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Upscaling xylem phenology: sample size matters

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• **Background and Aims** Upscaling carbon allocation requires knowledge of the variability at the scales at which data are collected and applied. Trees exhibit different growth rates and timings of wood formation. However, the factors explaining these differences remain undetermined, making samplings and estimations of the growth dynamics a complicated task, habitually based on technical rather than statistical reasons. This study explored the variability in xylem phenology among 159 balsam firs [*Abies balsamea* (L.) Mill.].
• **Methods** Wood microcores were collected weekly from April to October 2018 in a natural stand in Quebec, Canada, to detect cambial activity and wood formation timings. We tested spatial autocorrelation, tree size and cell production rates as explanatory variables of xylem phenology. We assessed sample size and margin of error for wood phenology assessment at different confidence levels.
• **Key Results** Xylem formation lasted between 40 and 110 d, producing between 12 and 93 cells. No effect of spatial proximity or size of individuals was detected on the timings of xylem phenology. Trees with larger cell production rates showed a longer growing season, starting xylem differentiation earlier and ending later. A sample size of 23 trees produced estimates of xylem phenology at a confidence level of 95 % with a margin of error of 1 week.
• **Conclusions** This study highlighted the high variability in the timings of wood formation among trees within an area of 1 km^2. The correlation between the number of new xylem cells and the growing season length suggests a close connection between the processes of wood formation and carbon sequestration. However, the causes of the observed differences in xylem phenology remain partially unresolved. We point out the need to carefully consider sample size when assessing xylem phenology to explore the reasons underlying this variability and to allow reliable upscaling of carbon allocation in forests.

Key words: Boreal forest, carbon allocation, cell production, *Abies balsamea*, tree growth, tree size, wood formation, xylem development, xylem differentiation, xylogenesis.

INTRODUCTION

The Intergovernmental Panel on Climate Change and the United Nations through the Paris Agreement recognize the role played by forest ecosystems in achieving climate change mitigation goals (IPCC, 2014, 2019; United Nations, 2016). By covering 31 % of the total land surface (FAO and UNEP, 2020), the forests store 861 ± 66 GtC (Pan *et al.*, 2011) and represent a net carbon (C) sink of 2.1 ± 13 GtC per year (Harris *et al.*, 2021). Plants use most of the assimilated C for metabolism and structural biomass (Hartmann and Trumbore, 2016). In trees, the largest part of the biomass accumulated is the result of C allocation during wood formation (Deslauriers *et al.*, 2016). Considering the crucial role of wood in tackling climate change, it is surprising to see that our understanding of its formation is still largely incomplete.

The need to assess climate–biosphere relationships has led to the development of vegetation models that use physiological and ecological principles to predict changes in the distribution of plant functional types or forest productivity over time (Prentice *et al.*, 2007). Models challenge our understanding of the factors influencing plant distribution or tree growth at regional to global scales (Prentice *et al.*, 2007). Several currently available models still lack an explicit representation of tree-level growth processes (Friend *et al.*, 2019). Plant growth is generally represented as the net primary production (NPP) and determined as the difference between the gross primary production (GPP) and the maintenance respiration. The main limitation of this approach is that NPP lacks a direct quantification of the timings of C allocation into the wood and its physiological processes (Cuny *et al.*, 2015). At the intra-annual scale, biomass accumulation in wood occurs after the increase in stem size (Cuny *et al.*, 2015). This lag helps to explain the

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differences observed between net ecosystem productivity and changes in tree size at short time scales (Zweifel *et al.*, 2010). However, more accurately, this approach to modelling growth has not been perceived as a problem until recently (Friend *et al.*, 2019), given the widespread understanding that plant productivity is limited by the input of C through photosynthesis (i.e. growth is C source limited). However, evidence that direct restrictions on sink activities and growth processes may occur before those on photosynthesis suggests the need to build growth models in which the demand for C plays a leading role (i.e. sink limitation) (Körner, 2015).

At the beginning of the growing season, cambial cells divide, and the new cells undergo differentiation, finally resulting in mature xylem (Rossi *et al.*, 2012). Cells enlarge by stretching the primary walls, then they produce, thicken and lignify secondary walls, and ultimately succumb to programmed cell death (Rossi *et al.*, 2012). Given the importance of wood formation in the process of C sequestration from the atmosphere to wood biomass, it is not surprising that climate–growth relationships have gained considerable interest worldwide. Models including xylogenesis are now available and promise to incorporate the intra-annual dynamics of the sink activity of cambium (Friend *et al.*, 2019). However, such models still lack data for their calibration and validation. Xylogenesis provides a picture of wood formation that can be directly used to test hypotheses or calibrate model parameters (Cuny *et al.*, 2013). Modelling xylogenesis would potentially allow reliable wood formation dynamics to be upscaled from tree to stand and, potentially, to ecosystem or biome. However, such a challenge requires answering the unsolved question about the variability in xylogenesis among individuals and the drivers of xylem phenology.

Wood formation: standards and limits

Interest in wood formation dynamics has grown worldwide in the last decades, leading to a substantial increase of studies available in the literature (De Micco *et al.*, 2019). Some standards have emerged, and microcoring and pinning have been adopted as sampling techniques (Rossi *et al.*, 2006*a*; Rossi *et al.*, 2006*b*; Gričar *et al.*, 2007). Most research groups use the same criteria to discriminate between phenological phases of the developing xylem and count cell numbers in conifers or measure the width of the differentiation zones in broadleaves (Rossi *et al.*, 2006*b*; Martinez del Castillo *et al.*, 2016). Thanks to these common standards, scientists can obtain comparable data (Rathgeber *et al.*, 2016), thus encouraging global studies to identify general patterns (e.g. Cuny *et al.*, 2015; Rossi *et al.*, 2016; Huang *et al.*, 2020).

Logistical and technical difficulties related to repeated sampling constrain the number of trees under investigation. As a result, the sample size for wood formation is based on a pragmatic decision involving a trade-off among cost, time and convenience (Buttò *et al.*, 2020) rather than ensuring an accurate representation of the process. The variability in wood phenology among individuals has been well known since the first pioneer studies (Wodzicki and Zajaczkowski, 1970) and has been demonstrated in many species (Rossi *et al.*, 2008; Gričar *et al.*, 2009; Linares *et al.*, 2009; Lupi *et al.*, 2010; Rathgeber *et al.*, 2011; Vieira *et al.*, 2014). In some cases, this variability has affected the success of some investigations (e.g. Lupi *et al.*, 2012). Although part of this variability can be related to the genetic differences among individuals, other factors such as tree size and age, and microsite conditions might influence xylem phenology (Lupi *et al.*, 2013). This methodological problem could finally raise doubts over the consistency of the results.

A preliminary assessment of sample size is a crucial step in statistics. Nowadays, most of the studies on wood formation use samples from five individuals (De Micco *et al.*, 2019), although the overall range is usually comprised between one and ten (Deslauriers *et al.*, 2015). To our knowledge, no study on wood formation in the literature involves sample size assessment, thus preventing an accurate generalization of the statistical estimations.

Power analysis is a tool for sample size assessment based on the standard deviation (s.d.) of the population. If the s.d. is unknown, researchers can estimate it or rely on comparable values available in the literature. As an example, according to the results reported in the literature (Lupi *et al.*, 2010; Rathgeber *et al.*, 2011), we run a power analysis with an s.d. of between 3 and 12 d for the estimated day of the year (DOY) of the occurrence of cell differentiation. To obtain a margin of error (ME) of ±1 d with an s.d. of 3, the sample size is estimated to be 37 trees (Supplementary data Fig. S1). An s.d. of 12 requires 200 trees to obtain the same ME (Supplementary data Fig. S1). These results are surprisingly high when compared with the sample sizes used in the literature.

This study explores the variability in xylem phenology among 159 trees of balsam fir [*Abies balsamea* (L.) Mill.] sampled weekly in 2018 in natural stands in boreal forest. We assess the variability in wood phenology among trees in an even-aged population by testing the spatial distribution of individuals, their size and the annual xylem production as explanatory variables for the timings of xylem phenology. We raise the hypothesis that xylem phenology is (1) affected by tree size; (2) spatially heterogeneous within a population; and (3) related to the rate of annual cell production. Once variability is assessed and explained, we quantify the thresholds of sample sizes that can be used as a guideline for sampling during the assessment of wood phenology in the field, and for building and calibrating intra-annual growth models.

MATERIALS AND METHODS

Study area

The study area is located at the Forêt Montmorency in Quebec, Canada (47°16′20″N, 71°08′20″ W), within the balsam fir–white birch bioclimatic domain (Fig. 1). According to the Köppen Classification System, the climate type is continental, with a short growing season characterized by cool temperatures and high humidity. Mean annual temperature is 0.4 °C. January is the coldest month with a mean temperature of –15.9 °C. July is the warmest month with a mean temperature of 14.6 °C. Mean annual precipitation is 1422 mm, of which 465 mm falls in the form of snow. Overall, the stands are composed of 80 % balsam fir [*Abies balsamea* (L.) Mill.], 10 % white birch (*Betula papyrifera* Marsh.) and 10 % spruce [*Picea glauca* (Moench)

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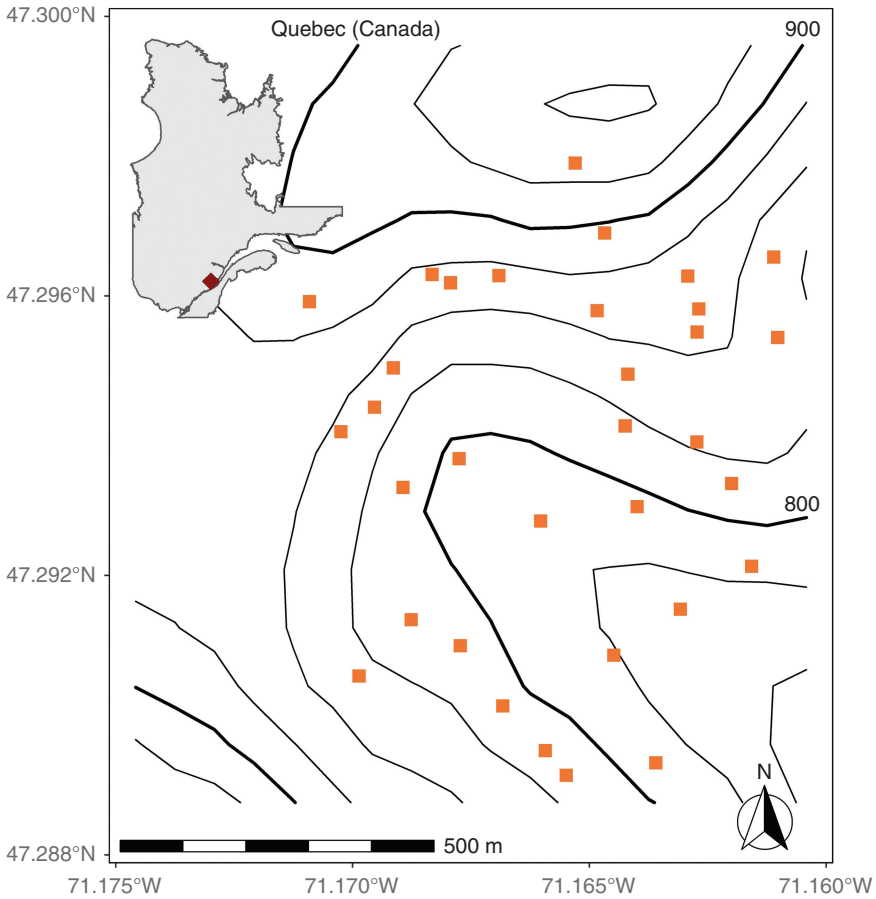

Fig. 1. Location of the experimental plots in the Montmorency Forest (QC, Canada).

Voss and *Picea mariana* (Mill.) B.S.P.]. The study area covers 218 ha that was submitted to clear cuts during 1993–1994. The actual age of the dominant and co-dominant balsam fir stands in this study ranges between 25 and 30 years.

Sampling and data collection

We selected 33 permanent plots of 20 × 20 m (Fig. 1). In each plot, we chose 4–5 dominant balsam firs with upright stems for a total sampling of 159 trees. Trees with polycormic stems, partially dead crowns, reaction wood or evident damage due to parasites were avoided. For each tree, we recorded height and diameter at breast height (i.e. 1.3 m above the ground).

Wood microcores were collected weekly from April to October 2018 on every tree using a Trephor (Rossi *et al.*, 2006*a*). The samples included mature and developing xylem of the current year, the cambial zone and adjacent phloem, and at least one previous complete tree ring. The microcores were dehydrated through successive immersions in ethanol and d-limonene, embedded in paraffin, cut into 8 µm cross-sections and stained with cresyl violet acetate (0.16 % in water) (Rossi *et al.*, 2006*b*). We discriminated between developing and mature tracheids under visible and polarized light at magnifications of ×400. Cells were counted across three radial rows and classified as (1) cambium, (2) enlarging, (3) wall-thickening and lignifying or (4) mature (Deslauriers *et al.*, 2003) (Fig. 2). Cambial cells were characterized by thin cell walls and small radial diameters. The enlargement zone was represented by the absence of glistening under polarized light, which indicates the presence of only primary cell walls. Cells undergoing secondary cell wall formation glistened under polarized light. Cresyl violet acetate reacts with lignin, turning from violet to blue in mature cells. Maturation was reached when the cell walls were entirely blue (Rossi *et al.*, 2006*b*) (Fig. 2).

Variability in xylem phenology

The onset and ending of the developmental phases were calculated and analysed for each tree. The onset and the ending of each cell developmental phase were determined as the date, expressed as the DOY calculated by interpolating two consecutive observations (Deslauriers *et al.*, 2018). Specifically, for each phenological phase, we defined the onset when the first cell was observed, and the ending when we observed the last cell. The duration of the growing season was calculated as the period between the onset of enlargement and ending of wall thickening and lignification. Cell production, i.e. the total number of cells in the tree ring at the end of the growing period, was computed with Gompertz functions using the equation (Rossi *et al.*, 2003):

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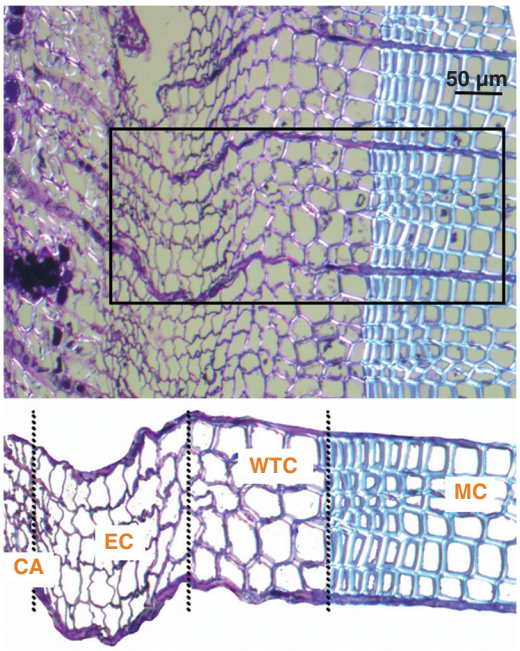

FIG. 2. Transverse section of a weekly sampled microcore, observed at ×400 magnification, for counting the developing tracheids, and classified as (CA) cambium, (EC) enlarging cells, (WTC) wall-thickening and lignifying cells and (MC) mature cells of the previous year.

$$y = \alpha e^{-e^{\beta-\kappa t}} \quad (1)$$

where y represents the number of weekly cumulative cells (enlarging, thickening and lignifying), mature at the time t, represented by a specific DOY, α is the upper asymptote representing the final cell production, β is the x-axis placement parameter and κ is the growth rate. The relationship between duration and timings of xylem formation and cell production was tested using standardized major axis (SMA) regressions. We used SMA regression as we cannot state which one of the variables is independent. Therefore, the aim is to test the relationship between the variables and estimate the line best describing the scatter.

The relationship between xylem phenology and tree size (i.e. tree height and diameter at breast height) was tested using linear regressions. We measured the distance among experimental plots and individual trees, and calculated Moran's index using the inverse of the distance to test for the spatial autocorrelation of xylem phenology and tree size at both plot and tree levels.

The margin of error and minimum sample size

Bootstrapping was performed 10 000 times, during which the mean and s.d. of each phenological phase were repetitively calculated by randomly resampling the original dataset for sample sizes ranging from two to 300 trees. We calculated the ME, i.e. the degree of error in results obtained by random sampling, on the outputs at different confidence levels (CLs) according to

$$\text{ME} = t \times \text{s.d.} \quad (2)$$

Where t (critical value) represents the two-tailed t-value for a given confidence level (i.e. from 70 to 99 %) at a degree of freedom of n–1, and s.d. represents the s.d. of the bootstrapped mean values (Moore *et al.*, 2012).

The ME for a given parameter expresses the maximum expected difference between the true population and the sample estimate. We obtained the minimum sample size for each phenological phase for a desired ME of ±1–5 d at 70–99 % CLs. We defined the minimum sample size as the minimum number of sample trees to meet an expected CL and ME for each phenological phase. All statistics were performed with R version 3.6.1 (R Development Core Team, 2015).

RESULTS

Timings of xylogenesis

The dormant cambium was composed of 3–5 narrow cells (Fig. 3). Only cell division occurred at the onset of the growing season (i.e. the beginning of May), and the cambial zone rapidly increased. The cells produced by the cambium moved into the phase of cell enlargement at the beginning of June. When the division rate slowed down (i.e. mid-June), the cambial zone began to narrow as the rate of cell differentiation was faster than cell division, and the number of enlarging cells increased to 7.5 ± 3.7 on 26 June (Fig. 3). The first wall-thickening cells were observed in mid-June, reaching 10.4 ± 4.8 on 10 July (Fig. 3). The first mature cells were observed at the beginning of July, reaching 42.4 ± 25.8 mature cells in mid-September.

Overall, xylem phenology described the annual pattern represented by three delayed bell-shaped curves (i.e. cambial, enlarging and wall-thickening cells) and a growing S-shaped curve (i.e. mature cells) (Fig. 3). These patterns result from the variation in time of the number of xylem cells passing through each differentiation phase. In contrast, the S-shaped curve is related to the gradual accumulation of mature cells in the tree ring.

Variability in xylem phenology

The onset of enlargement occurred from DOY 133 to 184, with 38 % of trees showing at least one enlarging cell between DOY 160 and 165 (Fig. 4). The onset of wall thickening and lignification occurred from DOY 158 to 193. However, 82 % of trees showed at least one cell in secondary wall formation between DOY 160 and 175. The first mature cells were observed from DOY 165 to 198, with 74 % occurring between DOY 175 and 185. Compared with the onset, the ending of cell differentiation exhibited a higher variability. The enlargement phase ended from DOY 191 to 244, whereas the end of wall thickening and the lignification phase was observed between DOY 205 and 266 (Fig. 4).

The length of the growing season ranged between 40 and 110 d. Cell production, i.e. total number of cells in the tree ring, varied between 12 and 93 cells. The relationships between cell production and the onset and ending of xylem formation were highly significant ($P < 0.001$), with an R^2 of 0.26 and 0.22, respectively (Fig. 5). The relationship between cell production

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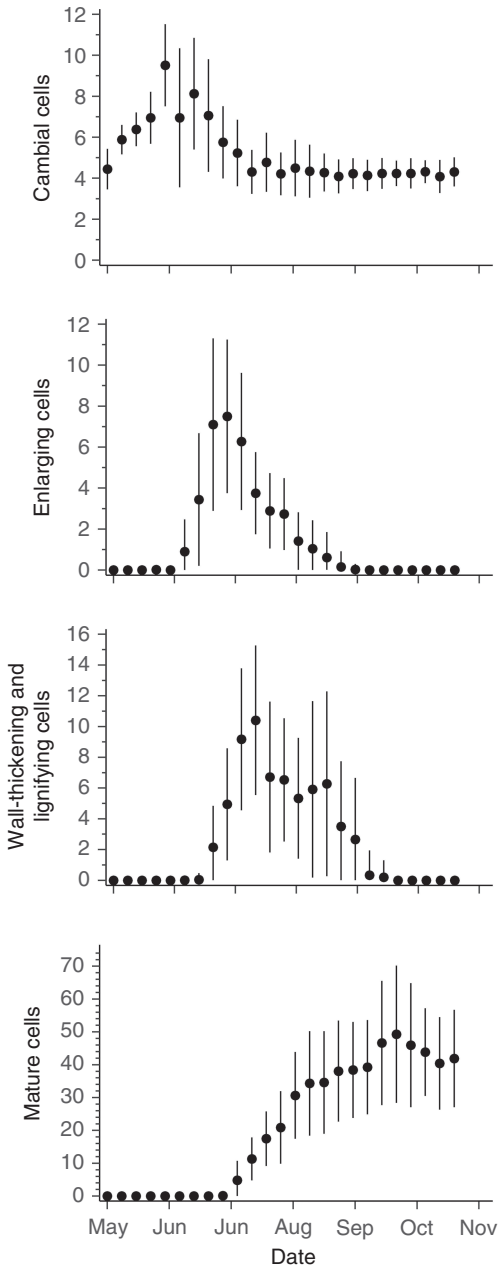

FIG. 3. Number of cambial, enlarging, wall thickening and lignifying, and mature cells observed in 159 balsam firs during 2018. Dots and bars represent the average and s.d., respectively.

and both duration of cell enlargement and cell wall thickening and lignification produced significant regressions ($P < 0.001$), with an R^2 of 0.29 and 0.41, respectively (Fig. 6).

Tree size and spatial variability

Stem diameter at breast height ranged between 58 and 194 mm among the 159 sampled trees (Supplementary data Fig. S2). Height varied between 4 and 13 m. Both variables had a bell-shaped distribution (Supplementary data Fig. S2). A total of 61 % of trees showed a diameter ranging from 75 to 135 mm, and 68 % of trees showed a height ranging from 8 to 12 m (Supplementary data Fig. S1). Moran's index indicated no spatial autocorrelation among experimental plots for either stem diameter or tree height, while it was significant for cell production (Supplementary data Table S1). The Moran's index was significant ($P < 0.001$) among individual trees, producing negative coefficients for stem diameter, tree height and cell production (Supplementary data Table S1; Fig. S3).

No significant relationships were detected between stem diameter and xylem phenology. R^2 of the regressions were low, between 0.002 and 0.0055 ($P > 0.05$) (Table 1; Supplementary data Figs S4 and S5). Also, the regressions between height and xylem phenology were non-significant, with R^2 ranging between 0.001 and 0.027 ($P > 0.05$) (Table 1; Supplementary data Figs S4 and S5).

The Moran's index calculated for each phenological phase and for either plots or trees was not significant, which indicated the absence of spatial autocorrelation in the timings of the phenological phases (Table 2; Supplementary data Fig. S5). When testing for the autocorrelation among plots, coefficients ranged between –0.043 and –0.014 ($P > 0.05$). The onset of all developmental phases had coefficients ranging between 0.014 and 0.057 ($P > 0.05$). When testing for the autocorrelation among trees, the coefficients ranged between 0.071 and 0.128 ($P > 0.05$, Table 2).

The margin of error and sample size

Sample size required to estimate parameters of the population increased at higher CLs and lower MEs (Figs 7 and 8; Table 3), allowing reliable parameters of the population to be estimated. The CL refers to the percentage of samples expected to include the true population parameter. In this study, considering average values, the sample size ranged from a minimum of four trees for an ME of ±5 d at a 70 % CL to approx. 300 trees for an ME of ±1 d at a 99 % CL (Table 3).

Considering average values for each ME among phases at a CL of 95 %, the minimum sample size ranged from 11 (ME ±5 d) to >220 (ME ±1 d) trees, with 29 trees being the suitable sample to obtain an estimation of the population at an accuracy of ±3 d (Figs 7 and 8; Table 3). A sample of 23 trees reaches a CL of 95 % and an ME of 1 week (ME ranging between ±3 and ±4 d).

The sample size required to assess the endings of both enlargement and lignification is bigger than that to determine the beginning of the phenological phases (Table 3). This reflects the above-mentioned larger considerable variability among trees observed for the end of cell enlargement and cell wall thickening.

DISCUSSION

This study investigated the timings of wood formation in 159 balsam firs from 33 permanent plots in Quebec, Canada. We used a large sample size from even-aged stands to assess the variability among individuals and the factors that could explain this variability. The dynamics of xylem formation have already been described in trees growing under different climatic conditions or characterized by different ages, sizes and vitalities

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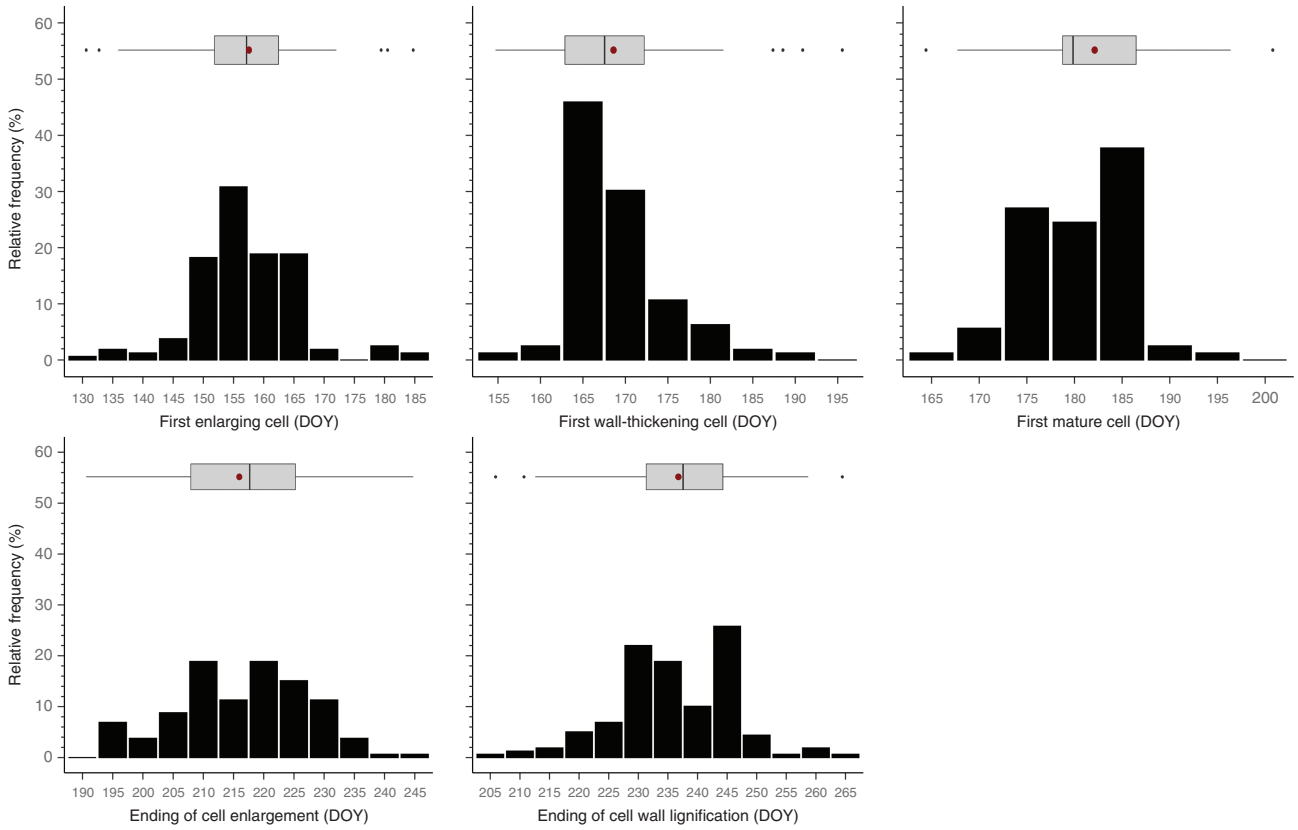

FIG. 4. Frequency distributions of the phenological dates determined in 159 balsam firs during 2018. Horizontal boxplots represent upper and lower quartiles, and the mean and median are drawn as orange dots and vertical black lines, respectively.

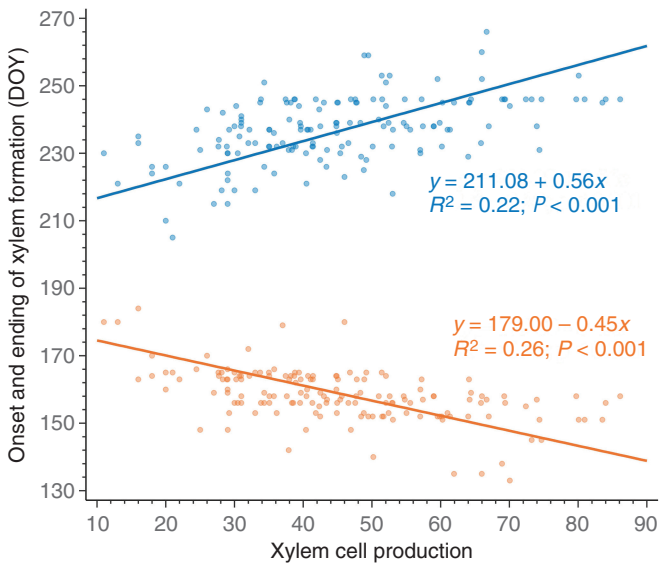

FIG. 5. Standardized major axis (SMA) regressions among timings of onset (orange dots) and ending (blue dots) of xylem phenology, and total number of cells in the tree ring at the end of the growing season in 159 balsam firs at Montmorency forest.

(Rossi *et al.*, 2008; Gričar *et al.*, 2009; Linares *et al.*, 2009; Lupi *et al.*, 2010; Rathgeber *et al.*, 2011; Vieira *et al.*, 2014). Despite such a vast literature, the causes of the observed differences in xylem phenology and length of the growing season remain partially unresolved. Our findings confirmed that trees of the same age exhibited a wide variation in the xylem formation timings and growing season duration. We confirmed the hypothesis that in a boreal forest, cell production is a main factor involved in such differences.

Does tree size shape tree growth dynamics?

In this study, the relationships between tree size (i.e. diameter or height) and the timings and duration of xylem formation were not significant. The question of whether size influences xylem development is a long-standing issue. As age and size are intrinsically coupled during a tree's life span, the main problem has historically been how to uncouple these two factors. An attempt to disentangle the effect of size from that of age was realized using grafting techniques (Mencuccini *et al.*, 2005, 2007; Abdul-Hamid and Mencuccini, 2009) or reducing population density by thinning (Martínez-Vilalta *et al.*, 2007). These manipulations demonstrated that photosynthesis and tree growth decline at increasing tree size (Martínez-Vilalta *et al.*, 2007; Abdul-Hamid and Mencuccini, 2009). However, while there is a general agreement that tree size, and in particular tree height, drives variation of xylem traits (Rosell *et al.*, 2017; Kašpar *et al.*, 2019), its role in xylem phenology remained uncertain (Rossi *et al.*, 2008; Li *et al.*, 2019).

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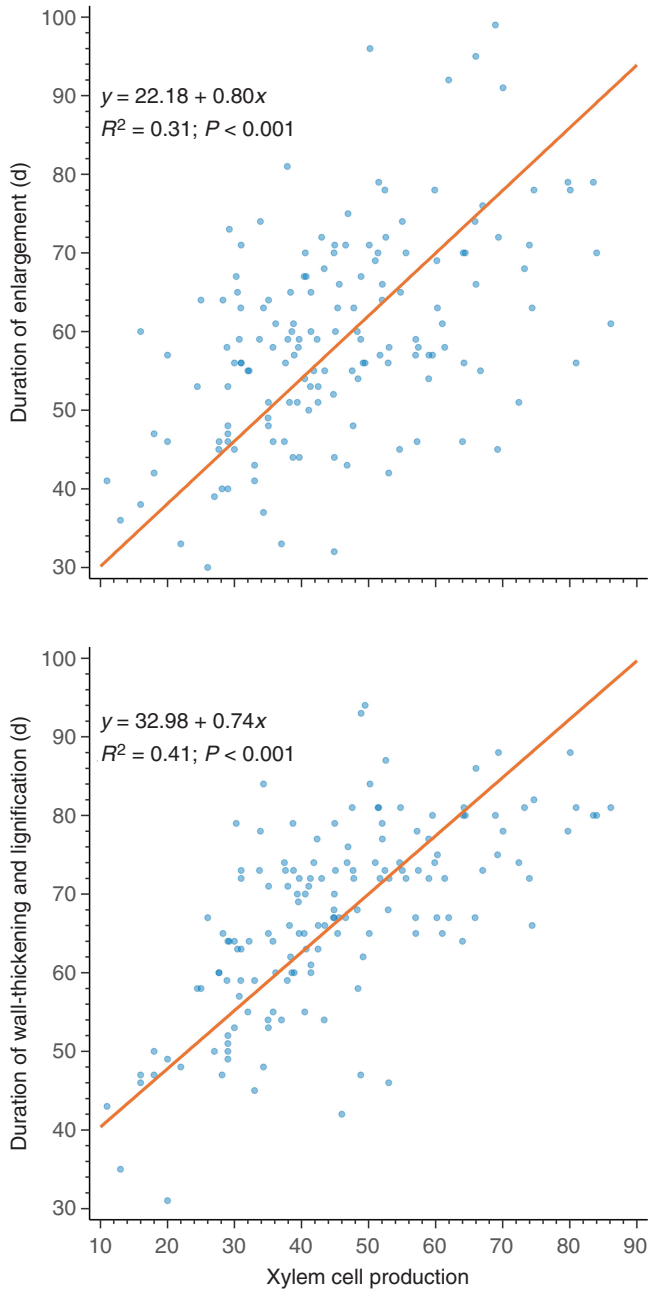

FIG. 6. Standardized major axis (SMA) regressions among total duration of cell enlargement and cell wall thickening and lignification, and total number of cells in the tree ring at the end of the growing season in 159 balsam firs at Montmorency forest.

Rossi *et al.* (2008) observed that xylem phenology is not constant throughout the tree life span. Older trees showed shorter periods of cambial activity and xylem cell differentiation. Nevertheless, the older trees considered in that study were also taller and larger; thus, the effect of age was not finally disentangled from tree size. Rathgeber *et al.* (2011) investigated xylogenesis in trees of the same age and of similar height but belonging to different social classes. Wood formation started earlier, stopped later, lasted longer and resulted in higher cell productivity in dominant individuals. However, since dominant trees also showed larger stem diameters and greater annual radial increments, the question of whether either or both factors affected xylem phenology remained unanswered. Li *et al.* (2013) monitored xylem phenology in two age classes and showed that xylem differentiation started earlier in young trees, resulting in a longer growing season. However, older trees were still also taller and larger. Zeng *et al.* (2018) selected trees according to their size and age, observing that small young pines exhibited earlier cambial reactivation and later end of cell differentiation compared with large old pines. However, these differences were not confirmed in junipers of different sizes but similar ages. The hypothesis that xylem phenology is size dependent could not be entirely rejected. Still, the results suggested that age plays an important role in the timings and duration of xylem formation.

Our sample is represented by an even-aged population with individuals of different sizes. No significant effect of stem size was observed on the timings of xylem formation, suggesting that the differences in dynamics of xylem formation detected in the previous studies resulted from an age effect or other factors (e.g. genetics, microsite or competition) not explicitly considered. Our results suggest that tree selection for tree growth dynamics may ignore the factor size when individuals have the same age and dominance. Senescence, the progressive loss of function accompanied by decreasing fertility and increasing mortality with advancing age (Kirkwood and Austad, 2000), in the previous studies emerged as an important factor in xylem phenology. This loss of function would also affect carbon sequestration at the stand level, which is a complex function of tree age (Kowalski *et al.*, 2004). This forest ecosystem effect results from a less efficient metabolism in old trees that leads to reduced growth (Bond-Lamberty *et al.*, 2004; Campbell *et al.*, 2004). Assuming that the differences in dynamics of xylem formation detected in the previous studies result from senescence, probably a function of a less efficient metabolism, it seems worthwhile to further explore the question to solve the chicken–egg dilemma in the relationship between phenological timing and C demand.

Should we sample closely located trees?

Xylem phenology is spatially heterogeneous. The variability has a similar magnitude within and between plots. Consequently, no spatial autocorrelation was detected by our analyses. Similar results were observed by Liang and Schwartz (2009) on the timings of bud phenology in a similar experimental design. Therefore, our results are not surprising given the synchronism between primary and secondary growth (Klein *et al.*, 2016; Buttó *et al.*, 2021). The spatial variability could reflect genetic differences within a population. The natural variation in phenology allows a part of the population to endure unfavourable climatic events and increases survival under changing conditions (Silvestro *et al.*, 2019, 2020; Guo *et al.*, 2021). However, variability in phenology is also reported among clones, i.e. individuals with the same genotype (Deslauriers *et al.*, 2009). Moreover, a part of the observed variation is the consequence of heterogeneous growth along the circumference of the stem (Lupi *et al.*, 2013).

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TABLE 1. *Linear regression between xylem phenology and stem diameter (DBH) and height*

Phenological phases	DBH		Height	
	R^2	q ± s.e	R^2	q ± s.e.
Onset of enlargement	0.002	–0.011 ± 0.02	0.001	–0.116 ± 0.34
Onset of wall thickening and lignification	0.002	–0.008 ± 0.01	0.011	–0.326 ± 0.25
First mature cell	0.006	–0.011 ± 0.01	0.027	–0.413 ± 0.21
Ending of enlargement	0.055	0.006 ± 0.03	0.009	0.548 ± 0.46
Ending of wall thickening and lignification	0.009	0.028 ± 0.02	0.019	0.715 ± 0.42

The 'q' represents the slope, s.e. the standard error. No significant results were observed.

TABLE 2. *Moran`s correlation coefficients testing for the spatial autocorrelation of xylem phenology for experimental plots and individual trees*

Phenological phases	Plots	Trees
Onset of enlargement	0.054 ± 0.035	0.099 ± 0.028
Onset of wall thickening and lignification	0.057 ± 0.035	0.109 ± 0.029
Mature cells	0.014 ± 0.035	0.071 ± 0.029
Ending of enlargement	–0.039 ± 0.035	0.071 ± 0.029
End of wall thickening and lignification	–0.030 ± 0.034	0.128 ± 0.029

No significant results were observed ($P < 0.05$). Values are indicated as Moran`s coefficient and s.d.

In contrast to phenological timings, we highlighted a significant spatial autocorrelation in the annual cell production. This result suggests that growth rates, and consequently cell production, are not exclusively dependent on the corresponding wood phenology and growing season length. Indeed, other studies have pointed out the plasticity and the complex interactions underlying the dynamics of wood formation in response to temperature (Cuny *et al.*, 2019) and water availability (Pasho *et al.*, 2012), factors that could probably also play a role at microsite scale.

The variation among trees is a scale issue in ecology, related to landscape and ecosystem patterns over time and space (Levin, 1992; Wu and Li, 2006). Our results show that while studying xylem formation dynamics of a population, the variability among trees assumes a leading role and should be carefully considered. At large scales (e.g. at ecosystem level or along environmental gradients), the variability within the stand seems to be usually lower than those among stands (Buttò *et al.*, 2019; Guo *et al.*, 2021). Nevertheless, at smaller scales (i.e. at population or community level), the variability in phenology among trees assumes a greater relevance even if it lacks spatial autocorrelation. These scale-dependent phenological behaviours therefore need to be considered when setting up an experimental design. Our results suggest that sampling for the determination of xylem formation dynamics may favour trees located in the same plot, given that the variability among individuals has a similar magnitude when compared within and among plots.

Is phenology an issue of carbon sink?

Our results showed that cell production is related to the period of xylogenesis. Trees with higher cell production start xylem differentiation earlier and end it later, thus resulting in a longer growing season. Moreover, durations of cell enlargement and cell wall thickening and lignification correlate with cell production. Several authors observed that the larger the amount of xylem produced, the longer the period of wood formation (Thibeault-Martel *et al.*, 2008; Gričar *et al.*, 2009; Rathgeber *et al.*, 2011; Vieira *et al.*, 2014). Cell production is composed of successive phases representing longitudinal data, i.e. a chain of consecutive events (Rossi *et al.*, 2012). Specifically, the timing of onset and the rate of cambial division affect the number of cells in the cambial zone which, in turn, influences the timing of cell differentiation (Lupi *et al.*, 2010; Rossi *et al.*, 2012). In the case of the occurrence of water deficit during the growing season, individual growth rate assumes a leading control on the annual cell production (Ren *et al.*, 2019). However, if water availability does not represent a constraint, the larger the number of xylem cells in differentiation the longer the time needed to complete their maturation.

Xylogenesis is a crucial component of plant metabolism, and it is likely to be affected by C dynamics at both plant and ecosystem scale. Wall thickening and lignification is the largest C sink in trees (Cuny *et al.*, 2015). Thus, if the number of cells produced during cambial activity affects the duration of each cell differentiation phenological phase, C availability could also potentially play a role in defining the duration of maturation of cell walls. Xylem cells start differentiation by stretching their thin primary walls (Cuny *et al.*, 2015). Most C is allocated during cell wall thickening (Cuny *et al.*, 2015). The high C demand required during formation of the cell walls can explain the strong correlation between cell production and the duration of this phase during cell differentiation.

Xylogenesis: sample size matters

Our results showed that estimates of xylem phenology at a CL of 95 % with an ME of ±1 d require samples larger than 100 trees, an excessive effort for a lab. Most studies on wood formation use samples with five individuals (De Micco *et al.*, 2019), with a broad range of between one and ten (Deslauriers *et al.*, 2015). The sample size for xylogenesis is closely related to the scaling, i.e. data collected at one scale and applied at another one (Seidl *et al.*, 2013). Data collection requires resources, and

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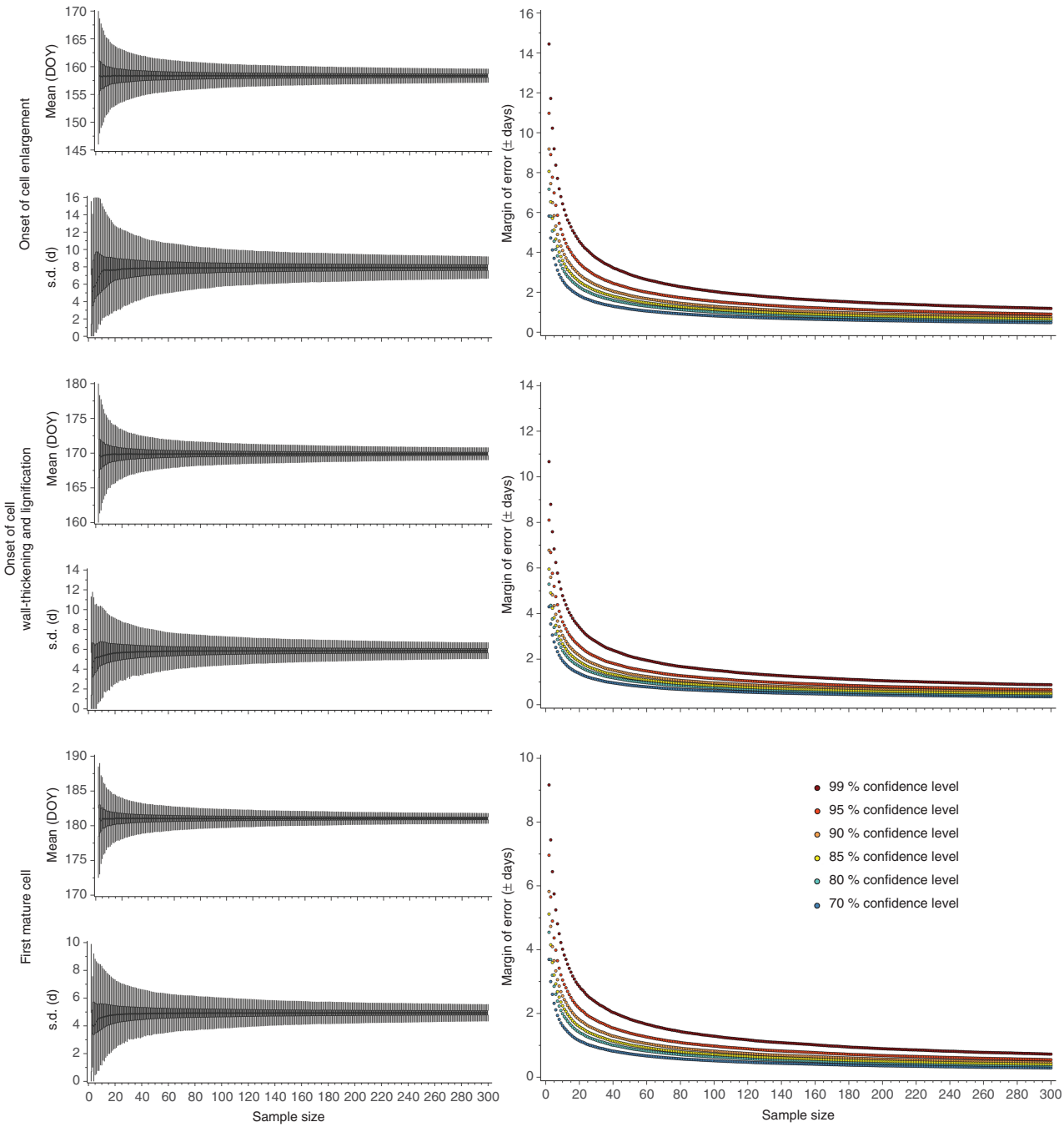

FIG. 7. Sample size for the onset of xylem formation. Boxplots represent upper and lower quartiles of bootstrapped mean and s.d. outputs for the onset of enlargement, wall thickening and first mature cell. The minimum sample size is calculated at different confidence levels and margins of error.

our analyses offer an accurate baseline for upscaling by linking local- (i.e. at tree level) with population-scale information (i.e. at the stand level). According to our results, the range in sample size reported in the literature (i.e. from 5 to 12 individuals) can reach, at best, a CL of 95 % with an ME of ±5 d. It is well known that precision and accuracy are, in part, a function of sample size, but cost and time to collect, prepare and analyse wood samples remain a severe constraint in studying wood formation. Therefore, the question defines a trade-off between accuracy (i.e. sample size) and resource investment (i.e. time and costs). According to our results, a sample size of 29 trees may increase the CL substantially at 95 % and maintain a low ME of ±3 d. However, a more reasonable sample size in terms of efforts and benefits is a sample of 23 trees reaching a CL of 95 % and an ME of a week (ME ranging between ±4 and ±3 d), the common sample time span used for sampling in xylem differentiation assessment.

Our estimates on the timings of phenological phases demonstrated that the sample size for spring events is defined by the beginning of cell enlargement, the spring phenological phase with

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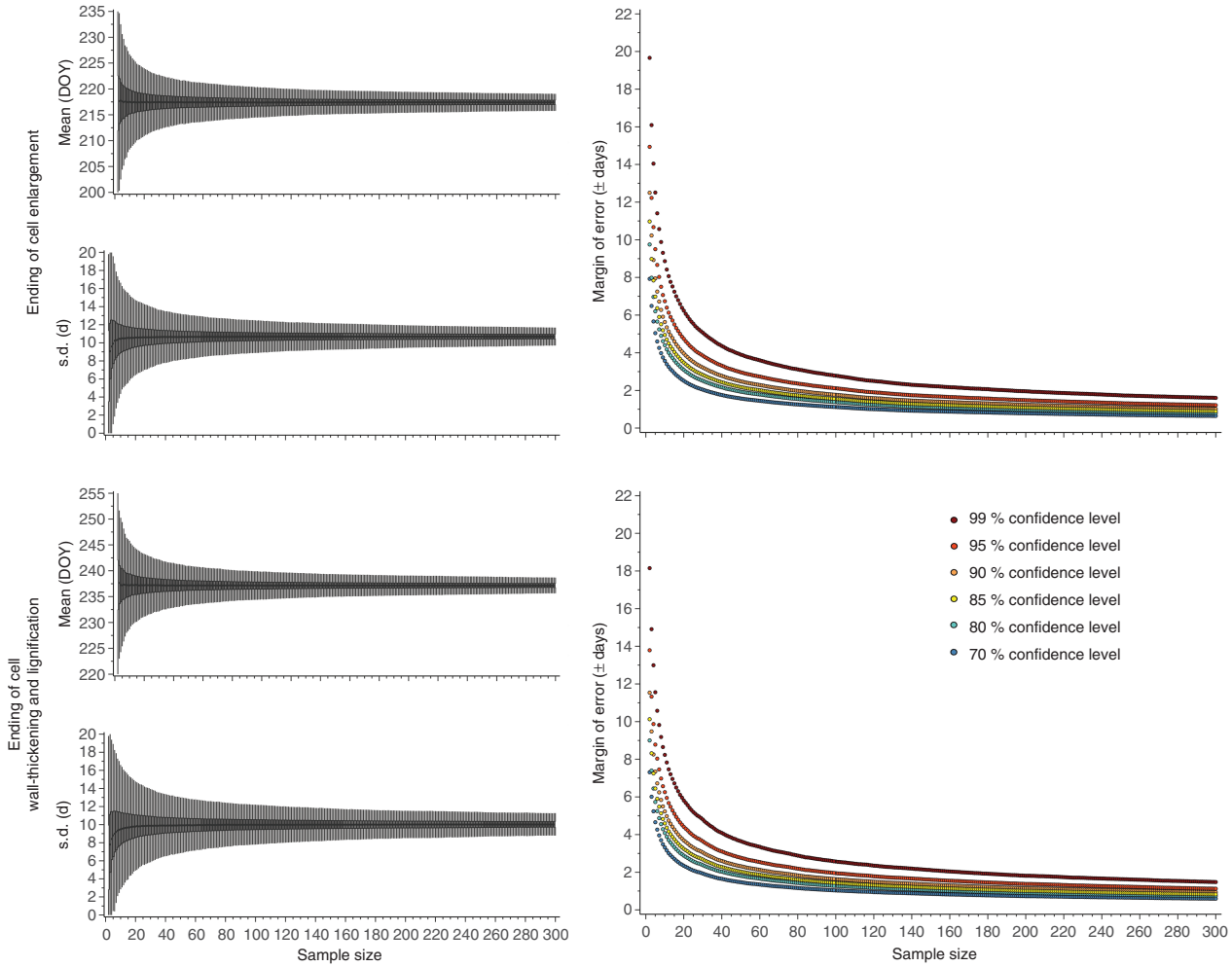

FIG. 8. Sample size for the ending of xylem formation. Boxplots represent upper and lower quartiles of bootstrapped mean and s.d. outputs for the ending of enlargement and wall thickening and lignification. The minimum sample size is calculated at different confidence levels and margins of error.

the widest among-tree variability. Also, above all, autumnal events require larger samples than spring events for a given accuracy level. This means that using a single sample size that results from an averaged value among phenological phases, we can estimate their beginning and ending with different levels of accuracy (i.e. different MEs at different CLs). In temperate and boreal regions, growth resumption is closely driven by environmental factors, mainly temperature and photoperiod (Linares *et al.*, 2009; Rossi *et al.*, 2012). In contrast, internal factors, such as the timings of growth reactivation and amount of cell production, are involved in the duration of the growing season and the ending of wood formation, as shown by our findings and previous studies (Rathgeber *et al.*, 2011; Li *et al.*, 2013). The larger the number of xylem cells produced, the longer the time needed to complete cell differentiation. When precise estimates at tree level have marginal importance, a practical solution could be that investigation on xylem formation should consider larger sample sizes for assessing autumnal phenology or gradually increasing the number of samples during the growing season. Even though further exploration is required to confirm this proposal, according to our results, it would allow maintaining a comparable ME among the phenological phases.

Estimates of xylem phenology obviously improve with larger sample sizes, as also demonstrated by our results. The literature indicates that experimental designs with more modest sample sizes were able to detect differences among ecosystems or species or along climatic gradients (Prislan *et al.*, 2016; Rossi *et al.*, 2016; Antonucci *et al.*, 2017, 2019; Zhang *et al.*, 2018; Buttò *et al.*, 2019). However, some manipulative experiments on xylogenesis failed to find conclusive relationships between wood formation dynamics and the environmental factors under investigation. Manipulations involved rain exclusion (Belien *et al.*, 2012; D'Orangeville *et al.*, 2013a), soil warming and acceleration of snow melting (Repo *et al.*, 2011; Lupi *et al.*, 2012), nitrogen fertilization (Lupi *et al.*, 2012; D'Orangeville *et al.*, 2013b; Camargo *et al.*, 2014) or thinning (Primicia *et al.*, 2013; Lemay *et al.*, 2017). These studies used between three and ten trees per group. The lack of significant results could be caused by the small sample size accompanied by the high among-tree variability in xylem phenology and the conservative behaviour of wood formation. The variability among trees (i.e. within a population) can vary according to a number of factors (e.g. species, tree age or the social status of the trees). For this reason, a preliminary assessment of the variability among trees can

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TABLE 3. *Minimum sample size for each phenological phase (both onset and end of enlargement, both onset and end of wall thickening and lignification, and onset of maturation) for each confidence level (CL, 70, 80, 85, 90, 95 and 99 %) and each desired margin of error (±1, ±2, ±3, ±4 and ±5 d)*

Phenological phases	Margin of error (± days)														
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
	CL 70 %					CL 80 %					CL 85 %				
Onset of enlargement	69	18	8	5	3	103	26	12	7	5	130	33	15	9	6
Onset of wall thickening and lignification	37	10	5	3	2	56	15	7	4	3	71	18	9	5	3
First mature cell	27	7	3	2	2	42	10	5	3	2	52	13	6	4	3
Ending of enlargement	122	32	15	8	8	189	47	22	13	8	239	61	27	16	10
Ending of wall thickening and lignification	109	29	13	7	5	165	42	19	11	7	209	52	24	14	9
Average among phases	**73**	**19**	**9**	**5**	**4**	**111**	**28**	**13**	**8**	**5**	**141**	**35**	**16**	**29**	**6**
	CL 90 %					CL 95 %					CL 99 %				
Onset of enlargement	169	43	19	11	7	243	61	27	16	10	>300	104	47	27	17
Onset of wall thickening and lignification	93	24	11	6	4	132	33	15	9	6	227	57	26	15	10
First mature cell	67	17	8	5	3	96	24	11	6	4	165	42	18	11	7
Ending of enlargement	>300	79	33	20	13	>300	110	49	28	19	>300	192	87	48	31
Ending of wall thickening and lignification	268	68	30	18	11	>300	96	43	25	16	>300	167	75	42	28
Average among phases	**179**	**46**	**20**	**12**	**8**	**214**	**65**	**29**	**17**	**11**	**258**	**112**	**50**	**29**	**19**

For average assessment, we used 300 when the value was >300.

help in improving the accuracy of the results and deepening our knowledge on the factors driving the growth dynamics of a population.

This study highlighted the existence of a high variability in the timings of wood formation among trees within an area of 1 km^2, although the causes of the observed differences in xylem phenology still remain partially unresolved. The correlation between the growing season length and xylem cell production suggests a close connection between the processes of wood formation and C uptake. A more in-depth analysis of the relationships between C source and sink processes could provide new insights to explain the variability in growth dynamics among trees and understanding of the physiological processes driving wood production. This work points out the need to consider sample size while assessing xylem phenology. Considering the huge variability within a population, increasing the sample size is a crucial step to further explore the reasons underlying this variability. Moreover, there is an ever-increasing interest in simulation models, developed mainly for research purposes in the past, and now applied in forest management planning and decision support. In this context, finding better ways to incorporate variability in growth dynamics and performance among individuals can help to improve the analysis of forest productivity and its changes under a warming climate.

SUPPLEMENTARY DATA

Supplementary data are available online at https://academic.oup.com/aob and consist of the following. Figure S1: sample size related to the margin of error according to a range of different standard deviations. Figure S2: relative frequency of diameter classes and height classes among the 159 individual trees sampled in Montmorency forest, Quebec, Canada. Figure S3: diameter at breast height, tree height and cell production in 159 trees at Montmorency Forest. Figure S4: linear regressions among timings of the onset of xylem developmental phases and stem diameter and tree height in 159 balsam firs at Montmorency forest. Figure S5: linear regressions among timings of the ending of xylem developmental phases and stem diameter and tree height in 159 balsam firs at Montmorency forest. Figure S6: timings of xylem phenology in 159 trees at Montmorency forest. Table S1: Moran's correlation coefficients tested for the spatial autocorrelation of diameter at breast height, tree height and xylem cell production for experimental plots and individual trees.

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R.S., V.B. and S.R. conceived the ideas and designed the methodology; J.D.S. and G.D. administrated the project and the data collection; R.S. and V.B. analysed the data; J.D.S., G.D.

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and I.A. validated the results; R.S. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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