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Research paper

Timeline of autumn phenology in temperate deciduous trees

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Cessation of xylem formation or wood growth (CWG) and onset of foliar senescence (OFS) are key autumn phenological events in temperate deciduous trees. Their timing is fundamental for the development and survival of trees, ecosystem nutrient cycling and the seasonal exchange of matter and energy between the biosphere and atmosphere, and affects the impact and feedback of forests to global change. A large-scale experimental effort and improved observational methods have allowed us to compare the timing of CWG and OFS for different deciduous tree species in Western Europe, particularly in silver birch, a pioneer species, and European beech, a late-succession species, at stands of different latitudes, of different levels of site fertility, for 2 years with contrasting meteorological and drought conditions, i.e., the low moderately dry 2017 and the extremely dry 2018. Specifically, we tested whether foliar senescence started before, after or concurrently with CWG. Onset of foliar senescence and CWG occurred generally between late September and early November, with larger differences across species and sites for OFS. Foliar senescence started concurrently with CWG in most cases, except for the drier 2018 and, for beech, at the coldest site, where OFS occurred significantly later than CWG. The behavior of beech in Spain, the southern edge of its European distribution, was unclear, with no CWG, but very low wood growth at the time of OFS. Our study suggests that OFS is generally triggered by the same drivers of CWG or when wood growth decreases in late summer, indicating an overarching mechanism of sink limitation as a possible regulator of the timing of foliar senescence.

Keywords: autumn phenology, chlorophyll, foliar senescence, sink limitation, wood growth, xylem formation.

Introduction

Phenology, the study of cyclic and seasonal plant and animal life cycle events, is important to understand the functioning of temperate deciduous forest ecosystems, especially in the context of global change (Lui 2016, Chen et al. 2018). Most phenological studies of deciduous forest ecosystems have investigated the onset of the growing season, mainly by characterizing the timing of canopy development and

cambium reactivation. Spring phenological events are relatively simple to observe and have clear responses to environmental change (Peñuelas and Filella 2001, Guada et al. 2019), which facilitates these studies. In contrast, autumn phenology has been largely overlooked (Gallinat et al. 2015), even though the timing of autumn phenophases affects not only tree development (i.e., winter survival) and growth potential for the next year (Estiarte and Peñuelas 2015), but also fundamental ecosystem dynamics such as nutrient cycling,

biomass production, CO₂ and H₂O fluxes between forest and atmosphere, albedo, seasonal weather and species interactions (Peñuelas et al. 2009, Richardson et al. 2010, 2013, Gallinat et al. 2015).

The paucity of attention and studies of autumn phenological events is not due to a failure of ecologists to recognize their importance, but rather due to assessment difficulties. Onset of foliar senescence (OFS) and cessation of xylem formation, i.e., the cessation of wood growth (CWG), are two key processes of deciduous trees in autumn. Leaves actively degrade their components during senescence, including chlorophyll and the photosynthetic apparatus. This degradation releases resources and relocates them into overwintering tree organs before foliar abscission. Average efficiencies of nitrogen, phosphorus and potassium resorption can be as high as 62, 65 and 70%, respectively (Estiarte and Peñuelas 2015). The timing of OFS is currently determined from ecophysiological, visual or remote sensing observations of canopy coloration, chlorophyll content or loss of foliar biomass (Mariën et al. 2019). However, a standard method for identifying this crucial autumn phenophase is not yet available.

Cessation of wood growth represents the end of the annual wood formation. More specifically, wood growth ends when the production and maturation of xylem cells is completed (Plomion et al. 2001). Cell maturation includes the deposition of a secondary cell wall and cell wall lignification, followed by a programmed cell death. In contrast to onset of wood formation in spring (cell production and expansion), which can be studied by changes in stem diameter, observation of cell maturation requires laborious microscopic anatomical analyses (Deslauriers et al. 2017).

The few studies on CWG, foliar senescence and related processes (specifically, chlorophyll degradation, canopy coloration and leaf fall) have not established a clear timeline for the onset and progress of these processes in temperate deciduous trees. These studies either did not measure CWG in detail (Fracheboud et al. 2009, Weih 2009) or included foliar senescence only peripherally (González-González et al. 2013, Prislán et al. 2013, Gričar et al. 2017). The time-course and causal relationship between these processes remains unclear. A better understanding of the timeline between CWG and OFS, however, is urgently needed. These events may serve as phenological cues, and knowing their timelines would be valuable to understand the functional priorities of trees at the end of the growing season. Moreover, future projections of forest production and forest climate services can be improved by the elucidation of the autumn timeline in deciduous trees.

We tested three plausible hypotheses to account for the causal sequence between OFS and CWG. The first hypothesis assumes that foliar senescence starts before CWG, because photosynthesis may be more limited than wood growth in autumn due to accumulated damage to foliar cells throughout

the season (Günthardt-Goerg and Vollenweider 2007). This classical approach (Gallinat et al. 2015) has been widely applied in forest models and in the land surface component of the Earth systems models, where tree growth is often modeled to depend on the available photosynthates (Fatichi et al. 2014). The second hypothesis assumes that foliar senescence starts after CWG as growth in autumn is more sensitive to environmental constraints than photosynthesis (Fatichi et al. 2014, Zuidema et al. 2018). The third hypothesis assumes that OFS and CWG occur concurrently, possibly because both phenophases respond to the same environmental cues, e.g., photoperiod. This hypothesis assumes that the triggering cues would not be perceived with different sensitivities by different tree organs. Three research questions were linked to these hypotheses: (Hypothesis 1) Do species follow the same hypothesis over their distributional range? (Hypothesis 2) Do populations growing on sites with a different level of fertility or on the same site, but during years with contrasting meteorological conditions, follow the same hypothesis? (Hypothesis 3) Do species with different traits respect the same hypothesis? To answer these research questions, we tested the validity of the hypotheses for (i) deciduous tree species of contrasting types, i.e., European beech (*Fagus sylvatica* L.), a late-successional species that produces leaves mainly in spring, and silver birch (*Betula pendula* Roth.), a pioneer species that produces leaves throughout the whole growing season, for European populations at three locations (southern Norway, Belgium and northern Spain) of their distribution, spanning 20 degrees of latitude; (ii) populations of these two species at the same location (Belgium), but with different soil fertilities and for 2 years (2017 and 2018) with different meteorological conditions and drought intensity; and (iii) populations of other key European species, i.e., pedunculate oak (*Quercus robur* L., population in Belgium), which can be either a pioneer or a late-successional species, with leaf flush mainly in spring, and common aspen (*Populus tremula* L., population in Norway), a typical pioneer species but with leaf flush mainly in spring for adult individuals.

The main analyses were based on detailed phenological observations of the timing of CWG and OFS for 64 mature trees during the autumn of 2017 and 16 trees during the autumn of 2018. Cessation of wood growth was determined through an improved method for an accurate determination of the end of xylem maturation based on microscopic analyses of wood cross sections (Gričar et al. 2017). Onset of foliar senescence was determined with a breakpoint analysis applied to standard observations of canopy coloration (determined by changes in foliar color and loss of color as a result of leaf fall; Vitasse et al. 2009; Materials and methods) and leaf chlorophyll degradation (Fracheboud et al. 2009; Materials and methods). For 2018, an additional comparison between CWG and the end of xylem enlargement was done. We also compared our estimates of the OFS from canopy coloration and chlorophyll degradation

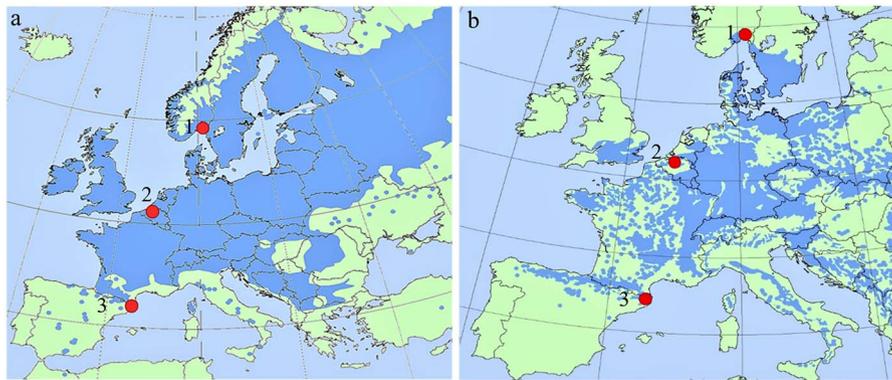


Figure 1. Maps of the distributions of (a) silver birch (*B. pendula*) and (b) European beech (*F. sylvatica*) in Europe (EUROGEN 2009, www.euforgen.org). Red dots indicate the locations of the sites: (1) southern Norway, (2) Belgium and (3) northern Spain.

to the timing of 50% canopy coloration and the timing when 50% of the leaves had fallen, which are commonly used proxies of OFS (Fracheboud et al. 2009, Vitasse et al. 2011, Possen et al. 2014; Materials and methods).

Materials and methods

Study species and sites

Stands of European beech (*F. sylvatica* L.) and silver birch (*B. pendula* Roth.) growing in moderately fertile conditions ('fertile stands') were selected at three locations within their distributions: Ås, Norway (59°40'N, 10°46'E; 86 m above sea level (a.s.l.); northern distribution), Brasschaat, Belgium (51°20'N, 4°30'E; 15 m a.s.l.; central distribution) and a mountainous area in Girona province, Spain (41°45'–42°11'N, 2°20'–2°28'E; 1075–1126 m a.s.l.; southern distribution; Figure 1). Additional stands of the European beech and the silver birch in Brasschaat were selected at sites with low fertility ('infertile stands'). We also studied one fertile stand of common aspen (*P. tremula* L.) in Norway and one fertile stand of pedunculate oak (*Q. robur* L.) in Belgium.

We selected four (co)dominant healthy individuals in each of the 10 stands where we monitored OFS and CWG in the year 2017. One birch tree in Norway was excluded from the dataset, because it was a suppressed understory and was erroneously selected. In the Belgian fertile stands of beech, birch and oak, four additional (co)dominant healthy individuals that neighbored the trees selected for the study of OFS and CWG were selected for the study of foliar senescence and related processes (chlorophyll degradation, canopy coloration and leaf fall). For the fertile beech stand and the infertile birch stand, these eight trees were studied during the year 2018.

The average daily temperature in Belgium was very similar over 2017 and 2018, respectively 11.1 and 11.8 °C. Both years were warmer than the long-term average of 10.1 °C (en.climate-data.org). In Belgium, 846 mm of precipitation fell in 2017 and 723 mm in 2018 compared with the long-term average of about 920 mm. In 2018, a severe spring/summer

drought took place in Belgium with only 112 mm of precipitation during May, June and July (compared with the long-term average of 212 mm). The precipitation in May 2018 and the number of days with precipitation in July 2018 were 'extraordinarily low' (recurrence time of 30 years). Both variables, precipitation and number of precipitation days, were extraordinarily low in June 2018 (meteo.be). Average temperature was respectively 7 and 11–13 °C for the stands in Norway and Spain, compared with long-term averages of respectively 5 and 11.1–15.5 °C. The total precipitation in 2017 was 980 mm in Norway (long-term average: 785 mm) and 820–850 mm in Spain (long-term average: 880 mm). Additional information about the stands, trees and meteorological data for late summer and autumn 2017 and 2018 is provided in Figure 2 and Table S1 available as Supplementary Data at *Tree Physiology* Online.

Canopy coloration

Canopy coloration at day t (X_t) in late summer and early autumn was estimated directly as a percentage. Later in the season, when the process became more intense, X_t was estimated by a combined rating of the percentage of leaves that had changed color (α_t) and the percentage of leaves that had fallen (β_t), as described by Vitasse et al. (2011):

$$X_t = \frac{\alpha_t \times (100 - \beta_t)}{100} + \beta_t$$

Observations were recorded weekly from mid-September until 100% X_t . Small proportions of the birch and aspen leaves in Norway were already colored (ca 10%) at the start of the monitoring, so we added a typical summer value (early August) measured from another year (2018) to the seasonal series of 2017.

Chlorophyll degradation

Chlorophyll content was estimated indirectly by measuring the relative chlorophyll content index (CCI) using a CCM-200 plus leaf absorption meter (Fracheboud et al. 2009; ADC Bioscientific Ltd, Hoddesdon, UK) every 2–3 weeks between 10:00 and

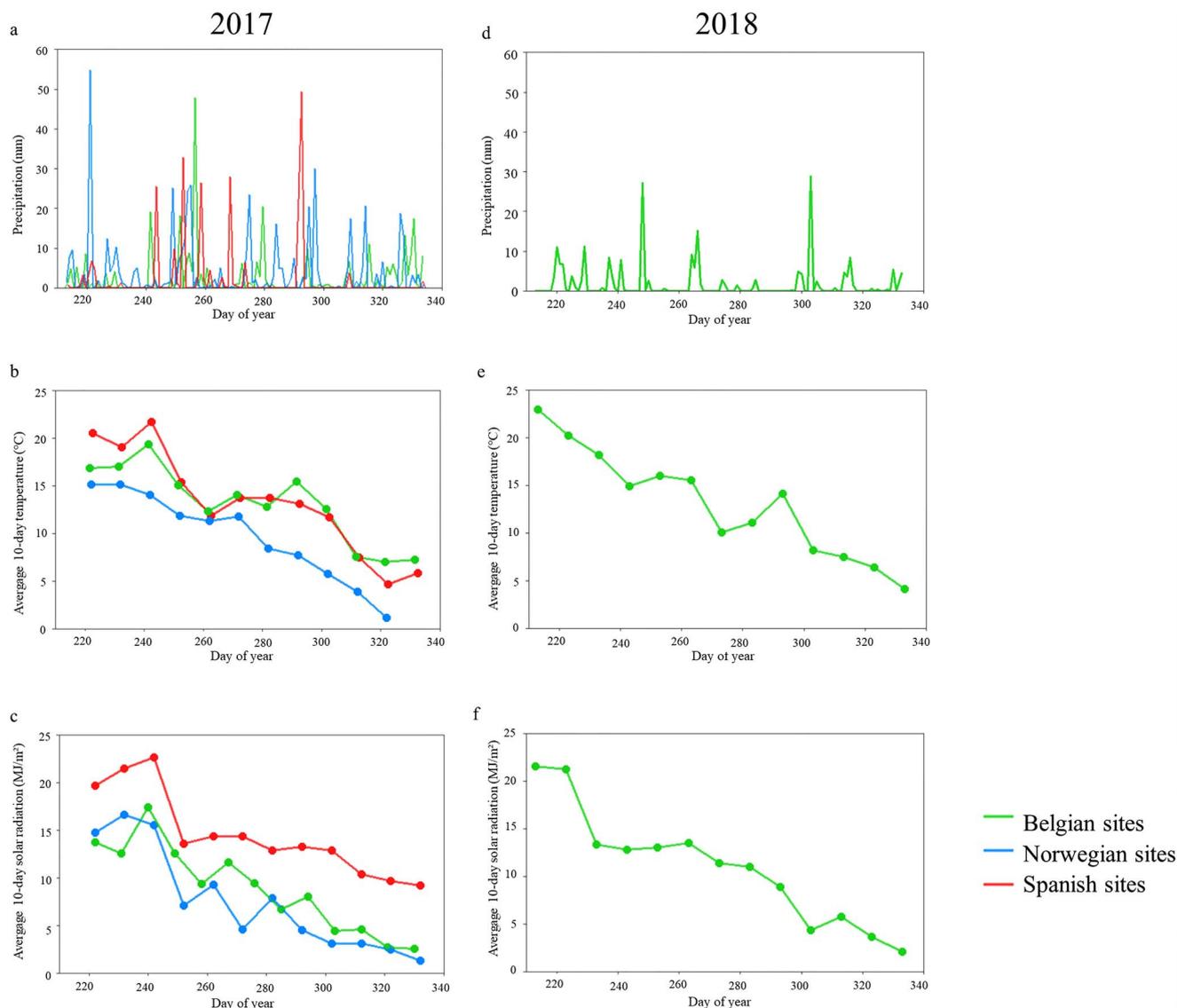


Figure 2. (a, d) Daily precipitation, (b, e) average 10-day mean temperature and (c, f) average 10-day solar radiation for the Norwegian, Belgian and Spanish sites in 2017 and Belgian sites in 2018.

16:00 h from late July to late November in 2017 and 2018. The CCI value was obtained by averaging measurements from leaves collected from the canopy by tree climbers. For beech and oak, five sun and five shade leaves were sampled, whereas for birch only five sun leaves were sampled as there was no clear difference between sun and shade leaves. Canopy coloration of the trees sampled for chlorophyll content was assessed as described above.

Determination of the onset of foliar senescence

The OFS was defined as the date when (i) seasonal canopy coloration sharply increased due to the start of natural foliar senescence (canopy coloration before this date had already slightly increased during the season due to stress damage to leaves) or (ii) chlorophyll content starts to decrease sharply. The two

phenophases are closely correlated, and their measurements provide similar dates of OFS, except in case of severe drought (see above). We determined OFS by a breakpoint analysis of the time series of mean canopy coloration or chlorophyll content (see above). The breakpoint analysis was performed with the R package 'segmented' (Mugge 2008). This package calculates multiple linear regressions for data with dependent variables that can be expressed by two or more straight lines with different slopes linked at a breakpoint and calculates these breakpoints (Figure 3).

Wood growth cessation

Seasonal wood growth was monitored weekly from late August to late November in 2017 and, for two stands in Belgium, also from mid-March to late November in 2018. We measured the

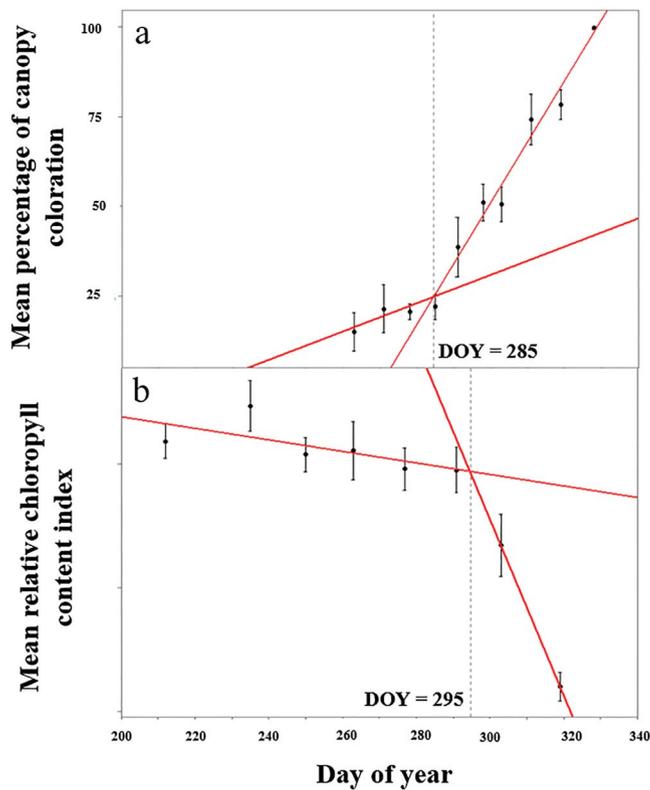


Figure 3. Example of the breakpoint analysis to determine the day of year (DOY) when onset of foliar senescence started according to (a) change in canopy coloration and (b) chlorophyll degradation for the infertile silver birch (*B. pendula*) stand in Belgium in 2017.

percentage of cells of the currently forming annual ring that were still in the wall-thickening phase (and thus still developing and operating as a sink for assimilates) through microscopic analysis of micro-cores of stem wood (small samples 15–20 mm long and 2 mm in diameter). In 2018 this analysis was extended to the cells in the enlarging phase (preceding thickening) and in the mature phase (after thickening).

Sampling The stem micro-cores were collected at breast height with a Trephor corer (Rossi et al. 2006). An upward spiral pattern of sampling was followed throughout the season to minimize wound reactions. The samples were stored in 1.5-ml Eppendorf microtubes containing a 70:30 EtOH:H₂O solution for conservation.

Slide preparation The micro-cores were cut to contain only the most recently created xylem rings (at least two), the cambium and the inner phloem. These segments were then dehydrated in a series of rising ethanol concentration, infiltrated with a clearing agent (UltraClear, J.T. Baker, Avantor Performance Materials, Center Valley, PA, USA) and paraffin (Paraplast plus, ROTH, Karlsruhe, Germany) and embedded in paraffin blocks. The paraffin blocks were cut into 5–10 µm slices with a microtome

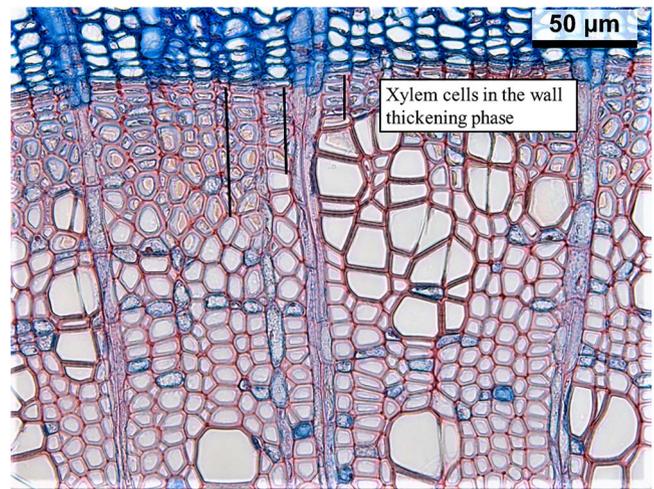


Figure 4. Example of a microscopic section prepared from a silver birch tree (*B. pendula*) near the end of the growing season, with a few cells still in the wall-thickening phase stained with Astra blue.

(Leica Microsystems, Wetzlar, Germany). Next, the paraffin was removed with a clearing agent (UltraClear) and ethanol. The sections were then stained in an aqueous solution of safranin (Merck, Darmstadt, Germany) and Astra blue (Sigma–Aldrich, Steinheim, Germany). The slices were fixed by mounting in Euparal (Waldeck, Munster, Germany) (Gričar et al. 2017).

Microscopic analysis The slides were examined under a light microscope (Leica DM 4000 B/M, Wetzlar, Germany) and a Leica LAS image analysis system to determine the end of the formation of wood, defined as the point when the cells of late wood are lignified and have thus completed cell wall thickening (Gričar et al. 2017). This stage is recognizable by a completely red cell wall through the safranin/Astra blue staining, whereas cells that have not completed wall thickening are colored blue (i.e., non-lignified) on the inner parts of the cell wall and red (i.e., lignified) on the outer part (Figure 4).

The percentage of wall-thickening cells (WTC) was estimated weekly by measuring the width of the current year ring and the width of the layer of WTC. The width of the layer of the WTC can also be used directly as an index of growth (see Figure S1 available as Supplementary Data at *Tree Physiology Online*), but we preferred to use the percentage of the WTC because of its value relative to annual growth. We defined CWG as the start of (at least) a 3-week period with the percentage of WTC <0.50%. This definition was adopted for multiple reasons. First, the 0.50% threshold is an indication of severely reduced allocation of carbon to wood growth. Second, using a 3-week period avoids associating the CWG with a sporadic short period when growth is limited due to a temporarily environmental constraint (e.g., drought). Third, this definition reduces the impact on the analysis of outliers and noise caused by minor heterogeneous growth patterns of the sampled tree stems. Cessation of wood

growth was determined at the tree level but then averaged ($n = 4-8$) to calculate the stand value. For 2018, we performed measurements also for the whole growing season, recording the width of the current forming ring section with enlarging cells (enlarging zone), thickening cells (wall-thickening zone) and mature cells (mature zone).

Statistical analysis

CWG and OFS Significant differences in the timing between CWG and OFS were determined by identifying overlaps in confidence intervals based on the uncertainty method. The 95% confidence interval of OFS was derived for each stand from the mean (\bar{x}) and standard error (SE) obtained from the 'segmented' package ($\bar{x} - 1.96 \text{ SE}$, $\bar{x} + 1.96 \text{ SE}$), assuming a normal sampling distribution for the onset of senescence. The confidence interval for CWG was also derived from the mean and standard error of the mean ($\bar{x} - 1.96 \text{ SE}$, $\bar{x} + 1.96 \text{ SE}$). The difference between CWG and OFS was significant at $P < 0.05$ when the two confidence intervals did not overlap. Significant differences among OFS dates for different stands and years were determined in the same way considering the confidence interval of each OFS value. On the other hand, significant differences in CWG dates among stands in 2017 were obtained by one-way ANOVA followed by a post hoc test with Tukey corrected P -values. For the Belgian stands, to check for CWG differences between the years 2017 and 2018, repeated measurements ANOVA were done.

Measurements methods of foliar senescence Repeated measurements ANOVA, with an HC3 correction for non-homogeneity of variance ('car' package), followed by a Tukey's post hoc tests were also performed to identify significant differences between OFS from canopy coloration data and OFS from the start of rapid chlorophyll degradation, 50% canopy coloration and 50% leaf fall, for fertile birch, beech and oak in Belgium.

Seasonal pattern of xylem cells still in the wall-thickening phase A pairwise t -test with HC3 correction was used for the seasonal change in percentage of WTC. Repeated measurements ANOVA, sometimes done after a log-transformation to ensure normality of the residuals and homogeneity of variance, were performed to compare the percentage of WTC for each sampling date. Similarly, t -tests were done to compare the percentage of WTC between fertile and infertile sites in Belgium. When necessary a Welch t -test was used or a log-transformation was performed to meet the assumptions.

The normality of residuals and homogeneity of variances were tested with a Shapiro-Wilk test and a `ncvTest` or `LeveneTest` ('car' package), respectively. Significance was set at $P < 0.05$. All statistical analyses and graph construction used RStudio version 3.2.2 (R Development Core Team 2016).

Results

Onset of foliar senescence

In 2017, for birch, OFS occurred in late September in Norway and Spain but in mid-October in Belgium ($P < 0.05$; Figure 5; see Table S6 available as Supplementary Data at *Tree Physiology* Online). For beech, OFS occurred in the same week in mid-October in Norway and Belgium and a week later in Spain (no significant difference). Onset of foliar senescence in Belgium occurred at about the same time at the infertile and fertile stands for both species (no significant difference). Finally, OFS for oak in Belgium occurred in late October (2.5 weeks later than the local beech and birch, $P < 0.05$) and in late September for aspen in Norway (same week as the local birch; no significant difference). In 2018, OFS for the infertile birch stand and the fertile beech stand in Belgium occurred in late October (no significant difference). For these stands, the difference in OFS between years was also not significant.

Cessation of wood growth

The CWG for both birch and beech occurred from late September to early October at all stands (no significant difference; Figure 5; see Table S6 available as Supplementary Data at *Tree Physiology* Online). The results were thus more similar among stands than for OFS. Cell maturation (i.e., thickening of the cell wall and lignification) for beech at the Spanish stand, however, continued throughout the whole monitoring period, which lasted until early December. Cessation of wood growth also occurred in late September for aspen in Norway (not significantly different from local birch), but only in early November for oak in Belgium, which was considerably later than for local beech and birch (significant difference with all other stands, $P < 0.001$). Cessation of wood growth in 2018 for the infertile birch stand in Belgium was at the end of September, with no significant difference compared with 2017. Similarly, no significant difference between years was found for CWG at the fertile beech stand in Belgium, also occurring at the end of September.

Comparison OFS and CWG

The OFS occurred concurrently with CWG for all fertile birch stands. On the other hand, beech populations on fertile sites presented a less consistent pattern, with concurrent OFS and CWG in Belgium, earlier CWG than OFS in Norway (2 weeks) and no detectable CWG in Spain (Figure 6; see Table S6 available as Supplementary Data at *Tree Physiology* Online). Onset of foliar senescence and CWG were also concurrent at the infertile birch and infertile beech stand in Belgium (Figure 6; see Table S6 available as Supplementary Data at *Tree Physiology* Online). For the two Belgian stands (infertile birch and fertile beech) studied in both 2017 and 2018, no difference was recorded between OFS and CWG in 2017. On the other hand, the

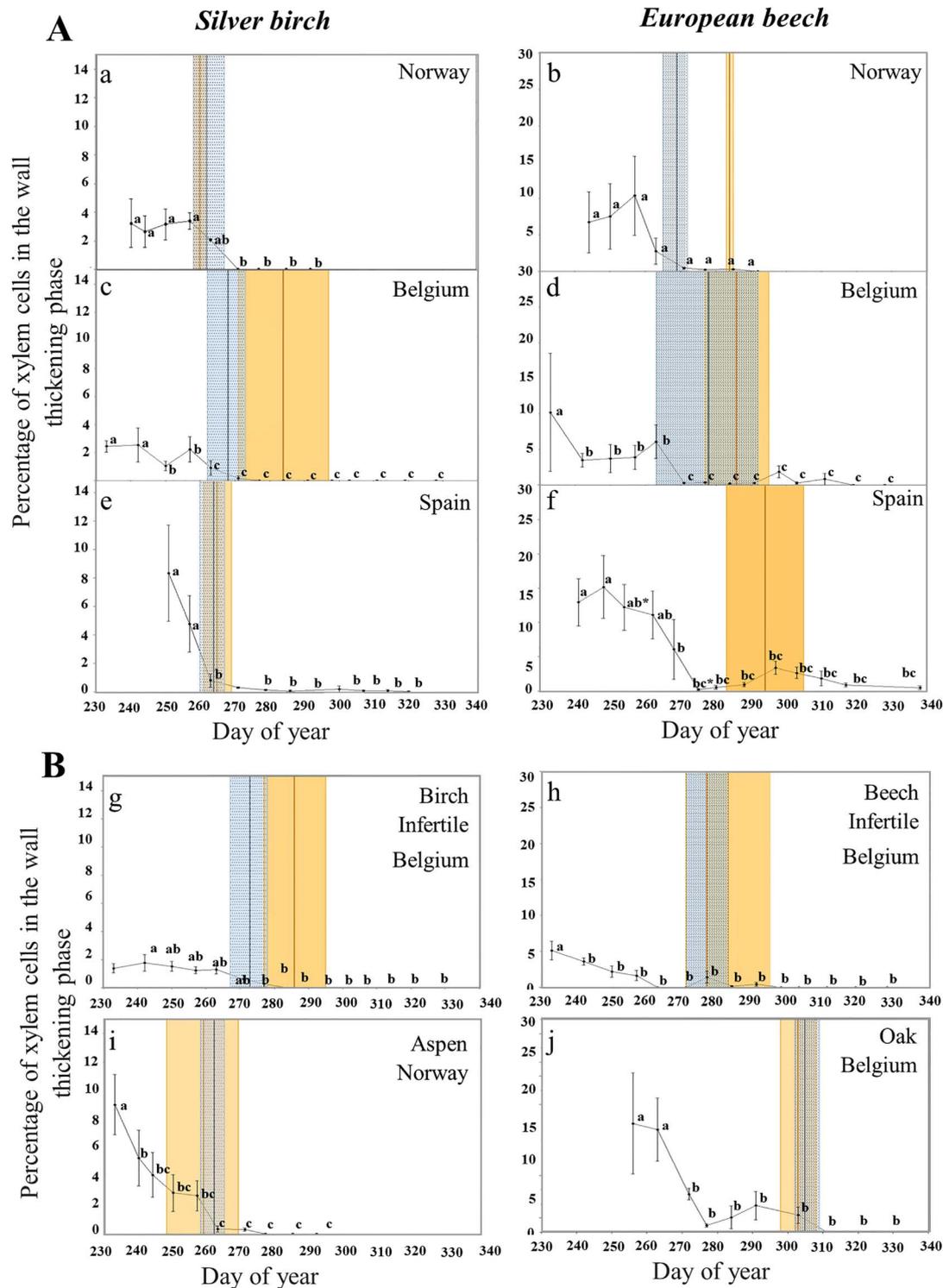


Figure 5. Percentage of stem cells in the wall-thickening phenophase in late summer and autumn and the timing of the onset of foliar senescence for temperate deciduous trees of different species, locations, site fertilities and years. Data points represent mean percentages of cells in the wall-thickening phase, and the associated error bars represent the standard errors of the means. (a) Graphs of fertile silver birch (*B. pendula*) and European beech (*F. sylvatica*) stands over latitudinal range (Spain, Belgium, Norway) in 2017; (b) graphs of infertile silver birch and European beech stands in Belgium, the common aspen (*P. tremula*) stand in Norway and the pedunculate oak (*Q. robur*) stand in Belgium in 2017; (c) graphs of the infertile birch stand and the fertile beech stand in Belgium in 2017 and 2018. The dark-gray lines indicates cessation of wood growth, and the dotted light-gray bands are its confidence intervals (methods). Cessation of wood growth was the intermediate value between the last sampling with >0.50% wall-thickening cells and the first sampling with <0.50% wall-thickening cells of a series of at least three consecutive sampling dates with wall-thickening cells <0.50%. The dark-orange lines (in A and B) indicate the OFS based on the canopy coloration method, and the light-orange bands indicate its confidence interval. The green lines (in C) indicate the OFS based on chlorophyll degradation and the light-green bands indicate its confidence interval. Different letters indicate significant differences in the percentage of xylem cells in the wall-thickening phase among the sampling events.

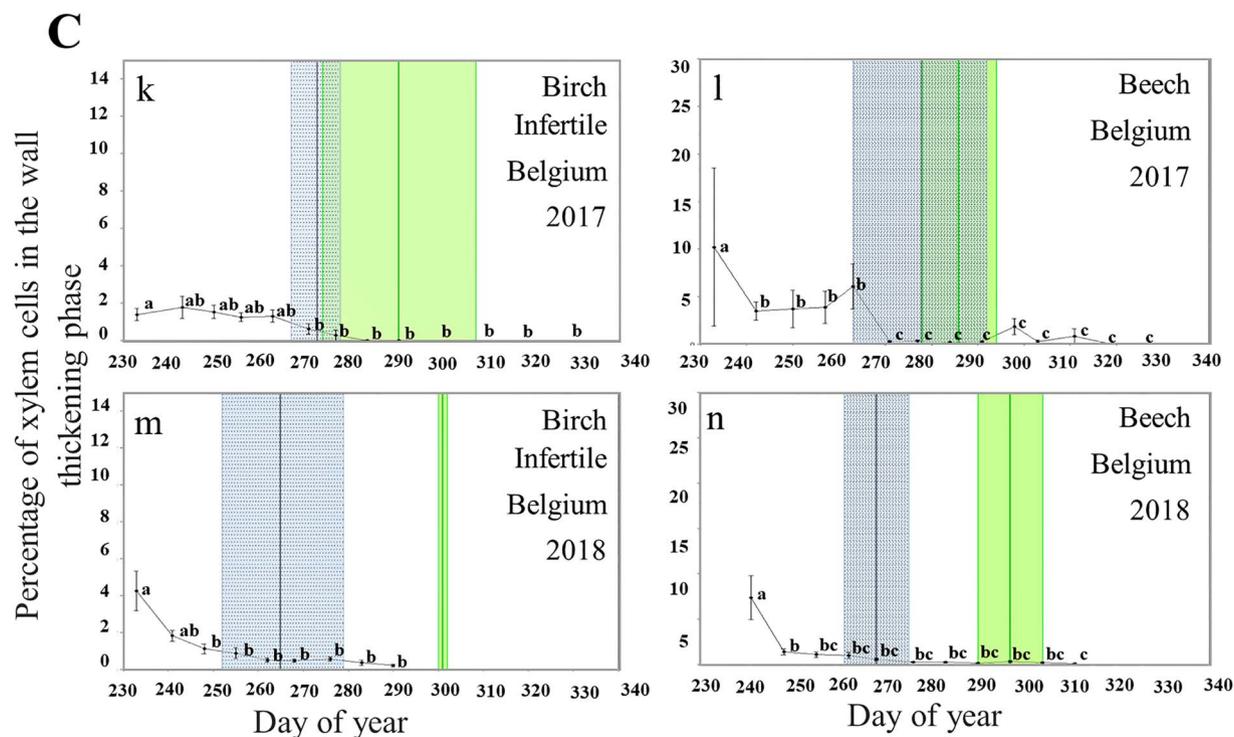


Figure 5. Continued.

difference between OFS and CWG became significant in 2018 at both stands, with OFS taking place 4–5 weeks after CWG (Figure 6; see Table S6 available as Supplementary Data at *Tree Physiology Online*).

Seasonal pattern of wood growth

In 2018, the seasonal progress of the xylem enlargement zone in the infertile birch stand and fertile beech stand in Belgium showed an increasing width until May to June and a declining width afterward, until complete enlargement in early August (Figure 6). The thickening zone showed a similar trend but shifted, with an increasing width until late June to mid-July, declining width until early September (Figure 6) and full cessation of thickening in late September (Figure 5). In more detail for the thickening phase in autumn and for all stands, the percentage of WTC generally decreased rapidly to <0.50% in about 1–2 weeks after a period with a larger, generally stable, percentage of WTC from late August to mid-September. The percentage of WTC was two- to threefold larger for beech than birch across locations before decreasing in mid-September. For each sampling date, no significant difference was observed in the percentage of WTC between sites of different fertility for both birch and beech with the exception of only a marginal difference in the first sampling event (DOY 233) at the birch site ($P = 0.094$; see Table S4 available as Supplementary Data at *Tree Physiology Online*). Similarly, no difference in percentage of WTC was observed between 2017 and 2018 in the monitored

birch and the beech stands, with only a marginal difference in the first sampling date for birch ($P = 0.070$; see Table S5 available as Supplementary Data at *Tree Physiology Online*). The seasonal pattern (and rates) of wood growth in late summer and autumn did not change when the width of WTC was analyzed instead of their percentage (see Figure S1 available as Supplementary Data at *Tree Physiology Online*).

Measurement methods of foliar senescence

In 2017, our detailed foliar measurements of autumn processes in Belgium indicated that OFS from data of chlorophyll degradation matched the estimates of OFS from data of canopy coloration (difference of 0.5–2.5 weeks; Figure 7; see Table S2a and b available as Supplementary Data at *Tree Physiology Online*). On the other hand, our analyses indicated significant differences between OFS from canopy coloration and 50% threshold in canopy coloration for birch ($P = 0.005$) and between the former and 50% threshold in leaf fall for all species ($P < 0.02$). Concerning the estimates of OFS from chlorophyll degradation, they were found to be different from the 50% threshold in canopy coloration for beech and oak ($P < 0.04$) and the 50% threshold in leaf fall for all species ($P < 0.01$). In 2018, OFS from canopy coloration was recorded significantly earlier than in 2017 ($P < 0.001$; see Tables S2b and S3 available as Supplementary Data at *Tree Physiology Online*), presenting a significant difference with the OFS estimates from chlorophyll degradation ($P < 0.001$).

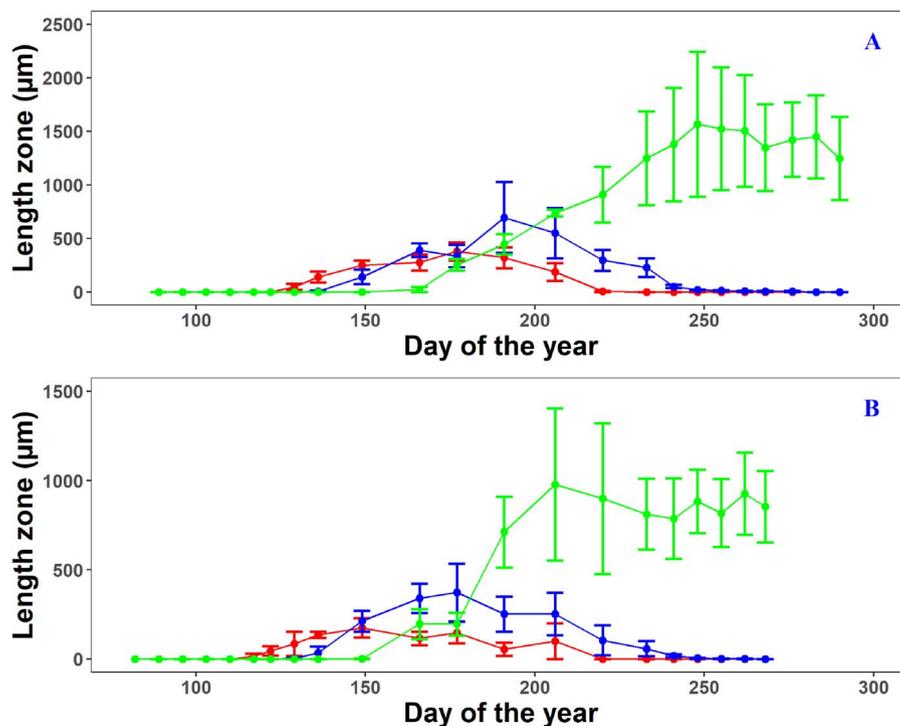


Figure 6. Seasonal development of wood formation at (A) the infertile silver birch site (*B. pendula*) and (B) the fertile beech site (*F. sylvatica*) in Belgium 2018, with the width of the current forming ring section with enlarging cells (enlarging zone) in red, with thickening cells (wall-thickening zone) in blue and with mature cells (mature zone) in green. Points are means ($n = 4$) and the associated error bars represent the standard errors of the means.

However, early coloration in 2018 was related to drought stress and not to the natural process of leaf senescence (Mariën et al. 2019). Therefore, OFS was derived from measurements of canopy coloration for the 2017 interpopulation comparison and from measurements of chlorophyll degradation for the 2017–18 interannual comparison.

Discussion

Distributional range

Based on the comparison between OFS and CWG for all fertile stands of birch and beech, Hypothesis 3 is respected in four cases out of six and always for birch. Beech in Norway follows Hypothesis 2, likely because wood growth is more sensitive to cold climate than leaf dynamics (Fatichi et al. 2014). The contrasting pattern of beech and birch in Norway might indicate that beech benefits more than birch from a prolonged period of photosynthesis, with later OFS of ca 3.5 weeks. In fact, beech needs more return for its higher investment in thicker and longer-living leaves (Martínez-Garza et al. 2005) and possibly a larger carbohydrate reserve to refoliate the entire canopy the next spring (Hoch et al. 2003, Michelot et al. 2012). Later OFS in beech than birch was also observed in Spain, but not in Belgium. The anomaly we observed at the beech stand in Spain (WTC at least until mid-November, with WTC still around 0.5% in early December) suggests a climatic influence due

to the location in the Mediterranean region, in agreement with Sass and Eckstein (1995), who suggested that the formation of wood in beech during the second part of the growing season is mainly influenced by external factors. However, sampling in spring of 2018 showed that the thickening phase of the 2017 ring was not fully completed by end March but only by early-mid May (data not shown). This indicates that the development of the latest formed cells in the previous ring was completed in the period of cambial reactivation in the following spring, i.e., end of April/early May. In other words, there are no indications of a slow-growth activity in winter, which is more likely in warmer Mediterranean regions (Cherubini et al. 2003, Vieira et al. 2017), and it is probable that CWG occurred shortly after our last sampling (or even shortly before it, as two sampling points were missed in late November 2017; Figure 5). Formally, the pattern observed for beech in Spain follows Hypothesis 1. Practically, the wood growth at OFS was very low, as the percentage of WTC (1–3.5%) was substantially lower than the corresponding percentage in early September (13–15%). The recorded pattern is inconsistent with the previous findings from a study on European beech in Moncayo Park, Spain (del Castillo et al. 2016), where CWG occurred already in early August. However, this stand is located on a drier location than our site (710 vs 820 mm annual precipitation, respectively), where wood growth may be constrained by drought. Also, as lignification is sensitive to temperature (Björklund et al. 2019)

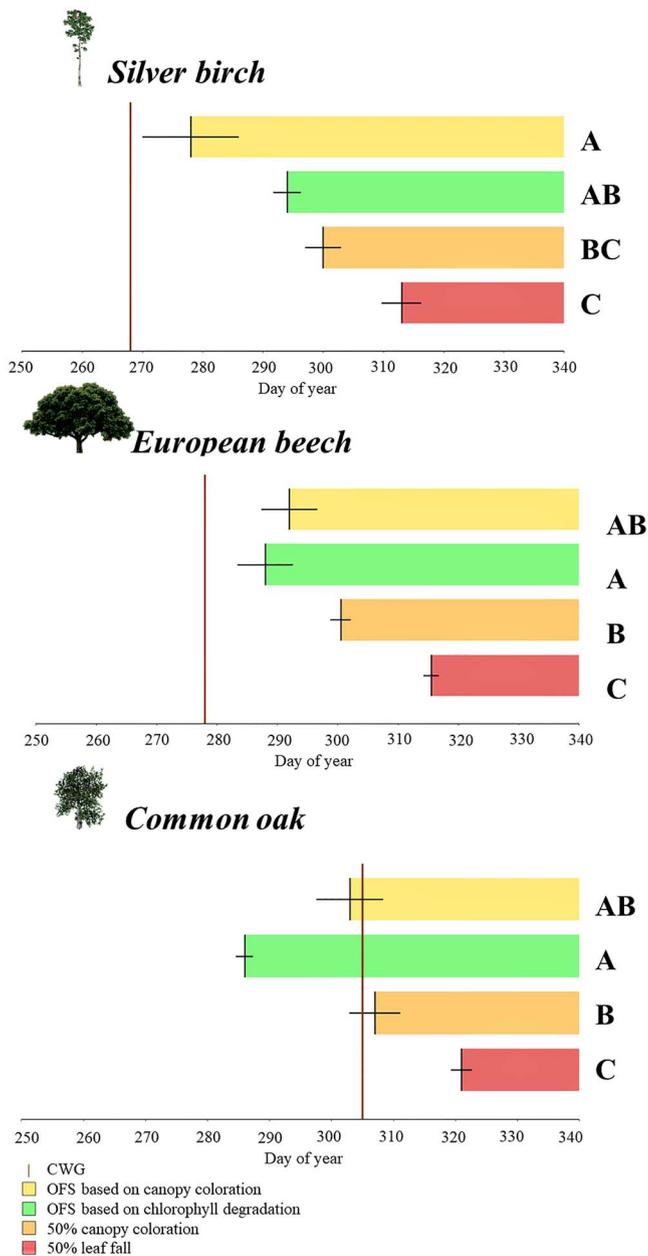


Figure 7. Timeline of key foliar autumnal processes of the silver birch (*B. pendula*), European beech (*F. sylvatica*) and pedunculate oak (*Q. robur*) at the fertile stands in 2017 under normal environmental conditions. For each process, the small vertical black line represents the start (with SE as the horizontal black line), and the color represents its progress. From top to bottom: (i) OFS based on canopy coloration (yellow), (ii) OFS based on rapid chlorophyll degradation (green), (iii) 50% canopy coloration (i.e., when 50% of the canopy is no longer green due to changes in foliar color and the loss of color from leaf fall) (orange) and (iv) 50% leaf fall (i.e., when 50% of the leaves have fallen) (red) relative to the CWG (brown vertical line). Capital letters indicate significant difference between dates.

and cold spells (Piermattei et al. 2015), significant interannual differences are possible, making comparisons between sites measured during different years difficult to interpret. At the same

sites, CWG of *Q. robur* and *Quercus pyrenaica* varied on average 20 days between 2 years (Pérez-de-Lis et al. 2017).

Site fertility and meteorological conditions

In Belgium, birch and beech had no different autumn phenologies at stands with different fertility, following Hypothesis 3 in both fertile and infertile conditions. Similar phenology under different fertility levels was unexpected. In fact, as supported by fertilization experiments, growth is limited, and foliar senescence is expected earlier, under sub-optimal nutrient conditions (Sigurdsson 2001, Weih 2009). However, as shown for other growth processes (Campioli et al. 2015), long-term adaptations might create differences between the influence of natural fertility and fertilization on plant growth. For the Belgian stands studied both in 2017 and 2018, drought did not have an effect on CWG and OFS. On the other hand, the difference between CWG and OFS became significant in 2018, following Hypothesis 2. As expected, the seasonal pattern of xylem production and enlargement was shifted compared with the seasonal pattern of xylem maturation, with the latter showing a later start and later end (Rossi et al. 2013). However, while it started 2 weeks earlier, enlargement ended 3–4 weeks before the large majority of the maturation zone was completed for both the infertile birch stand and fertile beech stand in Belgium. In other words, the phase of enlargement was shorter than the maturation phase. This aspect, together with the fact that the enlargement zone showed a declining width after June (for birch even during June), confirms that maturation is less sensitive to drought than enlargement (Arend and Fromm 2007). Concerning OFS, overall, our findings are comparable to the general range of values reported for the study species in multi-year field observations in areas comparable to our locations (e.g., Vitasse et al. 2011).

Traits

Aspen had the same autumn phenology as birch. In comparison to beech, autumn phenology of oak was substantially later, with OFS and CWG occurring respectively 2.5 and 4 weeks later. Nevertheless, in Belgium, oak also followed Hypothesis 3, as did beech. We speculate that the low sensitivity to low temperature of the oak populations in Belgium (Kint et al. 2012) allows a longer period of wood growth for this species. Deciduous oak species also showed a longer cambial activity than conifers under stress conditions (drought) (Fernández-de-Uña et al. 2017, 2018).

Addressing our three research questions demonstrated that temperate tree species along their distributions, in general, have common dynamics of autumn phenology. For example, across the latitudinal gradient considered, OFS of beech took place within 10 days in mid-late October and OFS of birch in late September to mid-October, while CWG for both birch and beech occurred from late September to early October at all stands. Notably we found that CWG and OFS were mostly concurrent

(Hypothesis 3), or OFS occurred after CWG (Hypothesis 2) in harsher conditions, e.g., during the very dry 2018 (for both birch and beech) or at the coldest location (for beech only). However, exceptions were observed, as the low but continued growth was recorded at the time of OFS at the beech stand in Spain. This case is particularly interesting as, due to the elevation, the seasonal temperature regime of this site in 2017 was not substantially different than the one of northern locations, e.g., in Belgium (Figure 2). On the other hand, it is possible that southern genotypes might have more flexible CWG timing, as CWG may be more related to temperature rather than to photoperiod at southern locations. In fact, for leaf dynamics, Gill et al. (2015) found that autumn phenology is more related to photoperiod at northern latitude and more to temperature at lower latitude. Also, we should note that only 2 years were studied and longer-term datasets (e.g., comprising a post-drought year, a year with an early cold spell) might provide more insights. Nevertheless, we are not aware of previous studies in which the final phase of seasonal wood growth and its link to leaf senescence has been analyzed with so much detail as in this analysis.

Even if Hypotheses 2 and 3 are logical alternatives to each other, both may rely on the well-known ecophysiological mechanism of sink limitation (Fatichi et al. 2014, Fu et al. 2014). This principle states that photosynthesis is sustained by a tree only if the newly produced assimilates are consumed by plant organs or other sinks, such as woody tissues, which are important carbon sinks in late summer and autumn (Cuny et al. 2015). The capacity of the carbon sink thus decreases when wood growth decreases, leading to the downregulation of foliar photosynthesis. Application of the sink limitation mechanism to autumn dynamics implies that foliar senescence is triggered when growth decreases in late summer (supporting Hypothesis 2 or 3). Our results are consistent with the mechanism of sink limitation (caused by CWG) for the timing of foliar senescence. Following a conservative interpretation, the anomalous case of the beech trees at the Spanish stand would represent an important exception to this pattern. However, the fact that wood growth was substantially reduced in late October -at time of OFS- compared to early September might not exclude a sink limitation even in this case. An experiment under completely different settings performed on birch and beech saplings in Belgium (Figure S2 available as Supplementary Data at *Tree Physiology* Online) further supports the sink limitation mechanism. Monitoring OFS and CWG in branches of saplings grown in well-irrigated and fertilized pots indicated that foliar senescence started in early to mid-September after the rates of wood growth had decreased significantly, even though (slow) growth continued until mid-October.

Our methodology allowed us to detect subtleties in the autumn phenologies of temperate deciduous trees and to observe the elusive temporal relationship between CWG and

OFS. Moreover, the overview of autumn phenology for birch, beech, aspen and oak provides the first detailed comparison of temperate deciduous trees for the timing of CWG and OFS. A better resolution of the timing of autumn processes is important for understanding tree functioning and potential causal relationships among autumn processes and for identifying their environmental drivers. These advances are key to improving simulations of tree growth in both forest models of biomass production and global models providing projections of climate change. In particular, modeling approaches simulating CWG after OFS should be amended. Improved modeling approaches should consider these phenophases as concurrent, with earlier CWG in case of extreme conditions.

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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