



**HAL**  
open science

## Timing of spring xylogenesis in temperate deciduous tree species relates to tree growth characteristics and previous autumn phenology

Lorène Julia Marchand, Inge Dox, Jožica Gričar, Peter Prislan, Jan van den Bulcke, Patrick Fonti, Matteo Campioli

### ► To cite this version:

Lorène Julia Marchand, Inge Dox, Jožica Gričar, Peter Prislan, Jan van den Bulcke, et al.. Timing of spring xylogenesis in temperate deciduous tree species relates to tree growth characteristics and previous autumn phenology. *Tree Physiology*, 2021, 41 (7), pp.1161-1170. 10.1093/treephys/tpaa171 . hal-03101749

**HAL Id: hal-03101749**

**<https://hal.science/hal-03101749v1>**

Submitted on 25 Feb 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## **Timing of spring xylogenesis in temperate deciduous tree species relates to tree growth characteristics and previous autumn phenology**

Lorène Julia Marchand<sup>1,2</sup>, Inge Dox<sup>1</sup>, Jožica Gričar<sup>3</sup>, Peter Prislan<sup>3</sup>, Jan Van den Bulcke<sup>4</sup>, Patrick Fonti<sup>5</sup>, Matteo Campioli<sup>1</sup>

<sup>1</sup>Research Group PLECO (Plants and Ecosystems), Department of Biology, University of Antwerp, 2160 Wilrijk, Belgium

<sup>2</sup> UMR 6553 ECOBIO (Ecosystèmes, Biodiversité, Evolution), Université de Rennes 1, CNRS, 263 Av. du Général Leclerc, 35042 Rennes, France

<sup>3</sup> Department of Yield and Silviculture, Slovenian Forestry Institute, Vecna pot 2, 1000 Ljubljana, Slovenia

<sup>4</sup> Laboratory of Wood Technology, UGent-Woodlab, Department of Environment, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, 9000 Gent, Belgium

<sup>5</sup> Swiss Federal Research Institute WSL, Zürcherstrasse 111, 8903 Birmensdorf, Switzerland

**Corresponding author's email:** [lorene.marchand@univ-rennes1.fr](mailto:lorene.marchand@univ-rennes1.fr)

## **Abstract**

We explored the timing of spring xylogenesis and its potential drivers in homogeneous mature forest stands in a temperate European region. Three species with contrasting leaf development dynamics and wood anatomy were studied: European beech, silver birch and pedunculate oak. Detailed phenological observations of xylogenesis and leaf phenology were performed from summer 2017 till spring 2018. Cambium reactivation (CR) occurred before the buds of oak and birch were swollen, whereas these two phenological phases were concurrent for beech. On the other hand, initial earlywood vessels were fully differentiated (FDIEV) after leaf unfolding for all three species. Timing of CR was correlated to average ring-width of the last 10 years (2017-2008), tree diameter, and, partially, with tree age. In addition, the timing of FDIEV was correlated to tree age and previous' year autumn phenology i.e. timing of wood growth cessation and onset of leaf senescence. Multivariate models could explain up to 68% of the variability of CR and 55% of the variability of FDIEV. In addition to the "species" factor, the variability could be explained by ca. 30% by tree characteristics and previous' years autumn phenology for both CR and FDIEV. These findings are important to better identify which factors (other than environment) can be driving the onset of the growing season and highlight the influence of tree growth characteristics and previous' year phenology on spring wood phenology, wood formation and, potentially, forest production.

**Keywords:** *Fagus sylvatica*, *Quercus robur*, *Betula pendula*, cambium, seasonal growth, hardwood species

## **Introduction**

Phenology is the study of event's occurrence in plant and animal's life cycles in interaction with the environment (Forrest and Miller-Rushing 2010). For trees, phenological observations are typically focussed on primary growth, for example, on the leaf phenophases (Koch et al. 2007). In temperate deciduous forests, the leaf life cycle starts in spring with bud-burst and ends in fall with leaf senescence. For secondary growth (indicated also as wood formation or xylogenesis), phenology is much less studied because of the complexity of its assessment (Zuidema et al. 2018, Friend et al. 2019). However, thanks to its seasonal pattern, secondary growth can also provide key information about tree functioning. In fact, the timing of the increasing number of cambial cells i.e. cambium reactivation (CR from here on), and of the full differentiation of the first row of earlywood vessels i.e. fully differentiated initial earlywood vessels (FDIEV from here on), are key events to characterize the timing of reactivation of the main carbon sink in the tree and the formation of its new hydraulic system (Begum et al. 2013). For ring-porous species, CR precedes bud-burst (González-González et al. 2013), whereas for diffuse-porous species the two events are concurrent (Čufar et al. 2008, Prislan et al. 2013). However, the relationships between spring xylogenesis and the other leaf phenophases (e.g. swollen buds, complete leaf unfolding) are less investigated. Accurate studies on CR and FDIEV (secondary growth phenology) and leaf development (primary growth phenology) are essential to better understand the drivers and mechanisms of the onset of the growing season and important tree (xylem) functional traits (Rathgeber et al. 2016).

Secondary growth represents one-third of the annual biomass production in temperate forest ecosystems (Capioli et al. 2016). Nevertheless, models of annual biomass production are largely based on primary growth variables (Friend et al. 2019). Therefore, the identification of determinants of secondary growth is important to predict vegetation growth and to increase

the accuracy of dynamic global vegetation models (Zuidema et al. 2018, Friend et al. 2019). For the main tree species in temperate European forests (e.g. European beech, pedunculate oak, sessile oak and Scots pine) secondary growth is influenced more by the growth duration (controlled by spring and autumn phenology) than the growth rate (Michelot et al. 2012, Pérez-de-Lis et al. 2017). Therefore it is important to gain more insights on CR and FDIEV.

For coniferous species, the onset of primary and secondary growth across the northern hemisphere is driven mainly by photoperiod and temperature (Piao et al. 2019, Huang et al. 2020). Concerning temperature, chilling (amount of cold experienced in late autumn-early winter) and forcing (amount of warmth experienced in late winter-early spring) are both needed to explain the beginning of xylogenesis (Delpierre et al. 2019). For angiosperm species, spring xylogenesis is also driven by climate, especially warm temperatures in late winter-early spring (Begum et al. 2013, 2018, Delpierre, et al. 2016). However, even with this knowledge, inter-individual variability of xylogenesis within a homogenous forest stand cannot be explained (Prislan et al. 2013). Comprehensive phenological studies of xylogenesis are therefore needed to understand tree functioning at individual scale within a stand.

At individual level, the timing of spring xylogenesis could be related to tree characteristics (e.g. age, size) or previous' year autumn phenology of leaves and wood (Rossi et al. 2007, Pérez-de-Lis et al. 2016). Multiple studies on coniferous species show that age and size affect spring xylogenesis. For example, younger individuals of *Larix decidua*, *Pinus cembra* and *Picea abies* have CR earlier than mature ones (Rossi et al. 2007, 2012). Furthermore, taller silver-firs have CR earlier than the smaller individuals (Rathgeber et al. 2011). However, less is known about the drivers of intra-stand variability of spring xylogenesis for angiosperm species as many of the existing studies on spring xylogenesis for angiosperms are mainly focused on hydraulic properties and wood anatomy (e.g. Pérez-de-Lis et al. 2016). Therefore, we hypothesize that, in a homogeneous mature stand, younger trees present earlier CR and

FDIEV than older trees. We expect for diameter the same trend as for age because of the consistent relationship between the two variables in homogenous stands. Timing of leaf autumn phenology in the previous year might affect the timing of current wood growth through the content of non-structural carbohydrates. In fact, later senescence likely assures more carbon uptake and more storage of reserves (Bréda et al. 2006, Estiarte and Peñuelas 2015) in a period (autumn) when growth is modest (Dox et al. 2020). More reserves might trigger fast spring reactivation because of greater fitness and the potential to mitigate risks such as, for example, late cold spells (Morin et al. 2007, Hartmann and Trumbore 2016, Tixier et al. 2019). In fact, the negative relationship between carbon reserves and spring phenology (i.e. the larger the carbon reserves, the earlier the spring reactivation) has been already suggested for primary growth (Delpierre et al. 2017, Pérez-de-Lis et al. 2017). Therefore we hypothesize that trees with later onset of leaf senescence also present earlier CR and FDIEV. Our hypothesis is further strengthened by the correlation between carbon storage in the previous year and width-ring variation in the following year, as observed for beech (Skomarkova et al. 2006). As in temperate angiosperms, autumn wood growth cessation is generally concurrent (or shortly before) onset of leaf senescence (Dox et al. 2020), we expect spring xylogenesis to be influenced in the same way by both the onset of leaf senescence and wood growth cessation. To our knowledge, the interaction between previous autumn phenology (leaf senescence and wood growth cessation) and the timing of spring xylogenesis (CR and FDIEV) has never been studied for angiosperm or coniferous tree species.

Here we postulate that under favorable climatic conditions (thus without stressors or extreme events), tree growth characteristics and previous' year autumn phenology can influence the timing of spring wood growth in addition to wood anatomy. The objectives of our study are (i) to determine the timing of CR and FDIEV in relation to spring leaf phenology for common deciduous forest tree species of the European temperate zone, (ii) study the timing of CR and

FDIEV in relation to tree growth characteristics and previous autumn phenological events, and (iii) build statistical models to determine the importance of such variables on the timing of CR and FDIEV. The study was performed on pedunculate oak (*Quercus robur* L.), European beech (*Fagus sylvatica* L.) and silver birch (*Betula pendula* Roth.), in two forests nearby Antwerp, Belgium, between summer 2017 and spring 2018. Whereas, (though with fewer details), previous studies have already compared leaf- and wood spring phenology of beech and oak, to the best of our knowledge, this is also the first study presenting a detailed description of leaf- and wood spring phenology for birch.

## **Material and methods**

### *Species and sites*

The species selected for this study (oak, beech and birch) are among the most common temperate forest tree species in Europe. Oak and beech are late-successional species with a determinate growth (*i.e.* they produce leaves in 1-2 flushes) whereas birch is a pioneer species with an indeterminate growth (*i.e.* it produces leaves continuously throughout the season). Regarding the wood type, beech and birch are diffuse-porous species, whereas oak is ring-porous. The study took place on two nearby (< 10 km) mature temperate forest sites in northern Belgium, specifically the park of Brasschaat (PB, 51°12' N, 4°26'E and 18m a.s.l) and the 'Klein Schietveld' (KS, 51°21'N, 4°37'E and 22m a.s.l). The region is characterized by a maritime climate with regular precipitation (778 mm year<sup>-1</sup>) and a monthly mean temperature ranging from +3°C (January) to 17°C (July) (Campioli et al. 2012). Both sites are characterized by sandy soil, but with a thicker organic layer (60-90cm) at PB compared to KS. On each site, we selected one homogeneous stand of each species. These were monospecific

for beech PB, oak PB, beech KS and birch KS, but with the presence of other species for birch PB (mainly *Quercus rubra*) and oak KS (mainly *Betula pendula*).

#### *Tree selection and their characteristics*

Dominant or codominant healthy trees of each species were selected. In total we selected 28 trees (4 oaks, 4 beeches and 4 birches) at each site, except for KS, where we selected 8 beeches trees. For each tree we determined the stem diameter at breast height and a tree density index to consider the effect of competition (Supp. Data Table I). The latter was calculated considering the total basal area of all (co)dominant trees within a circle centered at our target tree. Additionally, to assess the age and the average growth rate of the last decade, selected trees were cored on the north and south part with a Pressler corer. Tree-ring width measurements were performed with a Lintab measuring stage (0.01 precision) and TSAP-Win software (Table Supp. Data. 1).

#### *Wood phenology*

Wood xylogenesis was assessed from late August till late November 2017 and from early March till mid-June 2018. Weekly to biweekly stem micro-cores were collected using a Trephor (Rossi et al. 2006). To avoid any impact of previous sampling, sampling followed an upward spiral at a stem height between 1.3 m to 2 m. Sample processing to obtain micro-sections was performed at the Slovenian Forestry Institute (Ljubljana, Slovenia) according to the protocol described in Prislán et al. (2014). In short, immediately after removal from the trees, the samples were stored in 70 % of ethanol solution and further processed in the laboratory for preparation of permanent cross-sections (5-10  $\mu\text{m}$  in thickness) stained with safranin and Astra blue and embedded in Euparal. Histometric observations and image capturing were performed using the LAS image analysis software (LEICA Application Suite version 4.9.0, copyright 2003-2006) on images of the microsections collected with a LEICA

DMC 4500 camera connected to a LEICA DM 4000B light microscope (Leica Microsystems, Wetzlar, Germany) using both transmission and polarised light. The observation included five xylem phenophases (Puchałka et al. 2017, Gričar et al. 2017), specifically: 1: Dormant cambium (DC, cambium is non-productive); 2: Cambium reactivation (CR, increase of the number of cambial cells; production of new xylem and phloem cells); 3: Onset of xylem growth (OG, enlargement of newly formed xylem cells); 4: Appearance of initial earlywood vessels (IEV, enlargement of the first row of vessels of the current year-ring), and 5: Fully differentiated initial earlywood vessels (FDIEV, vessels and neighboring fibers are lignified, sap can be conducted through those vessels). During autumn, two additional phenophases were considered: 6: xylem cells in the wall-thickening phase and 7: last formed cells are mature except for very rare solitary terminal cells. Wood growth cessation dates were determined when latewood differentiation was completed which was visible thanks to the red colour of the cells under Safranin-Astra blue staining (Dox et al. 2020).

### *Leaf phenology*

Leaf phenology was assessed in autumn 2017 and spring 2018. Autumn phenology was monitored visually, following the method of Vitasse et al., (2009), from late August till late November, considering first only the tree canopy coloration and, later in the season, a combination of canopy coloration and leaf fall (decrease in canopy greenness). The date of onset of leaf senescence was determined at the appearance of a sharp decrease in canopy greenness (Mariën et al. 2019).

Spring phenology was monitored weekly or twice a week from early March to late May 2018 on 10 randomly selected buds, equally distributed from the upper and lower part of the crown, to account for variation within the crown (Davi et al. 2011). The considered phenophases included bud swelling, bud-burst, leave unfolding and were determined when 50% of the

observed buds or leaves have reached the corresponding phenophase. For detail see Marchand et al. (2020).

### *Statistical analyses*

Comparisons of timing between wood and leaf phenophases were performed for each stand and species with a permuted two-sample T-test (999 permutations;  $n=4$  except for KS- beech where  $n=8$ ). Relationships of CR and FDIEV along with tree characteristics and previous autumn phenology were quantified using univariate linear models. When the necessary assumptions of normality of the residuals (tested with Shapiro test, Royston 1982) and homogeneity of variance (tested with `ncvTest`, package *car*, Fox and Weisberg 2019) were not met, even after a *box-cox* transformation (using package *MASS*, Venable and Ripley 2002), a generalized linear model with quasi-poisson distribution was performed. The relationships between CR and FDIEV and categorical variables (site and species) were evaluated with one-way ANOVA, type III (package *car*). To consider nonlinear relationships as well, the Random Forest method (package *randomForest*, Liaw and Wiener 2002) was applied to rank the potential explanatory variables. Random Forest is a partitioning method that produces a large ensemble of regression trees considering the full dataset but with random subset of predictor variables. We set 5000 trees to have stable results and 3 (for FDIEV) or 4 variables (for CR) for each split, following the *floor* function (package *randomForest*). Finally, we used multivariate linear models (that can be applied with both continuous and categorical variables) to determine the most relevant explanatory variables and to build multivariate models. Because of the relatively small dataset, these models were built starting with the three most important variables identified with the univariate models. Interactions between variables were checked and were reported if significant. Tests for normality of residuals and variance homogeneity were done as reported above. Because these conditions were not met for FDIEV, instead of a linear model, we applied, in this case, a generalized linear model, with quasi-

poisson distribution. ANOVA, type III analyses, were done on each model to test categorical variables.

## Results

### *Wood and leaf phenology*

CR (mean date per stand) took place between DOY 77 - 87 for birch, DOY 108 - 109 for beech and on DOY 87 for oak (Fig. 1). FDIEV took place on DOY 162 for birch, DOY 160 - 162 for beech and DOY 134 - 143 for oak (Fig. 1). Both CR and FDIEV showed significant inter-species variation ( $p < 0.01$ ) but not significant inter-site variation (Fig. 1). Leaf phenology (mean date per stand) started with the 'swollen bud' stage on DOY 96 - 107, then bud-burst occurred on DOY 101 - 110 and finally, leaf unfolding took place on DOY 106 - 112 (Fig. 2). CR occurred 10 - 20 days before buds were swollen for birch and oak (Fig. 2, Table I), but after that (around budburst) for beech (Fig. 2, Table I). FDIEV occurred around a month or two after leaf unfolding (stand average between 22 - 55 days;  $p < 0.05$ ; Table I). Concerning previous autumn phenology, wood growth cessation varied between species and sites and onset of leaf senescence varied between species only (Fig. 1). Both events were concurrent ( $p > 0.10$ ) except for KS-beech ( $p = 0.02$ ) where an early wood growth cessation was observed.

### *Univariate relationships*

Cambium reactivation date was significantly related to species and also to 10 years average ring-width, with a later CR in case of narrower ring-widths (Fig. 3a, Table II). Furthermore, a weak positive relation ( $p = 0.060$ ) was found between CR and tree diameter (Fig. 3c, Table II). The Random Forest analysis confirmed that species was the most important factor and that tree diameter had also an important influence (Table III). Opposed to the linear models, the

Random Forest analysis ranked ‘age’ as a key variable and 10 years growth rate among the least important variables (Table III). Age was not significantly linearly related to CR date mainly because of the old trees in the oak-PB stand (Fig. 3e). Indeed, the average age of this stand was 115 years against an average age of 60 years for the rest of the studied trees. Without these 4 old trees the relationship between CR date and age is significant (T-value = 5.119; p-value < 0.01; Fig. 3e), with later CR for older trees until a certain age. The fact that the 10 years growth rate was ranked low by the Random Forest analysis only means that other variables (e.g. growth cessation and onset of senescence) had stronger non-linear relationships with CR (Table III).

FDIEV was highly related to species and also to age and previous autumn phenology (i.e. onset of leaf senescence and wood growth cessation) (Fig. 3b,d,f, Table II). Age was significantly related to FDIEV when all trees were considered (Fig. 3f, Table II). On the other hand, when trees older than 75 years were removed, the relation became non-significant (Fig. 3f). The relationships between FDIEV and onset of leaf senescence and wood growth cessation, were negative, meaning that a later FDIEV was found for trees with an early leaf onset of senescence or previous’ year early cessation of wood growth (Fig. 3b,d Table II). Moreover, a weak negative linear relation ( $p = 0.063$ ) was found between FDIEV and tree diameter (Table II). For FDIEV, Random Forest confirmed that species, age, onset of leaf senescence and wood growth cessation were the most important variables affecting FDIEV even when non-linear relationships were also considered (Table III).

#### *Statistical models of wood spring phenology*

For both spring wood phenological events, CR and FDIEV, a linear model was developed with the three most important variables obtained with univariate model analysis (Table IV and SI Table III; correlations between variables are shown in SI Table II). For CR, a multivariate model including ‘species’, ‘diameter’ and ‘age’ explained 68% of the variability (Table IV).

A model without the ‘species’ factor could still explain 28% of the variability (Table IV). For FDIEV, a model including ‘species’, ‘age’ and ‘onset of leaf senescence’ explained 55% of the variability (Table IV). Also in this case, a linear model without the species factor could explain ca. 30% of the variability (Table IV). An alternative model with ‘wood growth cessation’ instead of ‘onset of leaf senescence’, provided similar results (SI Table III).

## **Discussion**

### *Timeline of wood and leaf spring phenology*

For oak, our study aligns with previous observations of xylogenesis and bud-burst for this species and other comparable oak species (Sass-Klaassen et al. 2011, González-González et al. 2013, Pérez-de-Lis, Rossi, et al. 2016, Pérez-de-Lis et al. 2017, Lavrič et al. 2017, Gričar et al. 2017). For example, the timing of CR was similar to the one reported for pedunculate oak in The Netherlands (Sass-Klaassen et al. 2011). On the other hand, we could also show that CR occurs not only before bud-burst but also before the buds are swollen (the first stage of leaf spring phenology). This detail has not been reported before. In beech, CR occurred almost at the same time as bud-burst. Our data are in agreement with other studies on beech (Čufar et al. 2008, Prislán et al. 2013). Furthermore, our observation that earlywood vessels were fully formed in beech at the beginning of June is comparable to data from other sites in Germany (in the Bavarian Alps) and Slovenia (Čufar et al. 2008, Kraus et al. 2016). For birch, although diffuse porous as beech, CR already occurred in the second half of March, significantly before bud swelling. We speculate that for this pioneer species the cambial reactivation, uncoupled to leaf phenology, could be due to the temperature conditions in the spring of 2018 with an unusually cooler March that delayed bud-burst (see temperature and phenology comparison with 2019, when bud-burst occurred slightly before CR, SI Fig. 1).

Only few (and less detailed) studies on spring xylogenesis and wood phenology for birch exist. Therefore, such lag between CR and bud-burst for a pioneer diffuse-porous species is not supported by other research so far. It might indicate that, at least for the birch population investigated here, spring cold spells tend to block the leaf unfolding rather than the cambial activity.

#### *Factors influencing spring wood phenology*

In addition to the factor 'species', CR was related to the average ring-width of the last 10 years, with an earlier start of the growing season for trees with larger ring widths. A positive relationship between vessel density and ring width was already found for oak and beech (Delpierre et al. 2016, Prislan et al. 2018), suggesting that larger annual ring width is associated with an earlier start of the wood growth. Cambium reactivation was also related to tree age (at least till the age of 75 years), with younger trees starting CR earlier than older trees. Although the age range was limited (40-75 years), this follows the principle that young trees need to compete for light against the older larger trees as it was also reported for various conifers (Rossi et al. 2007, 2008, Li et al. 2013). Therefore, the first hypothesis is confirmed for CR but only for trees up to 75 years old. The fact that beech trees of 45 and 130 years in the same stands were reported to have the same CR (Giagli et al. 2015), might confirm that the positive relationship between CR and age is not relevant for older trees. Average ring width is more closely related than age to CR in homogenous stands. The relationship between tree-ring width and CR supports the idea that trees with better fitness and growth are the ones with earlier start of the secondary growth.

FDIEV was not related to age for trees up to 75 years old, but it occurred earlier for the oldest trees (oaks) considered in our study. The first hypothesis is therefore not valid for FDIEV that shows for old trees even the opposite pattern than expected. However, the findings indirectly agrees with Pérez-de-Lis et al. (2016), who found a negative relationship between FDIEV and

tree diameter for pedunculate oaks. The latter can be an indication that older, larger, trees (thus with more resources and reserves) can have a fast spring reactivation. We also found negative relationships between FDIEV and onset of leaf senescence and wood growth cessation in the previous autumn. To our knowledge, this is the first study to relate earlywood vessel differentiation with previous autumn phenology. The second hypothesis (i.e. that later autumn phenology might trigger earlier spring wood xylogenesis) is therefore confirmed for FDIEV but not for CR. Maybe the surplus of reserves gained by later onset of senescence is more important when the reserve storage is close to its minimum in June (the timing of FDIEV) rather than in March-April (the timing of CR) when the carbon reserves are still rather full (Barbaroux and Breda 2002).

#### *Statistical models of wood spring phenology*

Our models, which are based on simple correlations, captured about 70% and 55% of the variability of CR and FDIEV, respectively. Letting the species factor aside, about 30% of the variability was explained by growth characteristics and previous's year autumn phenology. This indicates that the difficult task to fully understand the inter-tree variability in spring xylogenesis still needs to be investigated and more potential drivers (e.g. non-structural carbon of reserves, nutrient content) need to be taken into account. In any case, our study is one of the few trying to elucidate the inter-individual variability in xylogenesis in homogenous stands of angiosperms. The model results, even without being mechanistic, are statistically significant and help us to better understand the tree functioning. For example, we showed that CR and FDIEV are not influenced by the same variables. Cambium reactivation is mainly related to growth characteristics (age, 10 years average ring-width and tree diameter), whereas FDIEV is also affected by previous autumn dynamics (onset of leaf senescence and wood cessation). Thus, FDIEV could be more sensitive to annual variations compared to CR. This is important as FDIEV can be a predictor of latewood formation

(Pérez-de-Lis, García-González, et al. 2016). As annual wood growth and forest ecosystem services are depending on the timing of both CR and FDIEV (Rathgeber et al. 2016), their relationships with tree growth characteristics and previous autumn phenology should not be overlooked.

## **Conclusions**

Our results demonstrate that CR can occur before any signs of spring bud activity (swollen buds) for birch and oak, while CR and the swollen bud phase occur concurrently for beech. Initial earlywood vessels were fully differentiated always after leaf unfolding. We show that the timing of CR correlates to tree diameter, ring-width and age. On the other hand, the timing of FDIEV was significantly correlated to age and previous' year wood growth cessation and onset of leaf senescence. The hypothesis that younger, smaller, trees present earlier CR and FDIEV has been proved true only for CR of trees up to 75 years old. The hypothesis that trees with later autumn phenology present earlier CR and FDIEV has been proved true only for FDIEV but not for CR. Our study might offer new perspectives in thinning strategies in very homogenous stands. In fact, in absence of major dendrometric differences, trees with earlier leaf senescence might be preferentially removed as they appear to have a later start of the wood growing season. However, our study is mainly fundamental, and the novel insights on inter-individual variability of xylogenesis open the door for further research. The observed relationship between previous year' autumn phenology and spring xylogenesis is particularly interesting as it indicates that stressors affecting autumn phenology during the current growing season (e.g. heat waves, severe drought, etc., all expected to increase in frequencies and intensity in the future) can have an impact on next year wood phenology.

## **Acknowledgments**

We acknowledge the funding support from ERC Starting Grant LEAF-FALL (714916). We thank also the Agency for Forest and Nature of the Flemish Government (ANB), the Military Defence of Belgium (Defensie), and the City of Brasschaat, that granted permission to conduct research in the study areas. We are also grateful to the Flemish Institute for Nature and Forest (INBO) and the Integrated Carbon Observation System (ICOS) for providing meteorological data. Special thanks are due to Stijn Willen (UGent) for analysis of the tree cores and to Dirk Leyssens (ANB) for the support.

Accepted manuscript

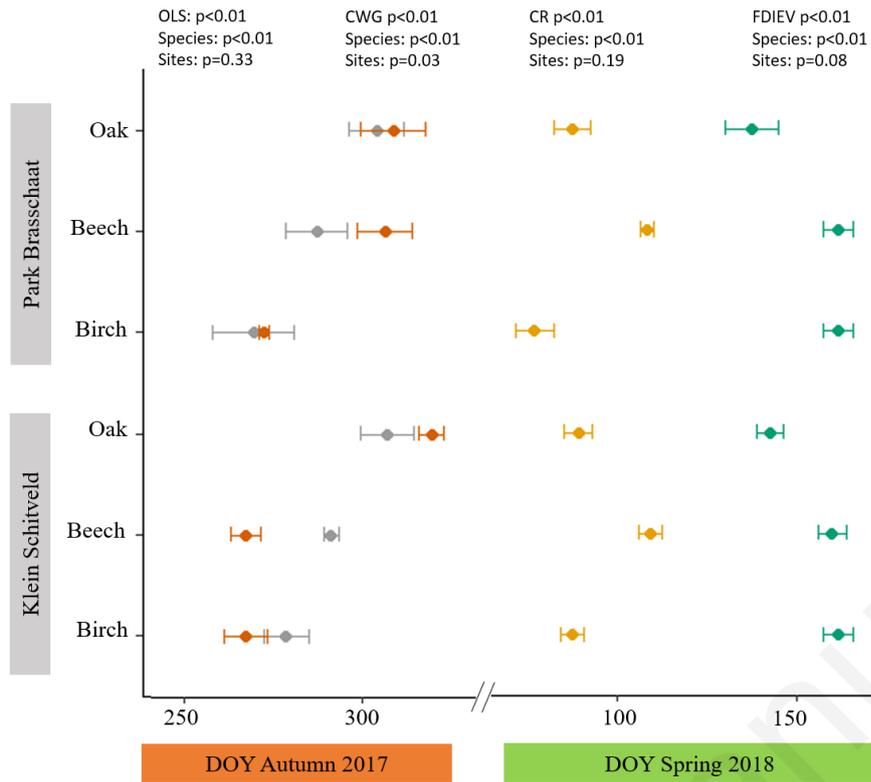
## References

- Barbaroux C, Breda N (2002) Contrasting distribution and seasonal dynamics of carbohydrate reserves in stem wood of adult ring-porous sessile oak and diffuse-porous beech trees. *Tree Physiology* 22:1201–1210.
- Begum S, Kudo K, Rahman MH, Nakaba S, Yamagishi Y, Nabeshima E, Nugroho WD, Oribe Y, Kitin P, Jin H-O, Funada R (2018) Climate change and the regulation of wood formation in trees by temperature. *Trees* 32:3–15.
- Begum S, Nakaba S, Yamagishi Y, Oribe Y, Funada R (2013) Regulation of cambial activity in relation to environmental conditions: understanding the role of temperature in wood formation of trees. *Physiologia Plantarum* 147:46–54.
- Bréda N, Huc R, Granier A, Dreyer E (2006) Temperate forest trees and stands under severe drought: a review of ecophysiological responses, adaptation processes and long-term consequences. *Ann For Sci* 63:625–644.
- Campioli M, Malhi Y, Vicca S, Luysaert S, Papale D, Peñuelas J, Reichstein M, Migliavacca M, Arain MA, Janssens IA (2016) Evaluating the convergence between eddy-covariance and biometric methods for assessing carbon budgets of forests. *Nature Communications* 7
- Campioli M, Vincke C, Jonard M, Kint V, Demarée G, Ponette Q (2012) Current status and predicted impact of climate change on forest production and biogeochemistry in the temperate oceanic European zone: review and prospects for Belgium as a case study. *Journal of Forest Research* 17:1–18.
- Čufar K, Prislan P, de Luis M, Gričar J (2008) Tree-ring variation, wood formation and phenology of beech (*Fagus sylvatica*) from a representative site in Slovenia, SE Central Europe. *Trees* 22:749–758.
- Davi H, Gillmann M, Ibanez T, Cailleret M, Bontemps A, Fady B, Lefèvre F (2011) Diversity of leaf unfolding dynamics among tree species: New insights from a study along an altitudinal gradient. *Agricultural and Forest Meteorology* 151:1504–1513.
- Delpierre N, Berveiller D, Granda E, Dufrêne E (2016) Wood phenology, not carbon input, controls the interannual variability of wood growth in a temperate oak forest. *New Phytologist* 210:459–470.
- Delpierre N, Guillemot J, Dufrêne E, Cecchini S, Nicolas M (2017) Tree phenological ranks repeat from year to year and correlate with growth in temperate deciduous forests. *Agricultural and Forest Meteorology* 234–235:1–10.
- Delpierre N, Lireux S, Hartig F, Camarero JJ, Cheaib A, Čufar K, Cuny H, Deslauriers A, Fonti P, Gričar J, Huang J-G, Krause C, Liu G, Luis M de, Mäkinen H, Castillo EM del, Morin H, Nöjd P, Oberhuber W, Prislan P, Rossi S, Saderi SM, Trembl V, Vavrick H, Rathgeber CBK (2019) Chilling and forcing temperatures interact to predict the onset of wood formation in Northern Hemisphere conifers. *Global Change Biology* 25:1089–1105.

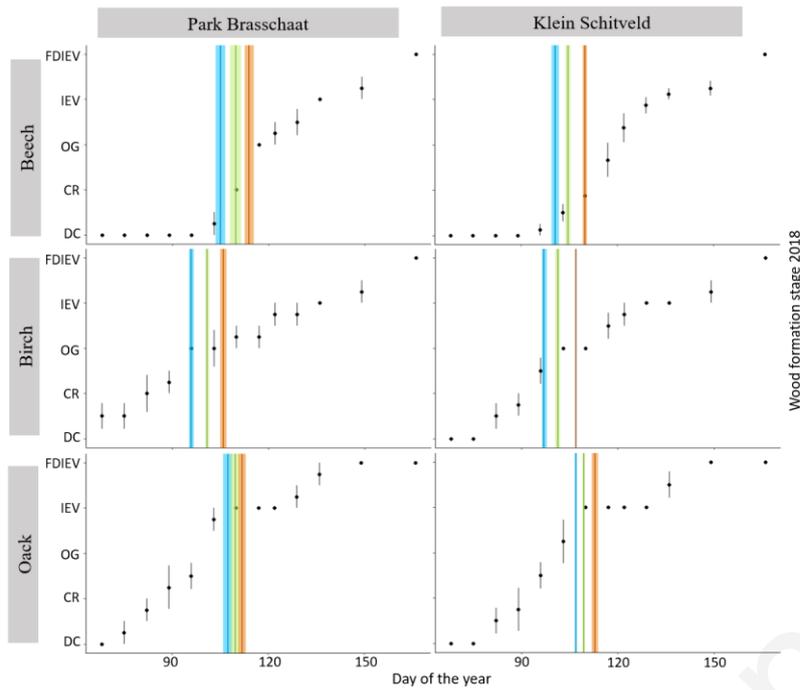
- Delpierre N, Vitasse Y, Chuine I, Guillemot J, Bazot S, Rutishauser T, Rathgeber CBK (2016) Temperate and boreal forest tree phenology: from organ-scale processes to terrestrial ecosystem models. *Annals of Forest Science* 73:5–25.
- Dox I, Gričar J, Marchand LJ, Leys S, Zuccarini P, Geron C, Prislán P, Mariën B, Fonti P, Lange H, Peñuelas J, Van den Bulcke J, Campioli M (2020) Timeline of autumn phenology in temperate deciduous trees. *Tree Physiology*:tpaa058.
- Estiarte M, Peñuelas J (2015) Alteration of the phenology of leaf senescence and fall in winter deciduous species by climate change: effects on nutrient proficiency. *Global Change Biology* 21:1005–1017.
- Forrest J, Miller-Rushing AJ (2010) Toward a synthetic understanding of the role of phenology in ecology and evolution. *Philosophical Transactions of the Royal Society B: Biological Sciences* 365:3101–3112.
- Fox J, Weisberg S (2019) *An R companion to applied Regression*, 3th edition. Sage publications.
- Friend AD, Eckes-Shephard AH, Fonti P, Rademacher TT, Rathgeber CBK, Richardson AD, Turton RH (2019) On the need to consider wood formation processes in global vegetation models and a suggested approach. *Annals of Forest Science* 76.
- Giagli K, Veteška O, Vavrčik H, Gryc V (2015) Monitoring of seasonal dynamics in two age-different european beech stands. *WOOD RESEARCH*:13.
- González-González BD, García-González I, Vázquez-Ruiz RA (2013) Comparative cambial dynamics and phenology of *Quercus robur* L. and *Q. pyrenaica* Willd. in an Atlantic forest of the northwestern Iberian Peninsula. *Trees* 27:1571–1585.
- Gričar J, Lavrič M, Ferlan M, Vodnik D, Eler K (2017) Intra-annual leaf phenology, radial growth and structure of xylem and phloem in different tree parts of *Quercus pubescens*. *European Journal of Forest Research* 136:625–637.
- Hartmann H, Trumbore S (2016) Understanding the roles of nonstructural carbohydrates in forest trees - from what we can measure to what we want to know. *New Phytologist* 211:386–403.
- Huang J-G, Ma Q, Rossi S, Biondi F, Deslauriers A, Fonti P, Liang E, Mäkinen H, Oberhuber W, Rathgeber CBK, Tognetti R, Trembl V, Yang B, Zhang J-L, Antonucci S, Bergeron Y, Camarero JJ, Campelo F, Čufar K, Cuny HE, De Luis M, Giovannelli A, Gričar J, Gruber A, Gryc V, Güney A, Guo X, Huang W, Jyske T, Kašpar J, King G, Krause C, Lemay A, Liu F, Lombardi F, Martinez del Castillo E, Morin H, Nabais C, Nöjd P, Peters RL, Prislán P, Saracino A, Swidrak I, Vavrčik H, Vieira J, Yu B, Zhang S, Zeng Q, Zhang Y, Ziaco E (2020) Photoperiod and temperature as dominant environmental drivers triggering secondary growth resumption in Northern Hemisphere conifers. *Proc Natl Acad Sci USA* 117:20645–20652.
- Koch E, Bruns E, Chmielewski F-M, Defila C, Lipa W, Menzel A (2007) Guidelines for plant phenological observations. :42.
- Koninklijk Meteorologisch Instituut van België (2018a) Klimatologisch maandoverzicht, maart 2018.
- Koninklijk Meteorologisch Instituut van België (2018b) Klimatologisch maandoverzicht, april 2018.

- Kraus C, Zang C, Menzel A (2016) Elevational response in leaf and xylem phenology reveals different prolongation of growing period of common beech and Norway spruce under warming conditions in the Bavarian Alps. *European Journal of Forest Research* 135:1011–1023.
- Lavrič M, Eler K, Ferlan M, Vodnik D, Gričar J (2017) Chronological Sequence of Leaf Phenology, Xylem and Phloem Formation and Sap Flow of *Quercus pubescens* from Abandoned Karst Grasslands. *Frontiers in Plant Science* 8.
- Li X, Liang E, Gricar J, Prislán P, Rossi S, Cufar K (2013) Age dependence of xylogenesis and its climatic sensitivity in Smith fir on the south-eastern Tibetan Plateau. *Tree Physiology* 33:48–56.
- Liaw A, Wiener M (2002) Classification and Regression by randomForest. *R News* 2:6.
- Marchand LJ, Dox I, Gričar J, Prislán P, Leys S, Van den Bulcke J, Fonti P, Lange H, Matthysen E, Peñuelas J, Zuccarini P, Campioli M (2020) Inter-individual variability in spring phenology of temperate deciduous trees depends on species, tree size and previous year autumn phenology. *Agricultural and Forest Meteorology* 290:108031.
- Mariën B, Balzarolo M, Dox I, Leys S, Marchand LJ, Geron C, Portillo-Estrada M, AbdElgawad H, Asard H, Campioli M (2019) Detecting the onset of autumn leaf senescence in deciduous forest trees of the temperate zone. *New Phytologist* 224:166–176
- Michelot A, Simard S, Rathgeber C, Dufrene E, Damesin C (2012) Comparing the intra-annual wood formation of three European species (*Fagus sylvatica*, *Quercus petraea* and *Pinus sylvestris*) as related to leaf phenology and non-structural carbohydrate dynamics. *Tree Physiology* 32:1033–1045.
- Morin X, Ameglio T, Ahas R, Kurz-Besson C, Lanta V, Lebourgeois F, Miglietta F, Chuine I (2007) Variation in cold hardiness and carbohydrate concentration from dormancy induction to bud burst among provenances of three European oak species. *Tree Physiology* 27:817–825.
- Pérez-de-Lis G, García-González I, Rozas V, Olano JM (2016) Feedbacks between earlywood anatomy and non-structural carbohydrates affects spring phenology and wood production in ring-porous oaks. *Biogeosciences* 13:5499–5510.
- Pérez-de-Lis G, Olano JM, Rozas V, Rossi S, Vázquez-Ruiz RA, García-González I (2017) Environmental conditions and vascular cambium regulate carbon allocation to xylem growth in deciduous oaks. *Functional Ecology* 31:592–603.
- Pérez-de-Lis G, Rossi S, Vázquez-Ruiz RA, Rozas V, García-González I (2016) Do changes in spring phenology affect earlywood vessels? Perspective from the xylogenesis monitoring of two sympatric ring-porous oaks. *New Phytologist* 209:521–530.
- Piao S, Liu Q, Chen A, Janssens IA, Fu Y, Dai J, Liu L, Lian X, Shen M, Zhu X (2019) Plant phenology and global climate change: Current progresses and challenges. *Global Change Biology* 25:1922–1940.
- Prislán P, Čufar K, De Luis M, Gričar J (2018) Precipitation is not limiting for xylem formation dynamics and vessel development in European beech from two temperate forest sites. *Tree Physiology* 38:186–197.
- Prislán P, Gričar J, Čufar K (2014) Wood Sample Preparation for Microscopic Analysis.

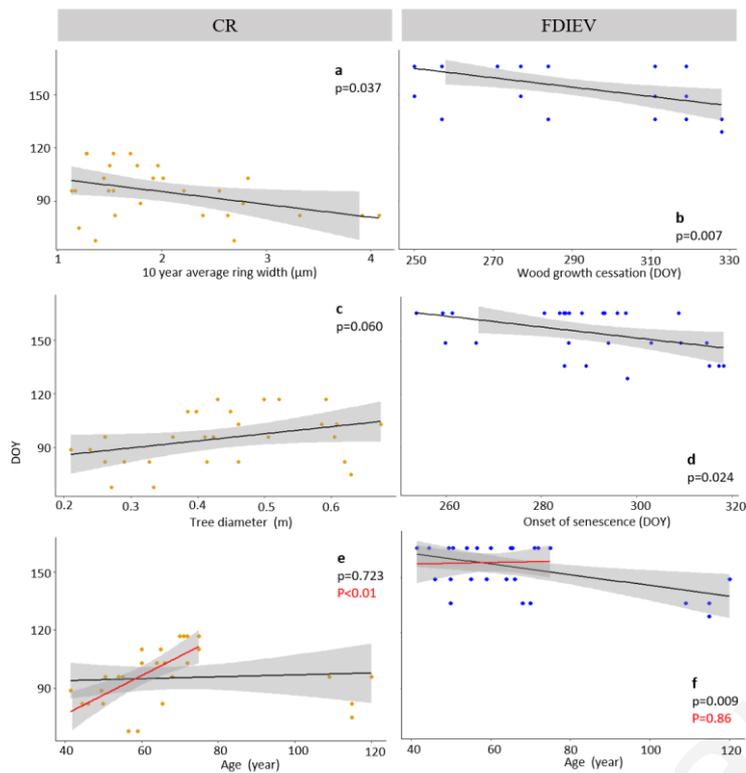
- Prislan P, Gričar J, de Luis M, Smith KT, Čufar K (2013) Phenological variation in xylem and phloem formation in *Fagus sylvatica* from two contrasting sites. *Agricultural and Forest Meteorology* 180:142–151.
- Puchałka R, Koprowski M, Gričar J, Przybylak R (2017) Does tree-ring formation follow leaf phenology in Pedunculate oak (*Quercus robur* L.)? *European Journal of Forest Research* 136:259–268.
- Rathgeber CBK, Cuny HE, Fonti P (2016) Biological Basis of Tree-Ring Formation: A Crash Course. *Frontiers in Plant Science* 7:734
- Rathgeber CBK, Rossi S, Bontemps J-D (2011) Cambial activity related to tree size in a mature silver-fir plantation. *Annals of Botany* 108:429–438.
- Rossi S, Deslauriers A, Anfodillo T, Carrer M (2007) Age-dependent xylogenesis in timberline conifers. *New Phytologist* 177:199–208
- Rossi S, Deslauriers A, Gričar J, Seo J-W, Rathgeber CB, Anfodillo T, Morin H, Levanic T, Oven P, Jalkanen R (2008) Critical temperatures for xylogenesis in conifers of cold climates. *Global Ecology and Biogeography* 17:696–707.
- Rossi S, Menardi R, Anfodillo T (2006) Trephor: A New Tool for Sampling Microcores from tree stems. *IAWA Journal* 27:89–97.
- Rossi S, Morin H, Deslauriers A (2012) Causes and correlations in cambium phenology: towards an integrated framework of xylogenesis. *Journal of Experimental Botany* 63:2117–2126.
- Royston P (1982) Algorithm AS181: the W test for Normality. *Applied Statistics* 31:176–180.
- Sass-Klaassen U, Sabajo CR, den Ouden J (2011) Vessel formation in relation to leaf phenology in pedunculate oak and European ash. *Dendrochronologia* 29:171–175.
- Skomarkova MV, Vaganov EA, Mund M, Knohl A, Linke P, Boerner A, Schulze E-D (2006) Inter-annual and seasonal variability of radial growth, wood density and carbon isotope ratios in tree rings of beech (*Fagus sylvatica*) growing in Germany and Italy. *Trees* 20:571–586.
- Tixier A, Gambetta GA, Godfrey J, Orozco J, Zwieniecki MA (2019) Non-structural Carbohydrates in Dormant Woody Perennials; The Tale of Winter Survival and Spring Arrival. *Frontiers in Forests and Global Change* 2:18.
- Vitasse Y, Porté AJ, Kremer A, Michalet R, Delzon S (2009) Responses of canopy duration to temperature changes in four temperate tree species: relative contributions of spring and autumn leaf phenology. *Oecologia* 161:187–198.
- Zuidema PA, Poulter B, Frank DC (2018) A Wood Biology Agenda to Support Global Vegetation Modelling. *Trends in Plant Science* 23:1006–1015.



**Figure 1:** Timing of observed phenophases (DOY  $\pm$  standard error) grouped by species and site. OLS = Onset of leaf senescence in 2017 (grey), CWG = cessation wood growth in 2017 (orange), CR = cambium reactivation in 2018 (yellow), FDIEV = fully differentiated initial earlywood vessels in 2018 (green). P-values are the result of the ANOVA test for ‘site’ and ‘species’ effect.



**Figure 2:** Progress of 2018 spring cambium activity (black dots; stand mean DOY  $\pm$  standard error) in comparison to the timing of the observed leaf phenophases (coloured bands, mean DOY  $\pm$  standard error) grouped by species and site. Blue = bud swelling, green = bud-burst, orange = leaf unfolding, DC = dormant cambium, CR = cambium reactivation, OG = onset of xylem growth, IEV = Appearance of initial earlywood vessels, FDIEV = fully differentiated initial earlywood vessels



**Figure 3:** Right panel: Relevant relationships between day of year (DOY) of 2018 cambium reactivation (CR, orange points) with dendrometric parameters or 2017 autumn phenophases: (a) 10 years average ring-widths, (c) tree diameter and (e) tree age. Left panel: relevant relationships between DOY of 2018 fully differentiated initial earlywood vessel (FDIEV, blue points) with dendrometric parameters or 2017 autumn phenophases: (b) wood growth cessation in previous autumn, (d) onset of senescence in previous autumn, and (f) tree age. Each point corresponds to a tree ( $n = 28$ ). Black lines and grey bands represent the linear models with the 95% confidence interval. Model p-values are reported on each panel. For panel (e) and (f) the red line (and red p-value) represents the linear model only considering trees < 75 years old.

**Table I:** The p-value of the student test with 999 permutations comparing the 2018 DOY of leaf- and wood spring phenological events for each species within each stand (see Figure 1). Bold values indicate whether the difference is significant (p-value <0.05)

site	specie	CR and swollen bud	CR and bud-burst	FDIEV and leaf unfolding
Park of Brasshaat (PB)	Beech	0.296	0.808	<b>0.024</b>
	Birch	<b>0.04</b>	<b>0.032</b>	<b>0.03</b>
	Oak	<b>0.022</b>	<b>0.032</b>	<b>0.036</b>
Klein Schietveld (KS)	Beech	<b>0.03</b>	0.194	<b>0.002</b>
	Birch	<b>0.03</b>	<b>0.03</b>	<b>0.04</b>
	Oak	<b>0.022</b>	<b>0.026</b>	<b>0.032</b>

Accepted manuscript

**Table II:** Characteristics of univariate linear models between 2018 cambium reactivation (CR) and fully differentiated initial earlywood vessels (FDIEV) with tree dendrometric parameters or 2017 autumn phenophases for all monitored trees at both sites (see Figure 3). Oak (n=8), beech (n=12) and birch (n=8). Significant models ( $p < 0.05$ ) are in bold; ‘\*’ indicates the use of a generalized linear model quasi-poisson instead of a linear model. The ‘ $\square$ ’ indicates the results of a one-way ANOVA.

Potential predictors	CR		FDIEV	
	T-value or F value ( $\square$ )	P-value	T-value or F-value ( $\square$ )	P-value
Onset of senescence	0.531	0.6	-2.388*	<b>0.024</b>
Wood cessation	-0.426	0.674	-2.927*	<b>0.007</b>
Age	0.358	0.723	-2.81*	<b>0.009</b>
10 years average ring-width	-2,198	<b>0.037</b>	-0.199*	0.844
Tree diameter	1.966	0.060	-1.942*	0.063
Tree density	0.235	0.816	-0.984*	0.334
species	30.296 $\square$	<b>&lt;0.01</b>	32.744 $\square$	<b>&lt;0.01</b>
site	2.269 $\square$	0.144	0.239* $\square$	0.625

**Table III:** Importance of predictors (indicated with an increase of mean square error IncMSE) obtained with Random Forest analysis for cambium reactivation (CR) and fully differentiated initial earlywood vessels (FDIEV) in 2018 for all observed trees (n = 28).

Potential predictors	CR	FDIEV
	% IncMSE	% IncMSE
species	133.61	61.94
Age	30.44	4.22
Tree diameter	19.81	-0.96
Cambium cessation 2017	8.48	6.26
Onset of senescence 2017	8.43	4.47
Tree density index	2.73	2.17
10 years average ring-width	1.95	-3.24
Sites	0.75	-0.41

**Table IV:** Characteristics of multivariate multi-species linear models of cambium reactivation (CR) and multivariate multi-species generalized linear models for fully differentiated initial earlywood vessels (FDIEV); models are presented with or without the ‘specie’ factor (CR n=28; FDIEV n=27). na: not applicable.

	CR model with age				FDIEV model with onset of senescence			
	F-value	P-value	F-value	P-value	LR Chisq	P-value	T-value	P-value
species	23.06	<0.01	na	na	14.97	<0.01	na	na
Tree diameter	3.19	0.09	11.24	0.03	na	na	na	na
Age	1.32	0.26	4.67	0.04	0.684	0.41	-2.12	0.04
Tree diameter : Age	na	na	6.61	0.017	na	na	na	na
Onset of senescence	na	na	na	na	0.001	0.97	-1.58	0.13
R <sup>2</sup> adjusted	68%		28%		55%		29%	