·40

CI indicates the bootstrapped 95 % confidence interval of the estimated parameters.
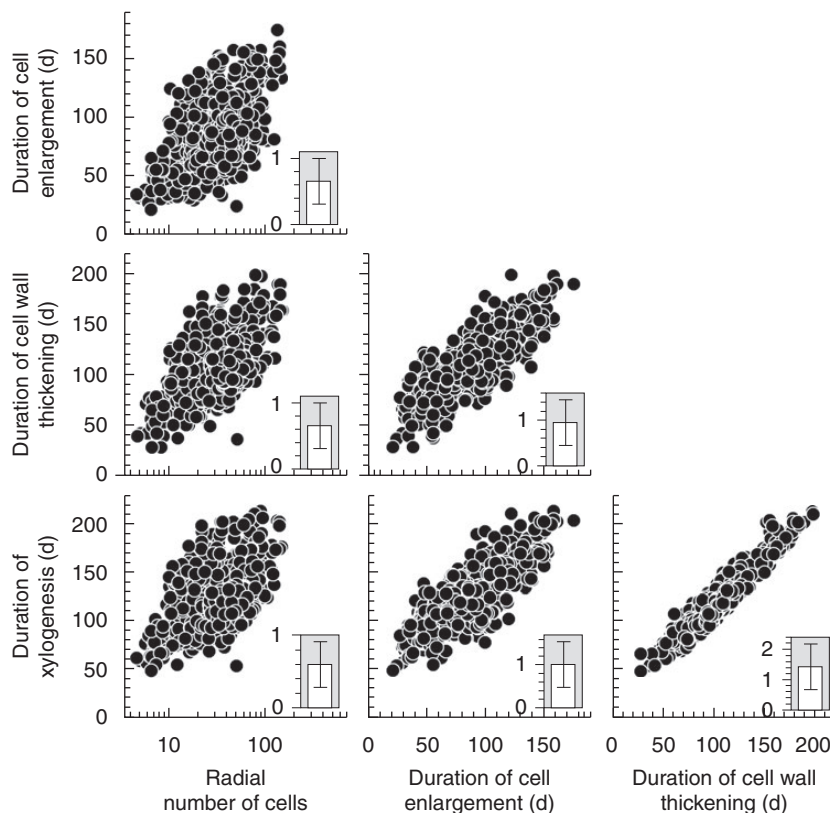The number of cells is transformed using the common logarithm.

FIG. 4. Correlation matrix between the durations of cell enlargement, cell wall thickening and xylogenesis and the radial number of cells produced annually by the cambium of nine conifer species of Europe and Canada. The enclosed vertical bars represent the mean and 95 % confidence interval based on the Fisher's *z*-transformation of the bootstrapped Spearman correlation coefficient calculated by 10 000 replications. The correlation is considered significant when both confidence intervals are either higher or lower than zero. Note the log_{10} scale on the axis for the number of cells.

according to either a gradual influence or a threshold effect (Rossi *et al.*, 2007; Seo *et al.*, 2008; Swidrak *et al.*, 2011). In contrast, xylem growth is a gradual accumulation of cells resulting from an integration of several mitotic processes lasting from a few weeks to several months. Earlier cambial resumptions are expected in sites or in species living where warmer spring temperatures occur. Thus, on the one hand, this allows more time for cambial cells to divide and produce new tracheids. On the other, the more favourable thermal conditions speed up cell production once dormancy has been broken, leading to higher rates of cell division being achieved. The latter mechanism explains

most of the variability in cell production (Rathgeber *et al.*, 2011; Cuny *et al.*, 2012). As a consequence, the exponential relationships observed between phenological timings and cell production could be the cumulative result of the length of the period of cambial activity and the rate of cell division. Moreover, the relationship between the length of the growing period and cell production could be complicated by the allocations of carbon observed within the different tree compartments. Trees in colder climates allocate higher proportions of their resources for the metabolism of below-ground organs, thus shifting the growth from stem towards roots (Gower *et al.*, 2001). The observed non-linear patterns could

TABLE 3. *Intercepts and slopes resulting from the RMA regressions comparing the durations of cell differentiation and the radial number of cells produced annually by the cambium of nine conifer species of Europe and Canada*

Dependent variable	Independent variable	Intercept	Slope	CI of intercept	CI of slope	R^2
Duration of cell enlargement	Log_{10} radial number of cells	−70·04	102·37	−108·91 to −32·93	76·25 to 129·51	0·32
Duration of cell wall thickening	Log_{10} radial number of cells	−44·52	102·03	−83·61 to −6·25	75·25 to 129·25	0·31
Duration of xylogenesis	Log_{10} radial number of cells	−33·23	104·89	−77·33 to 9·85	74·72 to 135·58	0·24
Duration of cell wall thickening	Duration of cell enlargement	25·28	0·99	9·09 to 41·32	0·80 to 1·19	0·65
Duration of xylogenesis	Duration of cell enlargement	38·53	1·02	23·55 to 53·12	0·84 to 1·21	0·66
Duration of xylogenesis	Duration of cell wall thickening	12·53	1·02	0·93 to 23·39	0·92 to 1·13	0·91

CI indicates the bootstrapped 95 % confidence interval of the estimated parameters.
The number of cells is transformed using the common logarithm.

therefore be in part explained because the shorter the growing period is, the higher the proportion of resource allocation to the roots.

The analysis compared species from different ecosystems, with a gradient of phenological timings representing long-term conditions of different growing seasons, here identified by the duration of xylogenesis, and defined by alternation of thermally favourable and unfavourable climate conditions during the year (i.e. summer vs. winter). It could be assumed that the observed reduction in the duration of xylogenesis from 220 to 50 d corresponds to a reduction in the temperatures of the sites. In most sites investigated for this study, the soil is frozen or covered by snow in winter, with temperatures close to 0 °C. At these temperatures, metabolism slows down as much as possible because only minimal physiological activity is allowed, and root activity and water uptake are inhibited (Decker *et al.*, 2003; Körner, 2003). Thermal conditions play a crucial role in decomposing soil organic matter and mineralizing soil nutrients, which affect the availability of nutrients for trees, mainly in the colder environments (Jarvis and Linder, 2000). The acquisition of both mobile and immobile soil resources increases with longer growing seasons (Nord and Lynch, 2009). Besides, snow cover, wintertime nutrient cycling and microbial processes strongly influence the annual transfer of nutrients in plants of cold ecosystems (Groffman *et al.*, 2009; Lupi *et al.*, 2013). Thus, the capture of nutrients from the soil, which are less available in colder sites and for a shorter period of time, could be an important constraint to growth. If the hypothesis of the effect of nutrient availability on cell production is confirmed, the adaptation of the phenological timings and xylem cell production could be asynchronous under a changing climate, at least at the highest latitudes or altitudes: shifts of cell production could be delayed in respect to cambial phenology, and might occur only when the biogeochemical processes (e.g. decomposition and mineralization) are adjusted to the new environmental conditions.

Linking onset and ending of xylogenesis

The diverse correlations observed between the phenological timings revealed the structure of the process of xylem formation and the close interconnection of all differentiation phases, from cell production to maturation. Our findings clearly demonstrated that the phenological phases are not episodic events that occur independently of each other, as also observed by Rossi *et al.* (2012). Although there is no evidence of a causal mechanism

in which a phenological phase necessarily affects or determines the successive one just because it occurs first, the observed correlations suggest that either such a causal link exists or, alternatively, the phenological timings are all related to the same internal or environmental drivers.

Earlier cellular differentiation was correlated with higher annual cell production, which in turn was associated with later maturation of terminal latewood tracheids. In contrast, the onset and ending of cell differentiation were not directly related (the correlations between the dates of the first differentiating cells and those of ending of cell enlargement and lignification were not significant; Fig. 3). It should be noted that onset and ending of xylogenesis involve different parts of the xylem, the first cells of earlywood and the last cells of latewood, respectively. Nevertheless, both phenological events showed significant correlations with total xylem cell production, which suggested the presence of possible common drivers of these phenological events. The results of this study definitively quantify the well-known phenomenon recorded along latitudinal and altitudinal gradients of temperate and cold climates in which reductions in wood production are associated with shorter periods of growth, regardless of species and biome (Rossi *et al.*, 2008; Moser *et al.*, 2010; Lupi *et al.*, 2012). The relationship between duration and amount of growth has been observed both in nature and in manipulation experiments. Cooling treatments reduced the width of tree rings and shortened the period of cambial activity (Gričar *et al.*, 2007). Similarly, roots with larger tree rings (i.e. a higher number of tracheids) than the stem showed later endings of cell wall lignification and thus longer periods of xylogenesis (Thibeault-Martel *et al.*, 2008).

Sparks and Menzel (2002) reported that earlier phenological events are more variable and change faster than later events. In the last decades, most of the reported lengthening of the growing season for both primary and secondary meristems is associated with an earlier onset rather than a delayed ending (Chmielewski and Rötzer, 2001; Boulouf Lugo *et al.*, 2012). An earlier spring phenology is more closely linked to more favourable conditions for photosynthesis than a longer autumnal growing season (Chuine, 2010). Our analysis across species confirmed these hypotheses by showing a greater variability in the spring onset than in the summer–autumn ending of differentiation. The coefficients of variation, dimensionless numbers independent of the mean of the distributions, indicated that the dispersion of the phenological timings gradually reduced from 11·7 for the first enlarging cell to 7·7 for the ending of cell wall lignification (data not shown). If the hypothesis of a chain of

intercorrelated phenological events based on causality during xylem formation is confirmed, this would indicate that the ending of cell differentiation represents more than just a direct response of trees to the transitory weather conditions. Ending of cell maturation may represent a conclusive event resulting directly or not from the previous phenological timings and integrating the weather signal over longer periods of time.

Concluding remarks and implications

Cambial activity is an intriguing biological system that in temperate and cold climates generates an annual sequence of phenological processes resulting in an accumulation of xylem cells representing the secondary growth. The phenological timings and xylem cell production of conifers of the Northern hemisphere are correlated. During the growing season and for the ranges observed in this study, the trees adjust their phenological timings according to linear patterns. Thus, shifts of one phenological phase are associated with synchronous and comparable shifts of the successive phases. In contrast, xylem cell production is related to the phenological phases by means of non-linear relationships. Lengthening of the growing season is associated with a more than proportional increase in the number of xylem cells. Thus, modifications of the environmental conditions that affect plant phenology could potentially engender disproportionate responses in secondary growth. Marginal consequences of environmental changes on the length of the growing season could be substantial in terms of cell production and carbon uptake by trees, and consequently forest productivity.

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