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Xylogenesis of *Pseudotsuga menziesii* in Canadian Rockies under the influence of age and the elevation gradient

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Abstract: We studied cambial activity (CA; onset, duration, cessation), cambium cells (CC; numbers during dormancy and throughout the growing season), and the process of xylogenesis (phases of xylogenesis: postcambial growth [PC], cell wall thickening and lignification [SW], mature cells [MT], and cambial productivity [CP]) in montane forest stands of Douglas fir (Pseudotsuga menziesii (Mirbel) Franco var. glauca (Mayr) Franco) in southern Alberta, Canada. We assessed the relationship between these dependent variables and exogenous factors (elevation and meteorological data) as well as endogenous regulation (tree age). Microcores were collected weekly throughout the growing season (April–October 2013) from 36 trees in two age categories located at three sites at different elevations (1480, 1670, and 1820 m a.s.l.). Samples were embedded, dehydrated, sectioned, and stained before counting cells and analysing xylogenesis in three randomly selected radial rows per sample. Gompertz growth function curves were generated for each site and age group, and linear regression was used to evaluate the relationship between cell production rate and meteorological variables. Location significantly impacted CA. Although tree age did not influence the onset of CA, it was associated with cambium cell numbers during the growing season. The duration of CA and the final xylem increment were related to location, while tree age showed a weaker relationship. The duration of CA, as calculated with the Gompertz function, increased with decreasing elevation and was prolonged in older trees. Overall, location strongly affects cambial activity and xylogenesis, while the impact of tree age was insignificant.

Keywords: cambial activity, wood formation, Gompertz growth function, douglas-fir, montane forest

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Introduction

Human-caused climate change is already affecting many weather and climate extremes in every region across the globe. Evidence of observed changes in extremes such as heatwaves, heavy precipitation and droughts, has strengthened. Hot extremes, including heatwaves, are now more frequent and intense, while cold extremes are less frequent and severe. Many impacts of climate change are projected to be more severe at higher latitudes due to mean and cold season warming rates exceeding the global average (IPCC, 2023). In addition, extreme weather conditions such as long-term drought or intense storms are predicted to increase with climate change. The limits of survival of every species or individual are determined by short-term, extreme events and long-term, unfavourable ecological conditions (Schweingruber et al., 2007). Understanding the phenology and growth of climate-vulnerable mid- to high-latitude boreal and montane forests, which cover vast areas of North America and are characterized by conifer-dominated stands with high carbon storage capacity, is essential for anticipating the impacts of climate change on these widespread ecosystems

In seasonal climates, global warming is expected to result in earlier cambial resumptions in spring, with a resultant lengthening of the growing season but with unknown consequences on forest productivity (Rossi et al., 2014). Projected increases in summer temperatures will likely cause greater soil moisture stress in many forested ecosystems. The potential of extended summer drought periods over decades may significantly alter spatial patterns of xylem productivity, thus impacting carbon storage (Case & Peterson, 2005). Differences in tree responses among sites, however, are only partially explained by different environmental conditions, suggesting that local adaptation may also play a decisive role as observed, for example, in adapted production of wood and phloem under local conditions (Gričar et al., 2014). Long-lived plants need to continuously adjust their structure to fit the changing environmental constraints. Xylem growth represents the means used to achieve these structural adjustments and a good indicator of plant success and performance (Deslauriers et al., 2017).

In regions of the world with seasonal climates, cambial activity follows a seasonal cycle. At the beginning of the growing season, when increasing temperature reaches a certain threshold during the spring months, the cambium resumes activity producing new vascular tissues (Rathgeber et al., 2016). A longer growing season (e.g., at lower altitudes) corresponds to greater annual cell production (Silvestro et al., 2023); the period of wood formation extends linearly with the mean annual temperature (Rossi et al., 2016). April–May temperatures yield the most accurate predictions of the timing of wood formation (Rossi et al., 2016). Chronologies of wood anatomical parameters also reflect multidecadal variability of regional climatic (Balanzategui et al., 2021).

Cell division in the vascular cambium is followed by xylem formation on the interior side, through the expansion of xylem cells (postcambial growth; PC), cell wall thickening (SW), and lignification, ultimately resulting in the production of mature cells (MT) and programmed cell death (Gričar, 2007; Schweingruber & Wimmer, 2007; Rossi et al., 2012; Fromm, 2013). The complex, plastic processes of radial tree growth are influenced by exogenous factors such as photoperiod, air temperature, precipitation, soil moisture, water potential, radiation, conditions associated with elevation, etc. For example, radial growth of trees in mountainous areas is subject to meteorological conditions associated with changes in elevation (Zhang & Hebda, 2004; Rossi et al., 2012; Gričar et al., 2014; Cabon et al., 2020; Antonova et al., 2023). Radial tree growth is also influenced by endogenous regulators such as phytohormones, tree age and vitality, and genetics (Panshin & De Zeeuw, 1980; Gričar, 2007; Schweingruber et al., 2007; Speer, 2010; Gryc et al., 2012; Fromm, 2013; Fajstavr et al., 2018). Interaction between the endogenous and environmental factors drives xylem phenology and xylem production. This interaction among multiple factors became even more complex when considering the weight of each factor and the period during the growing season (Silvestro et al., 2023). For example, every tissue and organ within a tree is subject to individual aging processes that take place at different rates. Optimal growing conditions (full light and deep, nutrient-rich, moist soils) give rise to rapid ageing of wood cells. Under these conditions, after only a relatively short time trees reach a size that exceeds their physiological and mechanical capacities (Schweingruber & Wimmer, 2007).

Montane forests are dominated by conifers (Family Pinaceae), of which Douglas fir (*Pseudotsuga menziesii* (Mirbel) Franco var. *glauca* (Mayr) Franco) is one of the dominant and most important timber species in western North America (Waring & Franklin, 1979; Howard, 2007). Douglas fir is an economically important species in Europe forests and is widely cultivated and planted (Schmid et al., 2013; Owens, 2015).

In this study we investigated cambial activity and wood formation in Douglas fir in montane forest stands in southern Alberta, Canada. This species was chosen because this variety of Douglas fir is widespread in the Rocky Mountain cordillera (Morin, 1994); and is found along a gradient of climate conditions, site conditions, and elevations (Waring & Franklin, 1979; Zhang & Hebda, 2004; Beedlow

et al., 2013). Douglas fir is considered a relatively fire-resistant species due to its large stem diameter and thick bark, which confer increased resistance to fire injury and mortality (Lachmund, 1921; Starker, 1932, 1934; Ryan et al., 1988; Hood et al., 2018). Stands have usually trees of various ages in contrast to many other boreal and montane conifers in North America. Douglas fir provides the opportunity to study the influence of elevation and meteorological conditions as well as the possibility of adaptation in trees of different ages. Previous studies suggest that the productivity, i.e. wood formation, of Douglas-fir will be lower at sites with shallow, excessively drained soils, south- and west-facing aspects, and steep slopes, and will decrease at high-elevation sites (Case & Peterson, 2005).

Hypotheses

(H1) It is assumed that the younger age group A1 will exhibit an earlier onset of cambial activity, a longer duration of cambial activity, a greater number of average cambial cells, higher productivity of xylem cells, and different timing of xylem cell production compared to older trees (A2).

(H2) Location is expected to influence cambial activity and xylem production across sites at different elevations. Locations at lower altitudes are anticipated to have an earlier onset of cambial activity, longer duration, and higher productivity of xylem cells compared to higher-altitude sites.

Materials and methods

We studied Douglas fir over the course of the growing season, 9 April–10 October 2013. We defined the beginning and end of the growing season as the date on which we first observed cambial activity and, on the date, when all wood cells were mature, respectively. Our research plots are located in montane forest in southern Alberta, Canada (Fig. 1) in an eastern spur of the Rocky Mountains where Douglas fir is the dominant coniferous tree species. This



Fig. 1. Map showing research plot locations

location has a typical continental climate with warm summers and extremely cold winters and is classified as Dfb (Warm-summer humid continental climate) by the Köppen-Geiger system (Arnfield, 2020). Mean annual air temperature is 5.7 °C and mean annual rainfall (snow converted to water equivalent) is 580 mm per year (https://en.climate-data.org/).

Research plots

We situated our research plots (Table 1) at three elevations: 1480 m a.s.l. (TC, Tennessee Creek), 1670 m a.s.l. (SR, Skyline Road), and 1820 m a.s.l. (PL, Porcupine Lookout). The distance between the Porcupine Lookout and Skyline Road plots is 2 km, and the Tennessee Creek (TC) plot is 20 km to the south (Fig. 1).

The forest in all research plots is natural, mature, closed-canopy forest dominated by the Douglas fir of different ages due to natural reproduction. Other tree species include lodgepole pine (Pinus contorta var. contorta Dougl. ex. Loudon), limber pine (Pinus flexilis E. James), trembling aspen (Populus tremuloides Michaux), engelmann spruce (Picea engelmannii Engelmann var. engelmannii), white spruce (Picea glauca (Moench) Voss). We selected plots that we judged to be unaffected by extreme factors such as open exposures with high winds or frost basins, in order to avoid stress conditions that can cause abnormalities in wood development (e.g., eccentric growth, reaction wood). Weather stations belonging to the Alberta Climate Information Service (ACIS; http:// agriculture.alberta.ca) located at Tennessee Creek and Porcupine Lookout were our source of meteorological data for these sites.

Tree selection

We selected trees that were healthy with a conical crown shape, avoiding any trees with apparent injury, reaction wood, polycormic stems, or partially dead crowns. We also chose trees that were spaced far enough apart such that they were not in evident competition with each other. We selected two sets of six trees in each plot representing two different age categories. The younger trees (A1) were 36–60 years old and the older trees (A2) were 82–103 years old (Table 1) as determined with cores from an increment borer (Pressler, 1866, 1883). Mean stem diameter at breast height (DBH) was 21.4 cm and 51.7 cm and mean tree height was 11.5 m and 19.5 m in A1 and A2 groups, respectively

Sample collecting and processing

We collected two microcores samples (one as a backup) 1.8 mm in diameter at approximately weekly intervals (5–9 days) using the Trephor handtool (Rossi et al., 2006). Samples were taken spirally around the trunk through the growing season starting at breast height and 10 cm apart (Deslauriers et al., 2003). The tissues were fixed in FAA (formalin-acetic acid-alcohol) solution immediately to interrupt metabolic processes, and after one week were moved to 30% ethanol for storing before processing in the laboratory. Samples were then dehydrated in an ethanol series before impregnating and embedding in paraffin (Rossi et al., 2006). Samples were sliced into 12 μ m thick sections using a rotary microtome (Leica Microsystems GmbH, Germany) and glued to slides using Albumin, then stained

Table 1. Research plot locations, ages of sampled trees, dendrometric characteristics (in cm)

Tennessee Creek				Skyline Road				Porcupine Lookout			
1480 m				1670 m				1820 m			
49°41'35.5"N 113°58'40.8"W				49°53'54.9"N 114°01'07.9"W				49°53'11.8"N 114°00'07.9"W			
Trees Age		Stem diameter	Trees		Age	Stem diameter	Trees		Age	Stem diameter	
A1	M1	42	22.9	A1	M1	45	25.2	A1	M1	38	16.6
	M2	50	20.4		M2	39	22.9		M2	46	21.7
	M3	47	21.0		M3	43	26.1		M3	39	19.7
	M4	54	20.7		M4	40	26.4		M4	36	17.2
	M5	55	17.5		M5	37	22.3		M5	38	19.1
	M6	45	21.3		M6	38	22.6		M6	60	22.3
	AVG	48.8	20.6		AVG	40.3	24.3		AVG	42.8	19.4
	Middle	stand age	12.5	Middle stand age 11.0 Middle		e stand age	11.5				
A2	S1	90	67.2	A2	S1	82	54.1	A2	S1	103	52.5
	S2	86	57.0		S2	85	61.5		S2	100	48.7
	S3	86	63.1		S3	91	56.7		S3	95	42.0
	S4	96	51.6		S4	90	63.1		S4	102	25.2
	S5	96	71.7		S5	82	59.6		S5	98	21.0
	S6	87	53.5		S6	82	62.7		S6	101	19.1
	AVG	90.2	60.7		AVG	85.3	59.6		AVG	99.8	34.8
Middle stand age 18.0		18.0	Middle stand age 19.5			19.5	Middle stand age			19.5	

with Safranin and Astra Blue to distinguish phases of cell differentiation before embedding in Euparal (Waldeck, Münster, Germany). The resulting permanent slides were observed using a Leica DMLS microscope with polarization and scanned with a Leica DFC 295 digital camera (Leica Microsystems GmbH, Germany).

Data processing

We assessed and counted cells of the cambial zone and wood tissue (tracheids) in the digital images. The following data were collected by examining three randomly selected radial rows:

Onset of cambial activity (CA) was determined as the date when the cambial cells (non-differentiated, unlignified, thin-walled cells) were actively dividing at least in two of the three radial rows (applies to further measurements).

At the moment when new PC phase cells were not observed, the CA was considered as finished and cambial activity duration was calculated (Gričar et al., 2005; Rossi et al., 2006). The number of cambial cells (CC) was counted in the three radial rows and averaged.

The onset of xylogenesis was the date when we could first distinguish developing tracheids. For the purposes of this study it coincides with the onset of CA.

Xylogenesis was defined as finished when all the cells had differentiated (completely matured) and the xylem growth increment was fully developed.

The phases of cell differentiation: (i) postcambial growth (PC), (ii) secondary thickening of cell walls (SW), and (iii) mature cells (MT). These phases were distinguished using staining techniques: non-lignified CC and PC cells stained blue but differ in shape and size; partially thickened SW cells exhibited both blue and red staining; and fully mature MT cells stained red (Gričar et al., 2005; Rossi et al., 2006). Tracheids at different stages of differentiation were counted in three radial rows and were averaged.

Statistical analyses

The effects of tree age and elevation on cambium productivity and wood formation were tested by two-way ANOVA. Before performing ANOVA, the normality and homogeneity of the data were assessed through residual diagnostics. These did not show serious violations of ANOVA assumptions, and the data were not transformed. Calculations were performed using STATISTICA[®] software, version 10.0 (StatSoft Inc., OK, USA), and significance was evaluated at the confidence level of 0.95 ($\alpha = 0.05$) throughout the whole study.

Gompertz growth function

The Gompertz growth function (Gričar et al., 2008) is commonly used in the study of xylogenesis to estimate the onset, duration, and productivity of cambial activity in cold climate according to the following equation:

$$\gamma = a. e^{-e^{b-k.t}}$$

- *y* the weekly cumulative number of cells,
- *t* time expressed as the day of the year,
- *a* regression coefficient (asymptote of the function),
- *b* regression coefficient (x-axis placement)
- *k* regression coefficient (rate of change)
- *e* Euler's number

Due to heteroscedasticity of data, power weights (Pinheiro & Bates, 2000) were used in calculations of the function parameters. The statistical language R (R Core Team, 2020) with nlme library (Pinheiro et al., 2020) was used for the calculations.

The cambial activity and xylogenesis data generated by the Gompertz function were compared with the selected meteorological data for the research plots, age categories of the trees, and elevations of the plots.

To describe the timing of biological processes and their potential relationship with meteorological conditions we used growing degree days (McMaster & Wilhelm, 1997). The basic equation used to determine Growing Degree Days (GDD) is if the value of GDD in the equation below is greater than 0:

$$GDD = \frac{T_{MAX} + T_{MIN}}{2} - T_{BASE}$$

- GDD growing degree day,
- T_{MAX} daily maximum air temperature,
- T_{MIN} daily minimum air temperature,
- T_{BASE} the temperature below which the process of interest does not occur; 5 °C used here.

The relationship between cell production rate and meteorological variables (Tavg, Tmin, Tmax, and sum of GDDs) was analysed with linear regression analysis. The meteorological data were averaged for each sampling period. Sums of GDD for a particular sampling period were divided by the number of days in that period. The numbers of cells produced in a particular sampling period was divided by the number of days in this period and logarithmically transformed to meet the assumptions of homoscedasticity and linearity. The adjusted coefficients of determination and the tests of significance of the slopes of the regression lines were calculated.

Results

Meteorological effects

Meteorological data were collected from the weather stations at two locations: TC (1480 m a.s.l.) and PL (1820 m a.s.l.). During the sampling year (January–October 2013) the average daily air temperature was 7 °C and 4.5 °C for TC and PL, respectively. Minimum air temperatures were -22.4 °C and



Fig. 2. Average, minimum and maximum temperature at the TC and PL weather stations



Fig. 3. Precipitation and Growing Degree Day values at the TC and PL weather stations

-24.9 °C at TC and PL, respectively. Maximum air temperature was reached at the beginning of July and was greater than 30°C at both locations. The minimum air temperature was generally above 0 °C at TC by the beginning of May and at PL by the second half of May (Fig. 2). Precipitation was well distributed and with cumulative values of 431 mm and 894 mm at TC and PL, respectively (Fig. 3). The value of GDD when cambial activity began at TC was 160 °C and 182 °C for A1 and A2, respectively. At PL GDD was lower: 123 °C and 133 °C for A1 and A2, respectively. The trees at the higher location required lower GDD for initiation of cambial activity and were more uniform within the age classes.

Cambium

Dormant cambium at the beginning of May contained 6–7 cells (CC) on average depending on location (p < 0.05). SR (mainly A2) had a higher average CC during dormancy (8 CC). However, a higher standard deviation at this site (SR) was observed (1.5). The influence of age on dormant CC was not proven (p > 0.05).

Cambial activity started DOY 143–158 (23 May– 07 June). The onset of cambial activity varied by the location (p < 0.001). The tree age did not significantly influence the start of cambial activity (p > 0.05). The cessation of cambial activity at the end of the



Fig. 4. Cambial activity duration in days according to location and age group. Boxes represent upper and lower quartiles, the whiskers show the 10th and 90th percentiles, and the median is drawn as a solid horizontal line. Different letters indicate statistically significant differences at the 0.05 level; identical letters denote non-significant differences. Location: TC (1480 m a.s.l.), SR (1670 m a.s.l.), PL (1820 m a.s.l.). Age groups: A1 (36–60 years old) and A2 (82–103 years old)

growing period was observed within one month for all trees (DOY 220–241; 08 August–29 August). The end of cambial activity was independent of tree age or location (p > 0.05).

The impact of age on the duration of cambial activity was not confirmed (p > 0.05). On the contrary, the effect of location was highly significant (p < 0.001), with the main differences at PL. Duration of cambial activity averaged 75 days (TC 81; SR 78; PL 66) and followed elevation gradient; the highest location, PL, had the shortest period of cambial activity (Fig. 4).

The average number of cambial cells (CC) during growing season was highest at SR (11 CC), followed by TC (10 CC), and lowest at PL (8 CC). The highest average standard deviation within the site was observed in age group A2. Across all sites, group A2 consistently had a higher number of CC than A1,



Fig. 5. Increment in terms of number of cells at the end of growing season 2013. Boxes represent upper and lower quartiles, whiskers show 10th and 90th percentiles, and the median is drawn as a solid horizontal line. Different letters indicate statistically significant differences at the 0.05 level; identical letters denote non-significant differences. Location: TC (1480 m a.s.l.), SR (1670 m a.s.l.), PL (1820 m a.s.l.). Age groups: A1 (36–60 years old) and A2 (82–103 years old)



Fig. 6. Duration of xylogenesis in days. Boxes represent upper and lower quartiles, whiskers show 10th and 90th percentiles, and the median is drawn as a solid horizontal line. Different letters indicate statistically significant differences at the 0.05 level; identical letters denote non-significant differences. Location: TC (1480 m a.s.l.), SR (1670 m a.s.l.), PL (1820 m a.s.l.). Age groups: A1 (36–60 years old) and A2 (82–103 years old)

though the difference was minor at PL. The maximum number of cambial cells was generally recorded in the second half of June and the very beginning of July. The maximum average number of CC ranged from 12 to 21 cells, depending on location and tree age, except at the PL site where differences between age groups were not significant. Cambial activity reflected in the number of CC throughout the growing season, was significantly influenced by location (p < 0.001), while the effect of age was not evident (Fig. 5).

Xylem increment

By the end of the growing season, trees produced 72 and 79 xylem cells per radial row on average at TC and SR, respectively. In contrast, the trees at the highest location (PL) produced about half as many cells on average (37). A statistically significant

Table 2. CC production and lengths of differentiation phases of xylem cells (D CC dormant cambial cells; AVG CC average cambial cells)

LOC	AGE	D CC (cells)	AVG CC (cells)	CC MAX (cells)	CC MAX (DOY)	Cam. Prod. (days)	Start of Cam. Prod. (DOY)	End of Cam. Prod. (DOY)	PC (days)	SW (days)	All cells in MT (DOY)
TC	A1	6.9	8.2	12.1	192	77	143	220	77	62	228
TC	A2	6.2	10.9	15.6	178	93	148	241	93	81	250
SR	A1	6.4	9.8	16.1	163	79	147	226	79	69	240
SR	A2	8.4	12.5	20.7	170	80	153	233	80	66	244
PL	A1	6.1	8.1	14.6	177	63	157	220	63	54	232
PL	A2	6.4	8.2	13.0	170	69	158	227	69	63	235

(p < 0.001) influence of location on the final wood cell increment was determined, with PL differing significantly from the other two sites based on a Tukey multiple comparison test. However, the effect of age was not significant (Fig. 5). The maximum average weekly increment first occurred in A1 at all sites (DOY 177–192; 26 June–11 July), followed by A2 (DOY 213–226; 01–14 August). Generally, the average increment was higher in group A1, except at PL where both age groups showed similar increments. During the period from late June to mid-July, the maximum daily increment of new xylem cells was observed, ranging from 0.43 to 1.0 cell/day. Presented results are based on the Gompertz function.

Xylem phases

The PC phase generally began earlier in the A1 age group, though the differences were minimal (not more than one week). Results indicate that cells remained in the PC phase longer (77 days on average) than in the SW phase (66 days). At the TC and PL locations, group A2 required a longer time to complete both the PC and SW phases, with the most pronounced difference observed at TC, where A2 took over two weeks longer than A1. In contrast, the difference between age groups was only a few days at SR and PL.

The differences at full cell maturity (MT phase) were statistically significant by location (p < 0.05) and tree age (p < 0.01). The time when all cells reached maturity (MT phase) was consistently earlier in the A1 group. The largest difference between A1 and A2 occurred at the TC location (22 days). But across all sites, group A2 reached the MT phase later. However, the difference between age groups was generally less than one week. On average, the time required to progress from PC to MT was 87 days, with the shortest duration recorded at PL (83 days).

The initiation of the PC phase was significantly influenced by location (p < 0.001); while tree age had no effect (p > 0.05). PC occurred earliest at TC and SR (low and middle elevations) and approximately one week later at PL (the highest elevation). However, for the subsequent differentiation phases (SW, MT) the effect of age was significant (p < 0.001).

Gompertz Function

The maximum production rate of xylem cells occurred between DOY 179-190 (end of June-first half of July 2013) based on Gompertz function (Fig. 7). The maximum number of cells produced per day was 0.63-1.47. The lowest value of the maximal increment was detected at PL in A2. In contrast, the highest value was at SR in A1. The average daily increment varied between 0.43-1.0 cells per day (Table 3). These results followed the same pattern as the maximum increment, with the lowest average daily increment in PL (A2) and the highest in SR (A1). On average xylogenesis lasted 93 days (Fig. 6), with the longest duration recorded at TC (113 days) and the shortest, 78 days, at the highest elevation (PL). The difference in the xylogenesis duration between these two locations exceeded one month. Cambial activity ceased earliest at the highest elevation (PL), though its timing closely matched SR, while TC continued cambial productivity approximately 20 days longer. All results based on the Gompertz function indicate that xylogenesis duration was independent of tree age and location (p > 0.05).



Fig. 7. Gompertz function results according to location and age group

Table 3. Cambial productivity and xylogenesis duration based on the Gompertz function

		-	=		
LOC	AGE	Max. Prod. (DOY)	Dur. Xyl. (days)	End of CP (DOY)	Growth Rate (cell/day)
TC	A1	181	112	257	0.59
TC	A2	187	113	267	0.79
SR	A1	177	92	239	0.83
SR	A2	182	83	238	1.00
PL	A1	179	74	229	0.54
PL	A2	190	82	241	0.43

Linear regression model

The linear regression model found a positive correlation of cell formation rate with independent variables GDD, T_{min} , T_{max} , and T_{avg} . This dependence was more significant for age group A2 than A1. For A2 the model explained 32–40% of variability of the data with a level of significance p < 0.01. For A2, p values ≤ 0.06 , and R²adj fluctuated between 16% and 23%. R²adj was highest with T_{max} and T_{avg} (Fig. 8), those variables had the strongest influence on the cell formation rate.



Fig. 8. Linear regression models

Discussion

Studying cambial activity in mountain environments reveals the responds of cambial zone to shifting weather patterns driven by accelerating climate change. Şevgin and Öztürk (2024) denote altitude as the key factor influencing warming rates, with elevation and terrain variability playing a greater role than latitude. Pepin et al. (2022) describe mountains as climate "hotspots", where complex topography and elevation gradients amplify or precede broader climate trends, leading to significant environmental impacts. This is especially relevant for Douglas

Table 4. Coefficients of determination, p-values

fir, which has the widest altitudinal and latitudinal range of any commercial conifer in western North America and is widely used as an introduced timber species in Europe.

Despite the initial hypothesis that tree age would influence cambial phenology, age did not have a significant impact. Although other authors have reported differences in the timing of cambial activity related to age (Rossi et al., 2007b; Li et al., 2012),

At the beginning of May dormant cambium consisted of 6-7 cells on average depending on the location. The results are confirmed by other authors (Rossi et al., 2007a; Rossi et al., 2007b; Hacura et al., 2014; Cocozza et al., 2016; Hacurová et al., 2020). The average number of CC at the TC site was higher (10 CC) compared to the PL site (8 CC). Cambial activity at TC started earlier and with a difference of approximately two weeks. Similar effects of elevation on the timing of cambial activity onset have been reported by other authors (Gričar & Čufar, 2008; Gričar et al., 2014; Rossi et al., 2014; Cocozza et al., 2016; Buttò et al., 2019; Antonova et al., 2023). The average duration of cambial activity was 75 days (TC 81, SR 78, PL 66), following an elevation gradient. The cambial activity at TC was prolonged (about three weeks). The effect location was highly significant (p < 0.001), with the main differences at PL. Useros et al. (2017) confirmed that, as a rule, increasing elevation results in a delayed onset, earlier cessation, and shorter duration of cambial activity.

Trees at SR (mainly A2) exhibited the most productive cambial zone, featuring the highest number of dormant CC (7.4) and an average of 11 CC during the growing season. Consequently, trees at SR produced the largest xylem increments by the end of the growing season, which aligns with expectations given their highly productive cambial zone. The results suggested several relevant factors, such as weather conditions, soil properties, soil moisture, incident radiation, rooting characteristics based on the type of subsoil, genetic disposition, and others (Panshin & De Zeeuw, 1980; Gričar, 2007; Speer, 2010; Gryc et al., 2012). However, due to a lack of data, we could not establish them definitively.

The threshold temperature for initiation of cambial activity was lower at the higher PL site (9.6 $^{\circ}$ C and 7.8 $^{\circ}$ C on average for TC and PL, respectively). Deslauriers et al. (2008) and Antonova et al. (2023)

LOC	ACE	T_{min}		T _{max}		T _{avg}		GDD	
LOC	AGE	R² adj.	p-value	R² adj.	p-value	R² adj.	p-value	R² adj.	p-value
TC	A1	0.23	0.02	0.21	0.02	0.23	0.01	0.21	0.02
	A2	0.33	< 0.01	0.38	< 0.01	0.37	< 0.01	0.36	< 0.01
PL	A1	0.16	0.05	0.23	0.02	0.23	0.02	0.14	0.06
	A2	0.34	< 0.01	0.40	< 0.01	0.40	< 0.01	0.32	< 0.01

both estimated the similar threshold value (8 °C) for CA initiation at higher elevations. Temperature variation is the key factor influencing phenology and cambial productivity at high elevations (Rossi et al., 2007b; Gričar et al., 2014). Higher-elevation sites require less heat accumulation to initiate cambial activity (Deslauriers & Morin, 2005). This phenomenon is caused by high variability and plasticity of cambial phenology in relation to elevation (Cocozza et al., 2016). Gričar et al. (2014) suggest that trees at the highest elevation site are adapted to initiate cambial activity at lower temperatures, thereby adjusting to the conditions imposed by elevation.

According to our hypotheses the extent of wood formation (number of tracheid cells produced in radial rows) significantly depended on elevation. Xylogenesis began earlier and lasted longer along an elevation gradient from lower to higher sites, which resulted in greater cell production. At lower locations, nearly twice as many wood cells were formed than at the highest locations. Similar results described Lupi et al. (2013), Cocazza et al. (2016) and Silvestro et al. (2023). Similar to cambial activity, trees can adjust the timing of xylogenesis to compensate for climatic constraints, enhancing their survival (Castillo et al., 2018).

The location (and connected characteristics) strongly influenced the onset of the PC phase, while tree age had no effect. However, tree age significantly affected the start of the subsequent differentiation phases (SW and MT), with younger trees beginning these phases earlier. Air temperature, linked to elevation, was the main factor initiating cell differentiation, but tree age influenced the later phases. According to Rossi et al. (2014), the relationship between temperature and most phenological phases of xylogenesis is linear.

According to the linear regression model, the cell formation rate increased with higher values of GDD, Tmin, Tmax, and Tavg, and this relationship was stronger for age group A2 than for group A1. This suggests that trees in group A2 responded more sensitively to changes in these factors than trees in group A1. Tmax (maximum temperature) and Tavg (average temperature) had the greatest impact on cell formation. While temperature alone explains the onset of tracheid production, water potential appears to be necessary for predicting both the cessation and the total annual production of tracheids (Cabon et al., 2020). It is necessary to consider other factors influencing the course of xylogenesis, such as the length of the growing season or precipitation (Zhang & Hebda, 2004). He et al. (2019) found that even 81% of radial growth is driven by soil moisture.

Conclusions

In this study, the elevation and its associated weather conditions (especially temperature), played a crucial role in cambial phenology. Although the tree age did not significantly affect the timing of cambial activity and the initial stage (PC) of wood cell development.

Trees showed adaptability in cambial activity and xylogenesis timing to compensate for climatic constraints, enhancing survival. At higher elevations, a lower threshold temperature was required to reactivate the cambium. Cambial activity also lasted longer at lower elevations. Prolonged cambial activity necessarily led to greater productivity and a resulting greater increase in xylem. The trees at higher elevations produced wood increments that were 50% smaller compared to those in lower locations, corresponding to the weak growth rate at this location.

The cell formation rate was positively correlated with temperature-related variables, with a stronger response in older trees. The age also became evident in the subsequent phases of xylogenesis (SW and MT).

It is encouraging to assume that trees can adapt to rising temperatures and other climatic changes. However, it is also essential to consider precipitation patterns and extreme weather events such as spring frosts, summer droughts, severe storms, and strong winds associated with climate change. Additionally, other factors influencing cambial processes, such as photoperiod, will remain unchanged and may moderate the impacts of temperature changes. Temperature changes will also affect the life cycle of tree pests, and air pollution, as well as other factors will play a role. Rising temperatures could also shift the optimal growth zones of trees to higher elevations.

The results are promising, but further research and an extended time series of observations are needed to strengthen the robustness of the findings and enhance their explanatory value.

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