Wood structure explained by complex spatial source-sink interactions

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Abstract

Wood is a remarkable material. It is responsible for the sequestration of significant anthropogenic CO₂¹, aids understanding of past climates², has unique acoustic, thermal, and strength properties, and is an endlessly renewable source of energy³. However, we lack a general integrated understanding of how its structure is created. A theoretical framework of wood formation is presented here that explains a diverse range of poorly understood observations, including: (i) the anatomy of growth rings, with a transition from low-density earlywood to high-density latewood; (ii) the high sensitivity of latewood density to temperature; (iii) cell-size regulation; and (iv) relationships between growth and temperature. These features arise from interactions in time and space between the production of cells, the dynamics of developmental zones, and the supply of carbohydrates. Carbohydrate distribution is critical for the final density profile, challenging current theory which emphasises compensation between the rates and durations of cell enlargement and wall thickening. These findings have implications for our understanding of how growth responds to environmental variability and the interpretation of tree rings as proxies of past climates. In addition, they provide a framework for the incorporation of explicit growth processes into models, such as those used to predict the role of vegetation in the future global carbon cycle. Finally, divergent responses in volume and mass with increasing temperature suggest caution in interpreting observations based on volume alone.

Main

Lignified cellulose, “wood”, is the largest store of carbon in terrestrial plants, and as such plays a major role in the global carbon cycle¹. Understanding controls on its formation is a major research priority^{4,5}. Unlike most metabolic pathways, the process of wood formation, xylogenesis, is a synthetic dead-end without the potential to recycle materials, and is therefore highly regulated to avoid wasteful

allocation of photosynthate⁶. This regulation results in conserved anatomical patterns, with tree rings in conifers and ring-porous species exhibiting transitions from low to high density wood each year. Nevertheless, these rings still carry the imprint of environmental conditions, a feature exploited in climate reconstructions, and as such underpin our ability to place contemporary climate change in an historical perspective². However, despite the importance of this imprint for understanding past climates, and the relevance of wood formation for carbon sequestration and tree physiology, our understanding of how tree-ring anatomy arises and controls on its variability at intra- and inter-annual timescales remains poor.

Insights derived from studies on wood formation at the intra-annual timescale^{7,8,9}, advances in understanding of cell size and growth regulation in meristems¹⁰, and high-resolution characterisation of biochemical and anatomical properties during wood development¹¹, are combined here in a theoretical framework to analyse and explain how wood formation processes result in final ring structures. The framework explicitly considers xylogenesis in time and space, and thereby provides a mechanistic basis for understanding and predicting how the rate at which carbon is sequestered into wood varies with environmental conditions at daily to inter-annual timescales.

Pinus sylvestris L. (Scots pine) is used as the model species due to the good availability of critical observations. As in most conifers, its growth rings normally consists of earlywood with large cells and thin secondary walls, which transitions to latewood with small cells and thick walls in the final third or so of the ring. The total annual carbon increment is the result of integrating this density distribution over the total ring width, and cell lumen areas and wall thicknesses determine wood properties such as conductivity to water and strength. However, the mechanisms that create this pattern are not understood⁹. Recent observations of intra-annual cellular kinetics have quantified the relative contributions of different processes, such as cellular enlargement^{7,12,13,9}, but have not yet been integrated into a general mechanistic explanation of how the final structures are created. A key question is why cellular density is relatively unresponsive to temperature, except in the final part of the developing ring. A paradigm that has emerged from statistical interrogation of intra-annual observations is that the lack of sensitivity in earlywood is due to compensation mechanisms between rates and durations, which somehow break down in latewood¹⁴.

The framework explored here combines observations, knowledge, and hypotheses for the fundamental mechanisms that underlie xylogenesis. The main assumptions are: (i) when not dormant, a

single bifacial initial (stem) cell in each radial file grows radially and undergoes periclinal divisions, producing phloem outwards and xylem inwards¹⁵; (ii) these divisions are stochastically assigned to xylem or phloem, with prescribed probabilities¹⁶; (iii) when not dormant, inward cells grow radially, and can themselves divide if in the proliferation zone, with division-size regulation intermediate between a constant size and constant increment¹⁰ (this assumes that the vascular cambium and the apical meristem share regulatory mechanisms¹⁷); (iv) division is asymmetrical, with the relative growth rates of the daughters conditioned on their relative sizes, maintaining homeostasis¹⁰; (v) initial cells and their derivatives elongate in the radial direction at a rate dependent on temperature (calibrated to¹⁸; other factors such as turgor and nutrients are not considered here, but could be incorporated as controls on radial growth); (vi) once cells are no longer in the proliferation zone they continue to enlarge at a temperature-dependent rate while in an enlargement zone (calibrated to¹¹); (vii) once they are no longer in the enlargement zone, secondary wall thickening occurs until they are no longer in the wall thickening zone, at which point they are assumed to lose their protoplasm and become mature, functioning, xylem; (viii) the rates of primary and secondary wall growth depend on temperature (calibrated to¹⁸), and the local availability of carbohydrates (calibrated to¹¹); (ix) carbohydrates diffuse from the phloem along the radial file, with a prescribed phloem concentration and constant resistance between cells, creating an equilibrium profile between supply and demand each day (calibrated to¹¹); (x) cells receive time-evolving positional information based on daylength that determines whether they are in the proliferation, enlargement, or wall thickening developmental zones (calibrated to¹¹); (xi) the proliferation zone enters dormancy at a prescribed daylength¹⁹, and exits dormancy when a chilling day-conditioned heat sum is reached²⁰ (calibrated to¹¹).

Overall density profile

The simulated distribution of cell-mass density across the growth ring at the end of the growing season compares favourably to observations (Fig. 1a), suggesting that its assumptions are highly plausible. No model parameter or underlying assumption was calibrated to observations of density distribution - the predicted pattern and absolute values are entirely an emergent property. In both observations and simulation, density remains low and fairly constant at c.0.4 g cm⁻³ for the first 40-50% of the ring, and then increases to a peak of c.0.85-1 g cm⁻³ in the final 10-30% of the ring, before declining in the final 10-20%.

Figure 1. Simulated cell-mass densities as functions of relative distance across annual rings in *Pinus sylvestris* growing at 64.25 °N, 19.75 °E (northern Sweden), 1976-1995 using the full model. 100 radial files were simulated, with a spin-up over 1951-1975, and proliferating cells carried over between years. Symbols are individual cells and orange lines are 0.06-fraction running mean over 0.02-fraction bins. **a**, Comparison with observations of the same species from: 1. France⁷, morphometric density; 2. France⁷, X-ray; 3. southern Finland²¹, X-ray; 4. northern Finland²¹, X-ray. **b**, Effects of changing model assumptions on density. The fixed-temperature response uses 10 °C for cell enlargement and wall thickening; the fixed zone-widths responses keeps developmental zones at their values on 20 June, with or without autumn dormancy; and the saturating carbohydrates response assumes $\Delta M = \Delta M_{max}$ (Eqn. 8).

The mechanisms responsible for the observed intra-annual density pattern are not known, but could involve, singly or in combination, climate variability (e.g. temperature and/or water availability directly affecting cellular development), changing developmental zone widths/activity (possibly related to daylength signalling), and/or carbon supply (possibly related to variability in photosynthesis and whole-plant source/sink dynamics). Removing the effect of temperature on cell enlargement and wall thickening does not change the overall pattern of low-density earlywood transitioning to high-density latewood, but does smooth the rate at which density increases towards the latewood, and removes the density decline in the final 20% of the ring (Fig. 1b). Therefore although temperature seasonality is not responsible for the overall density pattern, some features, such as the existence of a peak in the latewood, are temperature-controlled. Removing the effect of temperature on wall thickening only also removes the late-season peak, demonstrating that it is caused by low late-season temperatures reducing the rate of wall thickening.

The effect of changing zone widths was investigated by fixing all widths at their values on 20 June for the whole year (Fig. 1b). The resulting profile still has low-density earlywood and an increase in density in latewood, suggesting that zone width variability is not the cause of the early-latewood transition. However, in contrast to the full model, the transition is steeper and there is a rapid decline in density at c.85% of the ring. Analysis of cellular dynamics through the year (cf. Figs. S1; S4) shows that this decline is due to cells stuck in the enlargement zone when it becomes dormant, and hence do not undergo any wall thickening. Removing autumn dormancy but keeping the zone widths fixed produces very wide rings because many additional cells are produced and enlarge, but there is no longer any high-density latewood (Fig. 1b). Therefore although zone width variability *per se* is not the cause of the earlywood-latewood transition, the cessation of enlargement due to late-season dormancy is required, increasing the duration of wall thickening relative to enlarging.

The influence of carbohydrates on the density profile was investigated by allowing wall thickening to continue at its maximum, temperature-limited, rate (Fig. 1b). While there is still an increase in density across most of the profile, and a peak in the latewood, earlywood has a much higher density than in the full model and the earlywood-latewood transition is much smoother. The gradual increase in density across the earlywood with saturating carbohydrates is due to the narrowing of the enlargement zone, reducing cell size (density is constant across the first 50% of the ring if zone widths do not change but still increases if temperature is fixed). The low density in earlywood cells in the full model

is therefore due to their low carbohydrate concentrations during wall thickening as a result of their relatively large distance from the phloem (cf. Fig. S1). The role of compensation mechanisms in maintaining constant density constant across the earlywood is examined below. The densities across the latewood when carbohydrates are saturating and in the full mode converge towards the end of the ring. Late-season cells undergo wall thickening closer to the phloem than cells maturing earlier, resulting in lower carbohydrate limitation.

Taken together, these simulations demonstrate that the overall density profile in the ring, and hence its final carbon content, is determined by the spatial distribution of carbohydrates across the radial file, the contraction of the enlargement zone, and the imposition of autumn dormancy on the proliferation zone. The spatial distribution of carbohydrates is an emergent property of the developing ring, determined by the activities of the proliferation, enlargement, and wall thickening zones (Fig. S4).

Temperature and cell-mass density

The model was used to investigate mechanisms for the observed high sensitivity of cell-mass density to temperature within the latewood¹⁴. Adding a fixed temperature increment of 2 K to all daily temperatures (but keeping dormancy forcing unchanged) results in a wider radial file, with the increase due to more earlywood (Fig. 2), as is observed with respect to the relationship between ring widths and the fraction of earlywood in diverse tree species^{22,23}. Earlywood density is reduced with increased temperature, whereas latewood density is increased, consistent with observations²⁴. Applying the temperature increment to wall thickening but not enlargement results in no change in earlywood density (Fig. 2), showing that the reduction in earlywood density is due to its effect on cell elongation, leading to cell size increasing proportionally more than mass. However, latewood density is increased to the same extent as when temperature is applied to both thickening and enlarging, showing that the latewood temperature response is due to the effect on thickening alone. The control on earlywood density from cell enlargement and latewood density from cell wall thickening is consistent with anatomical analysis in different species across a broad range of locations²⁴.

The sensitivity of density to temperature, when applied to both enlarging and wall thickening, is negative in the earlywood but increases strongly through the latewood (inset in Fig. 2). This is consistent with the paradigm of compensation mechanisms occurring in the earlywood that eliminate

Figure 2. Effect of temperature on density across the simulated radial profile under different model configurations and forcings in *Pinus sylvestris* growing at the same site as in Fig. 1, 1995. Each point is an individual cell and solid lines are 120 μm running means over 40 μm bins. 100 radial files simulated for each model configuration and forcing. Temperature increment of +2 K applied to cell growth and wall thickening, or only wall thickening. Also shown is the result of applying the temperature increment to both cell growth and thickening but with the carbohydrate limitation to cell-wall growth removed. Inset shows profiles of ratio of change in density to change in temperature computed from the results in the main panel for the full model, only temperature effect on wall thickening, and with saturating carbohydrate and temperature effect on both enlargement and wall thickening.

the effects of temperature on density, with breakage of this compensation in the latewood¹⁴. The compensation mechanism has remained elusive, but has been interpreted as a consequence of tight negative coupling between rates and durations during xylogenesis⁹. However, if carbohydrates are assumed non-limiting, a different mechanism emerges. The relative effect of temperature on density under these conditions is nearly constant across the radial file, and equal to the maximum effect in the latewood for the full model (Fig. 2, inset). The low to negative effect of temperature in the earlywood

of the full model is therefore attributable not to compensation between rate and duration, but rather to the limited supply of carbohydrates, similarly to how carbohydrate supply controls absolute density (Fig. 1). In contrast, high concentrations of carbohydrates in the latewood, due to its proximity to the phloem during wall thickening (Fig. S1), mean that wall building is relatively more limited by the temperature kinetics of the relevant biosynthetic machinery (Eqn. 9) than the substrate concentration (Eqn. 8). The cellular and zone-width dynamics responsible for the final ring anatomy are show in Fig. S1. Earlywood cells spend much less time in the wall thickening zone due to the high rate of proliferation and large width of the enlargement zone, quickly pushing the phloem outwards as the radial file lengthens. As the enlargement zone narrows over the growing season, cells spend more and more time in the wall thickening zone and, crucially, remain closer to the phloem. The cell walls of the latewood cells therefore become imprinted with temperature signals, leading to their utility as a climate proxy.

Temperature and cell size

Higher temperatures lead to greater rates of cell enlargement and proliferation. The relative roles of these two processes in determining final cell sizes were quantified by comparing simulations in which temperature changes were applied to either the proliferation or enlargement zones, or to both.

Surprisingly, temperature increase results in a reduction in cell size across much of the ring (Fig. 3), demonstrating the existence of strong compensation mechanisms. The simulations in which temperature only influences the proliferation or enlargement zones shows that the compensatory mechanism is a subtle one. Higher temperatures in the enlargement zone indeed increase cell size, but higher temperatures in the proliferation zone decrease it. Higher rates of proliferation with increased temperature increase the rate at which cells are added to the enlargement zone (an average over the growing season of c.3 additional cells/file, +8%, for a 2 K rise), causing the duration of enlarging to decline, rapidly pushing the zones outwards (this can be envisaged in Fig. S1 as a steepening of the lines joining cells across the enlargement zone), compensating for the increased rate of enlargement.

Overall effects of temperature

A series of simulations was performed as above, but with step changes in temperature applied to the enlargement and wall thickening rates (Fig. 4). Ring width increases strongly and linearly with temperature across the entire range sampled. In contrast, total mass increases more strongly than ring

Figure 3. Effect of temperature on cell radial length across the radial profile under different model configurations and forcings in *Pinus sylvestris* growing at the same site as in Fig. 1, 1995. Each point is an individual cell and solid lines are 300 μm running means over 100 μm bins. 100 radial files were simulated for each model configuration and forcing. Temperature increment of +2 K applied daily to all cells, only proliferating cells, or only enlargement-zone cells. Inset shows radial profile of ratio of change in cell length to change in temperature over 0-2 K for the full model, only temperature effect on proliferating cells, and only temperature effect on enlargement-zone cells.

width at negative temperature anomalies, but for higher temperatures it saturates, not increasing >+5 K. As was shown in Fig. 2, the increase in ring width with temperature is due to an increase in the width of earlywood, which causes overall density to decrease for positive temperature increments (Fig. 4; density decreases have been observed in different species over recent decades²³). The increase in ring width is mainly due to an increase in the number of cells, rather than their size, which is consistent with the findings above of opposing effects of proliferation and enlargement rates on final cell sizes (Fig. 3). Removing the carbohydrate constraint on wall thickening causes total mass to increase very strongly with temperature as earlywood wall thickening is no longer limited by its distance from the phloem (this constraint increases with temperature due to the greater rate of increase

in the cell-phloem distance; cf. Fig. S1). Maximum density increases at the same rate as total width at negative temperature anomalies, but gradually saturates as temperature increases.

Figure 4. Temperature effects on simulated growth in *Pinus sylvestris* at the site used in Fig. 1, 1995. Each line is the 1.5 K running mean over 0.5 K increments for 100 radial files. Temperature anomaly applied as a step increment to the daily values used to compute cell enlargement and wall thickening.

The diverging responses of width and mass to temperature have significant implications for the measurement of tree mass growth and hence carbon uptake, which is usually inferred from volume increments¹. In addition, the saturation of maximum density with temperature has implications for its use as a proxy for past climate, and may well play a role in the “divergence problem”²⁵.

Conclusions

The mechanisms explored here suggest that conifer ring structure results from the interactions of the activities and spatial configurations of developmental zones with the distribution of carbohydrates across the radial file. These interacting factors buffer the response of anatomical features to temperature variation, such as cell sizes, the earlywood-latewood transition, and earlywood density. Crucially, the diffusion of carbohydrates from the phloem plays a major role in the distribution of mass across the ma-

ture tree ring and its response to temperature. These findings have implications for our understanding of the response of carbon sequestration in trees to changing temperature and atmospheric CO₂. Moreover, the framework presented here provides a mechanistic basis for the explicit treatment of tree growth in global vegetation models, enabling a balanced source/sink approach as advocated in a growing body of literature^{26,27,5}. Such reformulation has the potential to reconcile inconsistencies between current models and observations (e.g. in relation to increasing CO₂), with significant consequences for predictions of tree carbon storage, and hence the global carbon cycle.

Methods

Overall framework

Cells are arranged along radial files, with each cell in one of either the proliferation, enlargement, wall thickening, or mature zone, depending on its distance from the phloem. Only cells that contribute to the formation of xylem tracheids are treated. A daily timestep is used, on which cells in the proliferation and enlargement zones can enlarge in the radial direction if the zones are non-dormant, and on which secondary wall thickening occurs in the wall thickening zone. Cells in the proliferation zone divide if they reach a threshold radial length. Cell size control at division is intermediate between a critical size and a critical increment¹⁰. Mother cells divide asymmetrically, with the subsequent daughter relative growth rates inversely proportional to their relative sizes. Size at division and asymmetry of division are computed with added statistical noise¹⁰, and therefore the model is run for an ensemble of independent radial files with different initial conditions.

Equations and parameters

Cell enlargement and division

Cells in the proliferation and enlargement zones, when not dormant, enlarge in the radial direction at a rate dependent on temperature and birth size relative to their sister. A Boltzmann–Arrhenius approach for the temperature dependence is used²⁸:

$$\mu = \mu_0 e^{\frac{-E_a}{k} \left(\frac{1}{T_0} - \frac{1}{T} \right)} \quad (1)$$

where μ is the relative rate of radial cell growth ($\mu\text{m } \mu\text{m}^{-1} \text{ day}^{-1}$), μ_0 is μ at temperature T_0

(= 283.15 K), E_a is the effective activation energy for cell enlargement, k is the Boltzmann constant (8.617×10^{-5} eV K⁻¹), and T is temperature (K). μ_0 was calibrated to the observed mean radial file length at the end of the elongation period¹¹ (Table 1; see “Observations”), and E_a was calibrated to the observed temperature dependence of annual ring widths¹⁸ (Table 1; Fig. S3; see “Observations”).

Symbol	Description	Equation	Value	Units	Source
η	Resistance to carbohydrate flow between cells	15	3030	day ml ⁻¹	11
μ_0	Relative radial growth rate of cell at T_0	1	0.0559	$\mu\text{m } \mu\text{m}^{-1} \text{ day}^{-1}$	11
ω	Normalised rate of cell-wall mass growth	9	18.4	mg ml ⁻¹ day ⁻¹	11
E_a	Effective activation energy for cell enlargement	1	0.32	eV	18
E_{aw}	Effective activation energy for wall building	9	1.39	eV	18
K_m	Effective Michaelis constant for wall growth	8	1.2	mg ml ⁻¹	11

Table 1. Parameters calibrated to observations. Calibration of η , ω , and K_m uses predicted carbohydrate concentration at 257 μm from the phloem, μ_0 the final ring width, E_a the ring width response to temperature, and E_{aw} the maximum density response to temperature.

Radial length of an individual cell increases according to:

$$\Delta L_r = L_r(e^{\varepsilon r} - 1) \quad (2)$$

where ΔL_r is the radial increment of the cell ($\mu\text{m day}^{-1}$), L_r is the radial length of the cell (μm), and ε is the cell’s growth dependence on relative birth size, given by¹⁰:

$$\varepsilon = 1 - g_{asym}\alpha_b \quad (3)$$

where g_{asym} is the strength of the dependence of relative growth rate on asymmetric division (Table

235 2; unitless), and α_b is the degree of asymmetry relative to the cell¹⁰ (scalar):

$$\alpha_b = \frac{L_{r,b} - L_{r,b}^{sis}}{L_{r,b} + L_{r,b}^{sis}} \quad (4)$$

236 where $L_{r,b}$ is the radial length of the cell at birth (μm) and $L_{r,b}^{sis}$ is the radial length of its sister
237 at birth (μm), which are calculated stochastically¹⁰:

$$L_{r,b} = L_{r,d}(0.5 - Z_a) \quad (5)$$

$$L_{r,b}^{sis} = L_{r,d}(0.5 + Z_a) \quad (6)$$

238 where $L_{r,d}$ is the length of the mother cell when it divides (μm) and Z_a is Gaussian noise with
239 mean zero and standard deviation σ_a (Table 2; $-0.49 \leq Z_a \leq 0.49$ for numerical stability).

Symbol	Description	Equation	Value	Units	Source
f	Mode of cell size regulation	7	0.48	-	10
g_{asym}	Size growth dependence on division asymmetry	3	0.43	-	10
L_a	Cell axial length	11, 14	2680	μm	29
ρ	Cell-wall mass density	12, 13	1.54×10^3	$\text{mg}[\text{DM}] \text{ ml}^{-1}$	30
σ_a	Standard deviation of division asymmetry	6	0.105	-	10
σ	Standard deviation of birth size	7	0.227	-	10

Table 2. Parameters taken directly from literature.

240 Length at division is derived as¹⁰:

$$L_{r,d} = fL_{r,b} + \chi_b(2 - f + Z) \quad (7)$$

241 where f is the mode of cell-size regulation (Table 2; unitless), χ_b is the mean cell birth size (Table

242 3; μm), and Z is Gaussian noise with mean zero and standard deviation σ (Table 2).

Symbol	Description	Equation	Value	Units	Source
a_p	Proliferation zone intercept	17	-50.9	μm	11
a_e	Enlargement zone intercept	17	DOY ≥ 185 : -743.6 DOY < 185 : 1257	μm	11
a_t	Wall-thickening zone intercept	17	-427.3	μm	11
b_p	proliferation zone slope	17	7.47	$\mu\text{m hr}^{-1}$	11
b_e	Enlargement zone slope	17	DOY ≥ 185 : 51.7 DOY < 185 : -5.01	$\mu\text{m hr}^{-1}$	11
b_t	Wall-thickening zone slope	17	68.8	$\mu\text{m hr}^{-1}$	11
f_{phloem}	Probability of initial dividing to phloem mother	-	0.315	-	16
L_t	Cell tangential length	11, 14	50.1	μm	11
W_p	Primary cell-wall thickness	14	0.8	μm	11
χ_b	Mean cell radial length at birth	7	8.8	μm	11
θ_p	Phloem carbohydrate concentration	16	100	mg ml^{-1}	11

Table 3. Parameters calculated from observations.

243 The first cell in each radial file is an initial, which produces phloem mother cells outwards and
 244 xylem mother cells inwards. It grows and divides as other cells in the proliferation zone, but on division
 245 one of the daughters is stochastically assigned to phloem or xylem, the other remaining as the initial.
 246 The probability of the daughter being on the phloem side is f_{phloem} (Table 3).

247 **Cell-wall growth**

248 Both primary and secondary cell-wall growth are influenced by temperature, carbohydrate concentra-
 249 tion, and lumen volume. A Michaelis-Menten equation is used to relate the rate of wall growth to the
 250 concentration of carbohydrates in the cytoplasm:

$$\Delta M = \frac{\Delta M_{max} \theta}{\theta + K_m} \quad (8)$$

where ΔM is the rate of cell-wall growth ($\text{mg cell}^{-1} \text{ day}^{-1}$), ΔM_{max} is the carbohydrate-saturated rate of wall growth ($\text{mg cell}^{-1} \text{ day}^{-1}$), θ is the concentration of carbohydrates in the cell's cytoplasm (mg ml^{-1}), and K_m is the effective Michaelis constant (mg ml^{-1} ; Table 1).

The maximum rate of cell-wall growth is assumed to depend linearly on the lumen volume (a proxy for the amount of machinery for wall growth), and on temperature as in Eqn. 1:

$$\Delta M_{max} = \omega V_l e^{\frac{-E_{aw}}{k} \left(\frac{1}{T_0} - \frac{1}{T} \right)} \quad (9)$$

where ω is the normalised rate of cell-wall mass growth (i.e. the rate at T_0 ; Table 1; $\text{mg ml}^{-1} \text{ day}^{-1}$), V_l is the cell lumen volume (ml), and E_{aw} is the effective activation energy for wall building (eV; Table 1; e). ω and K_m were calibrated to the observed distribution of carbohydrates¹¹ (see next section). E_{aw} was calibrated to the observed temperature dependence of maximum density¹⁸ (Table 1; see “Observations”).

Lumen volume is given by:

$$V_l = V_c - V_w \quad (10)$$

where V_c is total cell volume (ml) and V_w is total wall volume (ml). Cells are assumed cuboid and therefore V_c is given by:

$$V_c = L_a L_t L_r / 10^{12} \quad (11)$$

where L_a is axial length (μm ; Table 2) and L_t is tangential length (μm ; Table 3). V_w is given by:

$$V_w = M / \rho \quad (12)$$

where M is wall mass (mg) and ρ is wall-mass density (Table 2; $\text{mg}[\text{DM}]/\text{ml}$).

Cells in the proliferation and enlargement zones only have primary cell walls. ΔM_{max} (Eqn. 9) is therefore constrained to:

$$\Delta M_{max} = \min(\Delta M_{max}, \rho V_{w_p} - M) \quad (13)$$

where V_{w_p} is the required primary wall volume:

$$V_{w_p} = V_c - (L_a - 2W_p)(L_t - 2W_p)(L_r - 2W_p)/10^{12} \quad (14)$$

where W_p is primary cell-wall thickness (Table 3; μm).

Carbohydrate distribution

The distribution of carbohydrates across the radial file is calculated from the balance of diffusion from the phloem and the uptake into cell walls. The carbohydrate concentration in the phloem is prescribed, and the resulting concentration in the cytoplasm of the furthest living cell solved numerically. It is assumed that the rate of diffusion is fast relative to the rate of cell-wall building, and therefore concentrations are assumed to be in equilibrium. Carbohydrate diffusion between living cells is assumed to be driven by the concentration gradient:

$$q_i = (\theta_{i-1} - \theta_i)/\eta \quad (15)$$

where q_i is the rate of carbohydrate diffusion from cell $i - 1$ to cell i (mg day^{-1}) and η is the resistance to flow between cells (calibrated to the observed distribution of carbohydrates¹¹, see next section; Table 1; day ml^{-1}).

At equilibrium, the flux into a given cell must equal the rate of wall growth in that cell plus wall growth in all cells further along the radial file. From this it can be shown that the equilibrium

282 carbohydrate concentration in the furthest living cell in the radial file is given by:

$$\theta_n = \theta_p - \eta \sum_{i=1}^n \sum_{j=1}^n \Delta M_j \quad (16)$$

283 where θ_p is the concentration of carbohydrates in the phloem (Table 3; mg ml⁻¹) and n is the
284 number of living cells in the file (phloem mother cells are ignored for simplicity). The rate of wall
285 growth in each cell depends on the concentration of carbohydrates (Eqn. 8), and therefore θ_n must be
286 found that results in an equilibrium flow across the radial file. This is done using Brent's method³¹ as
287 implemented in the "ZBRENT" function³².

288 **Zone widths**

289 The widths of the proliferation, enlargement, and wall thickening zones vary in time, and are fitted to
290 observations on three dates¹¹ (see Fig. S1 and "Observations"). Linear responses to daylength were
291 found, which are therefore used to determine widths for the observational period and later days:

$$z_k = a_k + b_k dl; DOY \geq 185 \quad (17)$$

292 where z_k is the distance of the inner edge of the zone from the phloem (μ m), k is proliferation
293 (p), wall thickening (t), or enlargement (e), a_k and b_k are constants (Table 3), dl is daylength (s), and
294 DOY is day-of-year. The proliferation zone width on earlier days when non-dormant was fixed at its
295 DOY 185 width (assuming this to be its maximum, and that it would reach its maximum very soon
296 after dormancy is broken in the spring). During dormancy, the proliferation zone width is fixed at its
297 value on DOY 231 (the first day of dormancy¹¹). The enlargement zone width prior to DOY 185 is
298 assumed to be a linear extension of the rate of change after DOY 185. The wall-thickening zone width
299 plays little role prior to DOY 185, and so was set to its Eqn. 17 value each earlier day. On all days the
300 condition $z_t \geq z_e \geq z_p$ is imposed. Fig. S1 shows the resulting progression of zone widths through the
301 year, together with the observed values.

Dormancy

Proliferation was observed to be finished by DOY 231¹¹, and so the proliferation and enlargement zones are assumed to enter dormancy then. Release from dormancy in the spring is calculated using an empirical thermal time/chilling model²⁰. It was necessary to adjust the asymptote and temperature threshold of the published model because the heat sum on the day of release calculated from observations in Sweden (see “Observations”) was much lower than reported for Sitka spruce buds in Britain in the original work:

$$dd_{req} = 15 + 4401.8e^{-0.042cd} \quad (18)$$

where dd_{req} is the required sum of degree-days (°C) from DOY 32 for dormancy release and cd is the chill-day sum from DOY 306. The degree-day sum is the sum of daily mean temperatures above 0 °C, and the chill-day sum is the number of days with mean temperatures below 0 °C.

Simulation protocols

Each simulation consisted of an ensemble of 100 independent radial files. Each radial file was initialised under dormant conditions by producing a file of cells with radial lengths $\chi_b (1 + Z_a)$, and then limiting these to only those falling in the proliferation zone on DOY 1. Values for ε (the relative growth of daughter cells) and $L_{r,d}$ (the radial length at division) were derived for each cell. The main simulations were conducted at the observation site in boreal Sweden (64.35 °N, 19.77 °E) over 1951-1995 to capture the growth period of the observed trees¹¹. Results are mostly presented for 1995 when the observations were made. Simulations for calibration of the effective activation energies (i.e. E_a and E_{aw}) were performed at 68.26 °N, 19.63 °E in Arctic Sweden over 1901-2004¹⁸. Daily mean temperatures for both sites were obtained from the appropriate half-degree gridbox in a global dataset³³.

Observations

Observations of cellular characteristics and carbohydrate concentrations¹¹ were used to derive a number of model parameters, and to test model output (model calibration and testing were performed using different outputs). Cell sizes, wall thicknesses, and positions in Figure 1¹¹, an image of transverse

sections on three sampling dates, were digitised using “WebPlotDigitizer”³⁴. These, together with the numbers of cells in each zone and their sizes given in their text, were used to estimate zone widths, which were then regressed against daylength to give the parameters for Eqn. 17 (Table 3), mean cell size in the proliferation zone on the first sampling date (used to derive χ_b ; Table 3), mean cell tangential length (Table 3), and final ring width (used to calibrate μ_0 ; Table 1). The thickness of the primary cell wall (Table 3) was derived by plotting cell wall thickness against time.

The distributions of carbohydrates along the radial files on the last sampling date for “Tree 1” and “Tree 3” (results for “Tree 2” are not shown for this date) shown in Figure 2¹¹ were calculated. The masses for each of sucrose, glucose, and fructose in each 30 μm section were digitised using the same method as for cell properties and then summed and converted to concentrations, with the results shown in Fig. S4. Mean observed carbohydrate concentration at a fixed point was used to calibrate values for the η , ω , and K_m parameters in Table 1. Calibration was performed iteratively across the three parameters using graphical analysis of the relationships between their values and the predicted carbohydrate concentration at 257 μm . Constraints were very tight for η and ω , but were poorer for K_m .

The calibration target for the effective activation energy for wall deposition was the observed relationship between maximum density and temperature over 1901-2004 in northern Sweden¹⁸ (Fig. S2), and for the effective activation energy for cell enlargement the relationship between ring width and temperature in the same study (Fig. S3).

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Supplementary figures

Figure S1. Simulated radial file development from the full-model simulation in Fig 1. Cells are means over 100 radial files. Cells positions are shown relative to the phloem, and are therefore represented as moving through development zones as they undergo differentiation. Filled circles are observed zone widths and total file length¹¹ (see “Observations”). Dormancy affects both proliferation and enlarging cells. Living cells are shown with green protoplasm and mature, dead cells with grey lumen. Cell walls are orange and are drawn to the y-axis scale. The influence of temperature (yellow zone in background) on wall thickness is limited when the proliferation and enlargement zones are active due to the wall thickening zone rapidly retreating and the large distance from the phloem, reducing carbohydrate concentrations. In contrast, wall thickening in latewood cells occurs closer to the phloem and for longer, leading to higher densities and strong temperature effects.

Figure S2. Observed and simulated relationships between annual maximum ring density and mean June-July-August temperature in northern Sweden. Simulation as for the full model in Fig. 1, except over 1901-2004, with latitude = 68.26 °N, and temperatures from the relevant half-degree grid-point in a global dataset³³. Observations are “Tree Ring Maximum Density Chronology, Regional Curve Standardization” from <ftp://ftp.ncdc.noaa.gov/pub/data/paleo/treering/reconstructions/europe/sweden/tornetrask-temperature2008.txt>¹⁸, scaled using the observed mean of 0.615 g cm⁻³¹⁸. Model parameter E_{aw} adjusted to achieve the same slope as observed (Table 1). Absolute densities were not calibrated.

Figure S3. Observed and simulated relationships between annual ring width and mean June-July-August temperature in northern Sweden. Simulation undertaken as in Fig. S2. Observations are “Tree Ring Width Chronology, Regional Curve Standardization” from <ftp://ftp.ncdc.noaa.gov/pub/data/paleo/treering/reconstructions/europe/sweden/tornetrask-temperature2008.txt>¹⁸, scaled using the observed mean of 0.796 mm¹⁸. Model parameter E_a adjusted to achieve the same slope as observed (Table 1). Absolute widths were not calibrated.

Figure S4. Observed¹¹ and simulated distributions of carbohydrates across the developing wood on DOY 231. Model results are values for individual cells across 100 files, as for the full-model simulation in Fig. 1. Shaded light green area is the observed proliferation zone, shaded light blue area is the observed enlargement zone, and shaded light red area is the observed wall thickening zone. Horizontal and vertical red lines show the calibration target at 256 μm .