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Effect of different stand densities on xylem and phloem formation in Norway spruce plantations

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Summary – Preliminary results of an investigation of the thinning effect on Norway spruce tree growth in terms of xylogenesis and phloemogenesis are presented. Three plots were selected where the stand densities were reduced by pre-commercial thinning to 1800 trees/ha (plot A; mild thinning) and 1300 trees/ha (plot C; heavy thinning) in February 2020. Plot B had no silvicultural intervention and represented a control variant (4500 trees/ha). Three dominant 14-year-old Norway spruce trees were sampled (micro-cored) for studying the xylem and phloem formation in the 2020 growing season. The total differentiation duration was determined to be around 169.7 ± 12.7–179.3 ± 4.0 days. The average number of xylem cells formed in control plot B was only 140 mature cells, plots C and A were determined to have 175 and 200 mature cells, respectively. Plot A had the fastest growth rate of all the plots studied. In all three plots (A, B, C), the beginning of the early phloem, late phloem sieve cells, and axial parenchyma cells coincided. Nonetheless, in terms of total phloem cell increase, plot C displayed the fastest growth rate among the three plots studied. The first results showed that the rate of total increment in both xylem and phloem cells, as well as the total number of produced tracheids and sieve cells, seem to be positively affected after the thinning application.

Keywords – phenological phases, phloem, *Picea abies* L. Karst., productivity, thinning intensity, xylem.

Introduction

Long-term and short-term anthropogenic climate changes are expected to be highly variable, having less or more severe impacts on the geographical shifts, habitat diversity, and vitality of many plant species (Villén-Peréz *et al.* 2020). The study of xylogenesis is an insightful approach to understanding mechanisms underlying growth patterns since it is controlled by several abiotic and biotic factors (Antonova & Stasova 1993; Vaganov *et al.* 2006). Monitoring seasonal cambial activity and intra-annual wood formation growth have been valuable tools to interpret direct relationships between tree-ring characteristics and climatic parameters (Seo *et al.* 2008), which allow us to develop scenarios on how and to which extent climate change will modulate plant phenology and morphology, species distribution and forest vegetation dynamics (Ziaco *et al.* 2016). Secondary-growth wood formation is regulated by a complex, dynamic mechanism controlled by several intrinsic factors combined with environmental ones (Battipaglia *et al.* 2014). Environmental factors, such as air temperature and precipitation or extreme weather events, such as drought,

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impact the rate and duration of cambial cell production and influence radial tree-ring growth (Cocozza *et al.* 2016). Thus, climate change will likely induce a plastic adaptive response in trees and is expected to influence wood formation production, and subsequently tree-ring growth and ultimately forest productivity (Güney *et al.* 2015).

The duration and rate of timing intervals through each cell differentiation phase determine the specific tree-ring anatomical characteristics of wood and, consequently, the physical and mechanical performance of timber (Rossi *et al.* 2006a). A warmer spring may induce an earlier onset of xylogenesis and a prolongated growing period (Deslauriers *et al.* 2008; Lupi *et al.* 2010). In contrast, spring and summer droughts or low precipitation could also play a major role in the onset of xylogenesis and wood cell production (Ren *et al.* 2015). Furthermore, a rapid decrease in air temperature during summer could be a critical factor in the cessation of cambial activity and latewood formation (Begum *et al.* 2018).

However, comprehending the wood formation processes and relationships with environmental factors is timely research that remains hardly understood (Pasho *et al.* 2012). Most xylogenesis investigation has not adequately elaborated on how the interaction between tree competition and microclimate might strongly influence wood development in closed forests (Deslauriers *et al.* 2008; Gruber *et al.* 2009; Primicia *et al.* 2013). Under this prism, alterations in inter-annual tree growth sensitivity to climate because of different competition levels have been reported for hardwoods (*Fagus sylvatica*) as well as softwoods (*Pinus nigra*) (Cescatti & Piutti 1998; Martín-Benito *et al.* 2010). Moreover, different canopy types affecting microclimate are reported to modulate xylogenesis (Primicia *et al.* 2013).

Thinning treatment is one of the most worldwide practices to manage forest stands (Oliver & Larson 1990). Over the years of practicing, a lot of general knowledge has been obtained about the effects of thinning on tree growth in monospecific stands (Primicia *et al.* 2013). Forest managers strongly count on using certain silvicultural treatments, which lead to manipulating the structure and dynamics of a forest stand to modify responses to climate change. Nevertheless, the impact of stand density on individual tree response to climate and drought is not a simply addressed question, while several approaches such as ecophysiology, dendroecology, and tree growth, might offer multiple insights (Misson *et al.* 2003; Moreno & Cubera 2008; Olivar *et al.* 2014, Giagli *et al.* 2019).

For the Czech Republic, it is predicted that the mean temperatures will rise between 1.0 and to 2.7°C during the summer and winter seasons by the year 2050 (Ministry of Environment of the Czech Republic 2015; Geletič *et al.* 2019). Climate change effects in the Czech Republic are considered to evoke significant weather events indicated by more common extreme events, such as frequent and rapid rains, longer droughts, heat waves, and warmer and wetter winters with fewer amounts of total precipitation (Ministry of Environment of the Czech Republic 2015, Rulfová *et al.* 2017). Norway spruce (*Picea abies* L. Karst.) is the most represented and valuably traded timber species in the Czech Republic, becoming more vulnerable to mortality as a consequence of its sensitivity to drought (Kučera *et al.* 2016). Gričar *et al.* (2014) demonstrated that cambial cell production in Norway spruce is highly variable and plastic over the years, depending on both phenotypic plasticity and local adaptation to environmental (seasonal air temperature and precipitation) conditions.

Understanding the seasonal and intra-annual wood formation dynamics of Norway spruce is crucial, to unraveling its growth response to the present and forthcoming local climate conditions (Hacurová *et al.* 2020). Nevertheless, to our knowledge, there is no information about how competition may modify the response of xylogenesis to microclimatic variation in Norway spruce forests at their low ecological valence border. The lack of respective data is unjustifiable for areas experiencing water deficit due to climate change, where thinning may be an appropriate management tool to counteract the negative effects of a warming-induced reduction in water availability (Linares *et al.* 2009; Primicia *et al.* 2013).

Respective studies have predicted that the response of wood formation will be influenced by forest composition and structure, which are profoundly affected by the forest management technique used over its lifespan (or rotation period). There is, however, a dearth of knowledge on how and to what degree forest management might help mitigate the effects of climate change on forest ecosystems. (Bosela *et al.* 2016). This study shows the preliminary results of a

project that attempts to bridge the gaps by investigating the thinning effect on rates of radial increment and tracheid number of the 14-year-old Norway spruce trees growing in three differently managed plots in the East-Bohemian Forest stand.

Materials and methods

Study site

In 2020, the presented study was conducted at the Křivina site, Czech Republic (50.2152100 N; 16.1140817 E) in 14-year-old small-pole pure Norway spruce stands. The site is located 402 m above sea level. It is characterised by 10.4°C and 606.3 mm of mean annual air temperatures and precipitation for the 2018–2020 period. The soil is cambisol, and the site typologically represents *Querceto-Fagetum illimerosum trophicum* (Viewegh *et al.* 2003). Daily air temperatures and precipitations were continuously measured during the observed growing season in the sufficiently extensive clearing located no more than 200 m away from all studied research plots using an EMS 33H sensor (EMS, CR) and two Pronamic Pro (EMS, CR) precipitation gauges, respectively (Fig. 1).

At the site, three research plots (40 × 65 m) with an initial stand density of 4500 trees/ha were established in 2018, and all trees in the plots are annually inventoried (DBH, H). In February 2020, stand densities were reduced by pre-commercial thinning to 1800 trees/ha (plot A; mild thinning) and 1300 trees/ha (plot C; heavy thinning). Plot B had no silvicultural intervention and represented a control variant with 4500 trees/ha (Table 1). Nine trees were selected (3 per plot) for the primary investigation of the thinning effect on the xylem and phloem growth in the same year (2020).

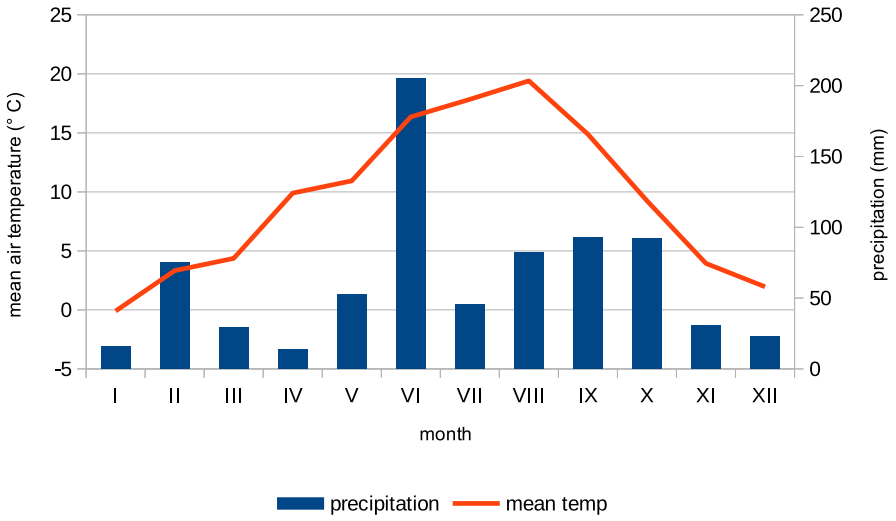

Fig. 1. Average monthly air temperature and monthly precipitation.

Table 1. Descriptive dendrometric characteristics of the studied Norway spruce small-pole stands.

Variant	A	B	C
Age (years)	14	14	14
Stand density (trees/ha)	1800	4500	1300
DBH (cm)	10.40 ± 2.15	8.46 ± 2.52	10.01 ± 1.83
H (m)	8.55 ± 1.11	8.33 ± 1.13	8.34 ± 1.04

Sample collection and preparation

Three dominant and healthy 14-year-old Norway spruce trees were selected per plot (A, B, C) in the year 2020 for monitoring (9 trees in total). Wood microcores with a thickness of 1.8 mm were taken circumferentially from the breast height of the stem (1.3 m above ground) at weekly intervals (mid-March to mid-November). Wood microcores were collected with the Trephor tool (Rossi *et al.* 2006b). Each microcore contained phloem (non-collapsed and collapsed), vascular cambium, and at least two years of the last-formed xylem growth rings. The spacing between two neighbouring microcores was set at 2 cm to prevent traumatic tissue formation in the samples (Vavrčík *et al.* 2013). Immediately after extraction from the trees, the microcores were immersed in formalin-alcohol-acetic acid (FAA) solution for a week and later in ethanol-water (30%, w/w) solutions. Wood microcores' processing for microscopic analysis was performed according to Prislan *et al.* (2014) with the following steps: (i) microcore preparation, marking of transverse plane, (ii) dehydration in ethanol series (70, 90, 95 and 100%) and embedded in paraffin by tissue processor (Leica TP1020), (iii) moulding of microcores with paraffin blocks (Leica EG1120 paraffin dispenser), (iv) cutting of transverse sections (8–12 μm) with a rotary microtome (Leica RM2235), (v) drying in the laboratory oven (70°C for 20 min), (vi) removal of paraffin content (BioClear — 2 cycles of 20 min each), (vii) staining with safranin (0.04%) and Astra Blue (0.15%) and (viii) preparing permanent slides (Euparal mounting medium). The radial increment of the xylem and phloem was measured in 3 radial cell rows per growth ring with a Leica DM 2000 microscope, connected with a Leica DFC 295 digital camera (Leica Microsystems) and the public-domain image processing program ImageJ (Abramoff *et al.* 2005).

Measurements and data processing

The timings of the phenological phases (Fig. 2) of Norway spruce trees' xylem production during one growing season were recorded in days of the year (DOYs), as well as total duration (days) based on: the onset and cessation of cambial cells (CC) activity, the onset and cessation of cell enlargement (PC), the onset and cessation of secondary cell wall thickening with subsequent lignification (SW), the occurrence of the first fully matured cells (MT), (Rossi *et al.* 2006a, 2009; Deslauriers *et al.* 2008; Gryc *et al.* 2011; Vavrčík *et al.* 2013, Gričar *et al.* 2014; Dickson *et al.* 2017; Nanayakkara et al. 2019).

The CC and newly formed xylem and phloem cells were counted and averaged along three radial files on transverse sections (Deslauriers *et al.* 2008). The CC (Fig. 2A) was defined as cells with almost equal small radial diameters and thin tangential walls (Dickson *et al.* 2017). The cambial zone included the cambial initials as well as their mother and daughter cells. The apparent beginning of radial diameter expansion to radial widths at least twice that of the cambial cells (Rossi *et al.* 2009) was attributed to the PC cells (Fig. 2A). During cell enlargement, the tracheids have a protoplast that is still surrounded by a thin primary wall but have a much greater radial diameter than a CC. The lowest radial width stretches across the expanding zone to the point where cell wall thickening begins to grow from the minimal value designated at the start of the cell enlargement zone (Nanayakkara *et al.* 2019). CC and cells undergoing radial expansion (astra blue) revealed only primary walls, which were dark under polarized light, unlike secondary walls.

The rounding of the cell corners and a colour shift of the cell wall from light blue to red marked the last increase in radial cell diameter and the start of secondary wall production with subsequent lignification (SW) according to Vavrčík *et al.* (2013). The red colour was brought on by safranin staining (Fig. 2B). The MT (Fig. 2C) was identified by the continuous visible lack of tracheid cell contents (Treml *et al.* 2015; Dickson *et al.* 2017). This stage was associated with lumina lacking protoplasmic material, revealing the completion of lignification and cell death as mature tracheid features. At this point, xylem formation appeared to be complete. Cell divisions were completed and cambial activity was thought to have terminated when no more radially enlarging cells were detected. When the last produced cells finished forming their secondary walls, xylem formation was complete. The red staining of all the wall layers distinguished the MT cells (Vavrčík *et al.* 2013).

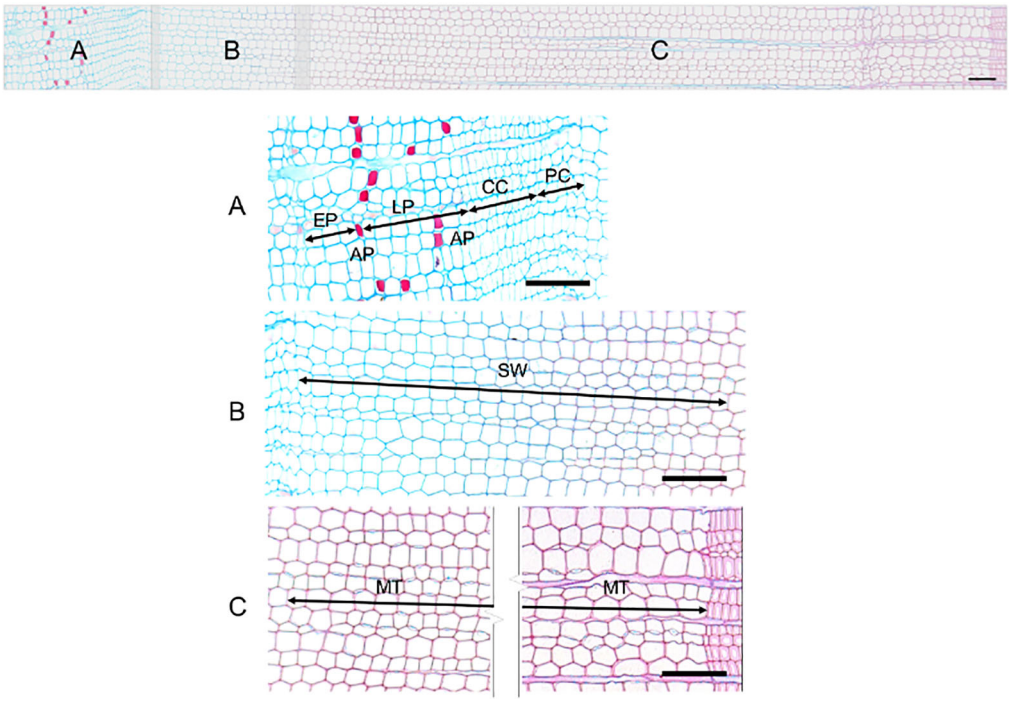

Fig. 2. Structure of xylem and phloem of a completely formed growth ring in 2020 in *Picea abies* L. Karst on the transverse section. A, Cambium and first formed xylem cells in post cambial growth; phloem sieve cells and axial parenchyma cells; B, secondary cell wall thickening with subsequent lignification; C, mature tracheids; CC, cambial cells; PC, tracheids in the phase of post cambial growth; SW, synthesis of secondary cell wall and lignification; MT, mature tracheids; EP, early phloem sieve cells; LP, late phloem sieve cells; AP, axial parenchyma cells. Scale bar A–C = 100 μm.

Respectively the formation of the early phloem sieve cells (EP), the late phloem sieve cells (LP), and the axial parenchyma cells (AP) were monitored (Fajstavr *et al.* 2020). The mean values of terminal LP sieve cells (i.e., the last tangential row of cells next to the cambium) and initial EP sieve cells (i.e., the first tangential row of cells at the phloem growth ring border) were computed. The emergence of the first AP cells dividing the two tissues marked the shift from EP to LP (Gricar *et al.* 2015). The distinguished border between EP and LP sieve cells (Fig. 2A) was determined by the layer of AP (red-filled cell lumens as dyed by safranin solution).

The dynamics of xylem formation were analysed by the Gompertz function (Rossi *et al.* 2003; Hacurová *et al.* 2020) according to Eq. (1):

$$y = A \cdot \mathrm{e}^{-e^{B-k\cdot t}} \tag{1}$$

where y is the weekly cumulative cells, t is the day of the year, A the upper asymptote, representing the maximum number of cells, B is the place on the x-axis, estimating the beginning of the cambial activity, and k is the inflection point on the curve.

The approximate date of entrance and the number of days spent in the PC and SW differentiation phases per consecutive tracheid formed during the respective growing season were calculated according to Wodzicki (1971). The methodology is based on differences between (1) total cell number (radial elongation + wall thickening + mature cells), (2) wall thickening + mature cells, and (3) mature cells at a 7-day sampling interval (Deslauriers *et al.* 2003). To calculate the duration (days) of the PC and SW phase for each tracheid, the WCDcalc R-script (Vavrčík & Gryc 2011) based on Wodzicki's algorithm (Wodzicki 1971) was used.

As ANOVA assumptions appeared to be violated in data samples Kruskal-Wallis one-way analysis of variance was used as a non-parametric method to analyse the differences among means. *Post hoc* testing Dunn's test was used to assess differences among groups.

Results

Xylogenesis and phloemogenesis timings

The beginning of the PC phases in the plots occurred between DOY 121.3 ± 4.0 and 126.0 ± 0.0, and the beginning of the SW phase was recorded almost ten days later (DOY 130.7 ± 4.0–137.7 ± 4.0, and the first mature cells were formed about two weeks later (DOY 144.7 ± 8.1–154.0 ± 7.0. The PC phase lasted approximately 160.3 ± 11.2–165.0 ± 4.4 days; 21.9 days in total, as well as the SW phase 158.0 ± 8.7–167.7 ± 4.0 days, while the whole differentiation period was calculated at around 169.7 ± 12.7–179.3 ± 4.0 days.

The pre-commercial thinning performed intensively in plot C and with mild intensity in plot A showed no influence on the timings of the phenological phase of xylem formation neither between them nor in comparison with the untreated plot B (control trees) (Table 2). The beginning of the PC, SW, and MT, as well as the ending of the PC and SW, in all 9 studied trees, seem to co-occur without any apparent effect from the thining or other factor. The Kruskal–Wallis test analysis showed no significant differences in the duration of the phenological phases of xylem formation in the three examined plots.

Similar behavior was noticed in the timings of the phloem formation (Table 3). According to our findings, the beginning DOY of the EP, LP and AP coincided in all three plots examined (A, B, C). Only a difference of approximately 20 days was observed at the beginning of the LP between the two treated plots, where the first LP was formed earlier in the Norway spruce trees growing in the heavily thinned plot C. However, the control trees (plot B) started to form the first LP in the meantime, maintaining a 10-day difference in timing from both treated plots (C and A).

Examining the timing lag occurrence between the xylogenesis and phloemogenesis phases (Table 4) in all three plots, there is no difference in the case of EP-PC between the treated plots and the control one. Referring to the AP-SW,

Table 2. Timing (DOY) and duration (days) of xylogenesis phases in the three plots examined.

Stand	Beginning (DOY)			Ending (DOY)		Duration (days)		
	PC	SW	MT	PC	SW	PC	SW	Differentiation
A1	126	133	147	289	303	163	170	177
A2	126	140	154	296	303	170	163	177
A3	119	133	147	281	303	162	170	184
A mean ± SD	123.7 ± 4.1	135.3 ± 4.0	149.3 ± 4.0	288.7 ± 7.5	303.0 ± 0.0	165.0 ± 4.4	167.7 ± 4.0	179.3 ± 4.0
B1	126	140	161	296	303	170	163	177
B2	126	133	147	274	281	148	148	155
B3	126	140	154	289	303	163	163	177
B mean ± SD	126.0 ± 0.0	137.7 ± 4.0	154.0 ± 7.0	286.3 ± 11.2	295.7 ± 12.7	160.3 ± 11.2	158.0 ± 8.7	169.7 ± 12.7
C1	126	133	154	267	274	141	141	148
C2	119	133	140	303	310	184	177	191
C3	119	126	140	289	303	170	177	184
C mean ± SD	121.3 ± 4.0	130.7 ± 4.0	144.7 ± 8.1	286.3 ± 18.1	295.7 ± 19.1	165.0 ± 21.9	165.0 ± 20.8	174.3 ± 23.1

PC, cell enlargement phase; SW, secondary wall formation phase; MT, maturation phase; plot C, heavy thinning; plot A, mild thinning; plot B, untreated control.

Table 3. Timing (DOY) of phloemogenesis in the three plots examined.

Stand	Beginning		
	EP	AP	LP
A1	105	126	161
A2	105	133	147
A3	84	126	154
A mean ± SD	98.0 ± 12.1	128.3 ± 4.0	154.0 ± 7.0
B1	98	133	154
B2	92	133	140
B3	98	140	147
B mean ± SD	96.0 ± 3.5	135.3 ± 4.0	147.0 ± 7.0
C1	105	126	133
C2	84	126	140
C3	92	119	133
C mean ± SD	93.7 ± 10.6	123.7 ± 4.0	135.3 ± 4.0

EP, early phloem; AP, axial parenchyma; LP, late phloem; plot C, heavy thinning; plot A, mild thinning; plot B, untreated control.

Table 4. Timing lag (days) between phloemogenesis and xylogenesis phases in the three plots examined.

Stand	Timing lag		
	EP–PC	AP–SW	LP–MT
A1	−21	−7	14
A2	−21	−7	−7
A3	−35	−7	7
A mean ± SD	−25.7 ± 8.1	−7.0 ± 0.0	4.7 ± 10.7
B1	−28	−7	−7
B2	−34	0	−7
B3	−28	0	−7
B mean ± SD	−30.0 ± 3.5	−2.3 ± 4.0	−7.0 ± 0.0
C1	−21	−7	−21
C2	−35	−7	0
C3	−27	−7	−7
C mean ± SD	−27.7 ± 7.0	−7.0 ± 0.0	−9.3 ± 10.7

EP, early phloem; AP, axial parenchyma; LP, late phloem; PC, cell enlargement phase; SW, secondary wall formation phase; MT, maturation phase; plot C, heavy thinning; plot A, mild thinning; plot B, untreated control.

there is a difference noticed between the treated plots (A: −7.0, C: −0.7) and the control plot (B: −2.3). Furthermore, plot C demonstrated the highest difference (C: −9.3) when it comes to the LP-MT timing lag.

Xylogenesis and phloemogenesis number of produced cells

In both treated plots (plot C: heavy thinning, plot A: mild thinning), the production of the xylem cells seems to increase dramatically compared to control plot B (Figs 3 and 4). While the number of the produced xylem cells in the control plot B hardly reached 140 mature cells on average, plots C and A were calculated, on average, 175 and 200 mature cells, respectively.

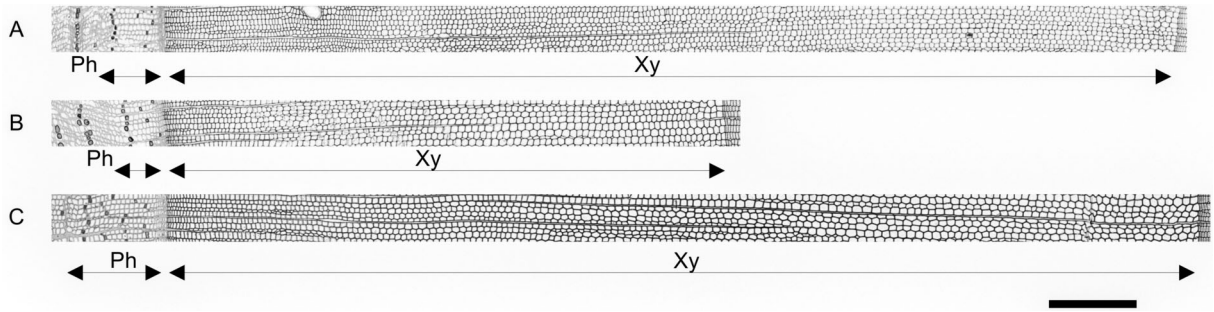

Fig. 3. Final tree growth rings — phloem (Ph) and xylem (Xy) increments in the plots examined (A, B and C) as viewed in the transverse section. Scale bar = 500 μm.

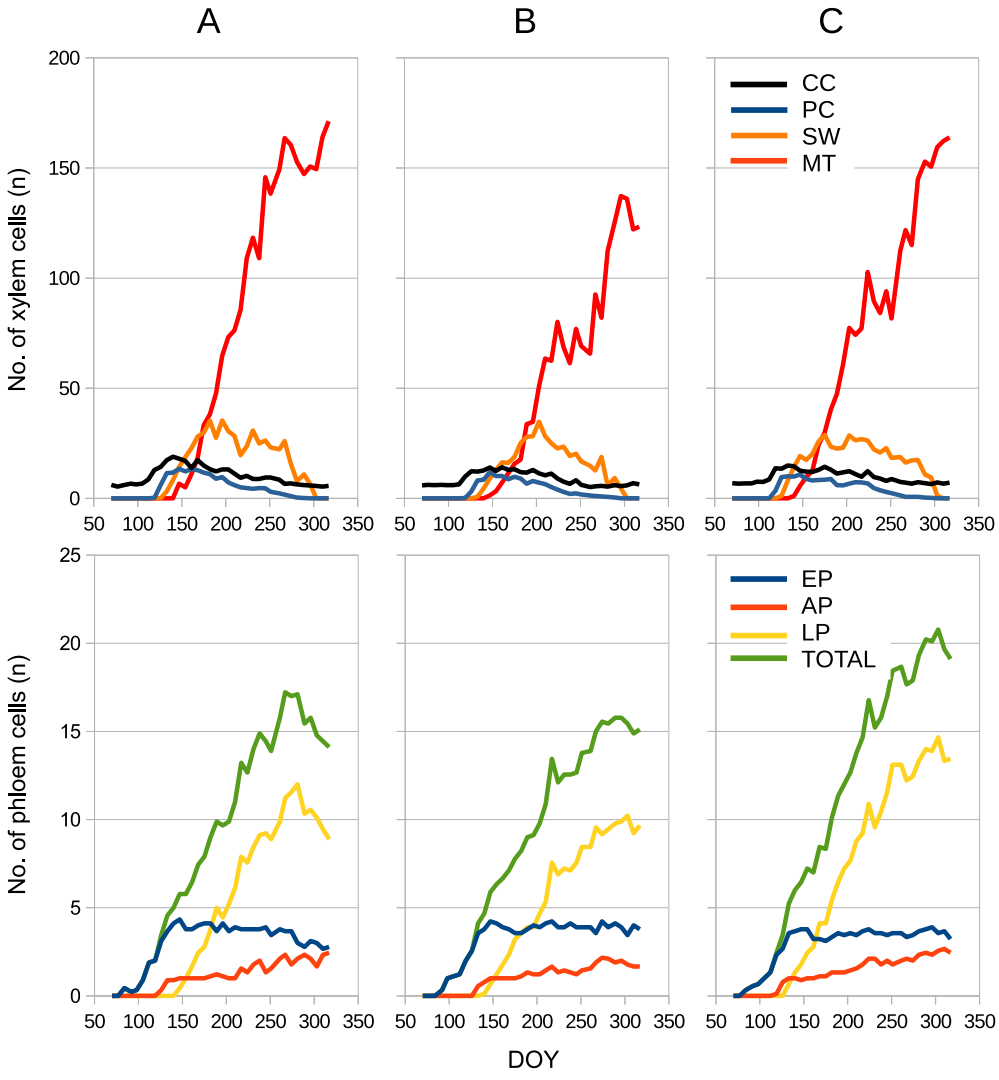

Fig. 4. The number of xylem- and phloem-produced cells in the three treated plots examined (plot C, heavy thinning; plot A, mild thinning) and the untreated plot (plot B, control).

In the same frame, the number of phloem cells produced in the same growing period revealed a noticeable increase of sieve cells (more than 20 on average) in the Norway spruce trees growing in plot C where the thinning treatment was the most intensive. Inversely, the average number of phloem cells produced in plot B (control) in the respective growing period hardly exceeded 15 sieve cells.

Duration of PC and SW phases per tracheid

Radial enlargement of the tracheids (Fig. 5, Table 5) was relatively uniform among all three plots examined, i.e., the treated plots (plot C: heavy thinning, plot A: mild thinning) and the untreated plot (plot B: control). The overall growing season of 2020 may almost be the same length (comparing the beginning and the end) but of variable progressions in the cell division rates and cell wall thickening periods. Kruskal-Wallis and *post hoc* Dunn's tests (Table 6) proved a statistically significant difference in the mean values found between A-B plots and C-B plots ($\alpha = 0.05$). Plot B showed a higher mean value of the duration of both PC and SW phases per tracheid. Namely, the control plot (plot B) showed a higher mean value of the duration of both PC (7.67 days) and SW (23.90 days) phases per tracheid, while the total number of formed tracheids was lower (plot A: 171.2; plot B: 123.3; plot C: 163.9).

Rates of xylem and phloem radial increment

Both treated plots (plot C, heavy thinning; plot A, mild thinning) demonstrated higher total increment rates of xylem cells in comparison with the untreated control plot (plot B), where the control trees performed less strongly (Fig. 6). Based on our findings, plot A showed the highest growth rate of all the plots examined. Nevertheless, referring to the total increment of the phloem cells, plot C demonstrated the highest growth rate of the three plots examined.

Discussion

Trees' radial growth response to climate change becomes more complicated, apart from the regional climatic variations, due to the forest ecologies parameters, such as forest management practices, topography, or tree competitions (Treml *et al.* 2012). A few studies have directly investigated the effects of modified or different stand structures or canopy positions on climate–growth relationships (Carnwath *et al.* 2012; Steckel *et al.* 2020), and even less especially in pure Norway spruce stands (Černý *et al.* 2020). In this study, CCP began in early May and lasted through the end of August in line with previous research on Norway spruce trees growing in the area (Gričar *et al.* 2015; Giagli *et al.* 2016). Our results from the examined 14-year-old Norway spruce trees showed no differences in the timings of the xylogenesis and phloemogenesis, demonstrating no effect of the thinning treatments on the occurrence of the phenological phases (DOYs and duration). Lemay *et al.* (2017), investigating mature even-aged black spruce and balsam fir trees, commented that the intensity of the thinning was sufficient to increase the light availability significantly for remaining trees but not enough for modifying soil temperature and water content to the extent that would drastically affect the timings or durations of xylogenesis. Also, an absence of a significant increase in radial growth after thinning has been recorded as well, mainly after mild thinning from below (Martín-Benito *et al.* 2010). On the other hand, in this study, we found that the number of the produced cells (xylem and phloem), as well as the growth rates of the total increment, were impacted favorably by rising in the intensively thinned plots A and C. This is in line with other examined wood species showing that thinning often leads to higher rates of radial increment and/or tracheid number (Mörling 2002; Jaakkola *et al.* 2005; Corcuera *et al.* 2006; Linares *et al.* 2009; Primicia *et al.* 2013). Olivar *et al.* (2014) reported that growth rates before thinning proved to be not significantly different among the experimental units, suggesting that the thinning intensities were sufficient to cause differences in diameter growth rates. Lemay *et al.* (2017) found that thinning increased the cell production rate of the stem and roots, increasing the total number of cells produced by the cambium in the black spruce and balsam fir trees.

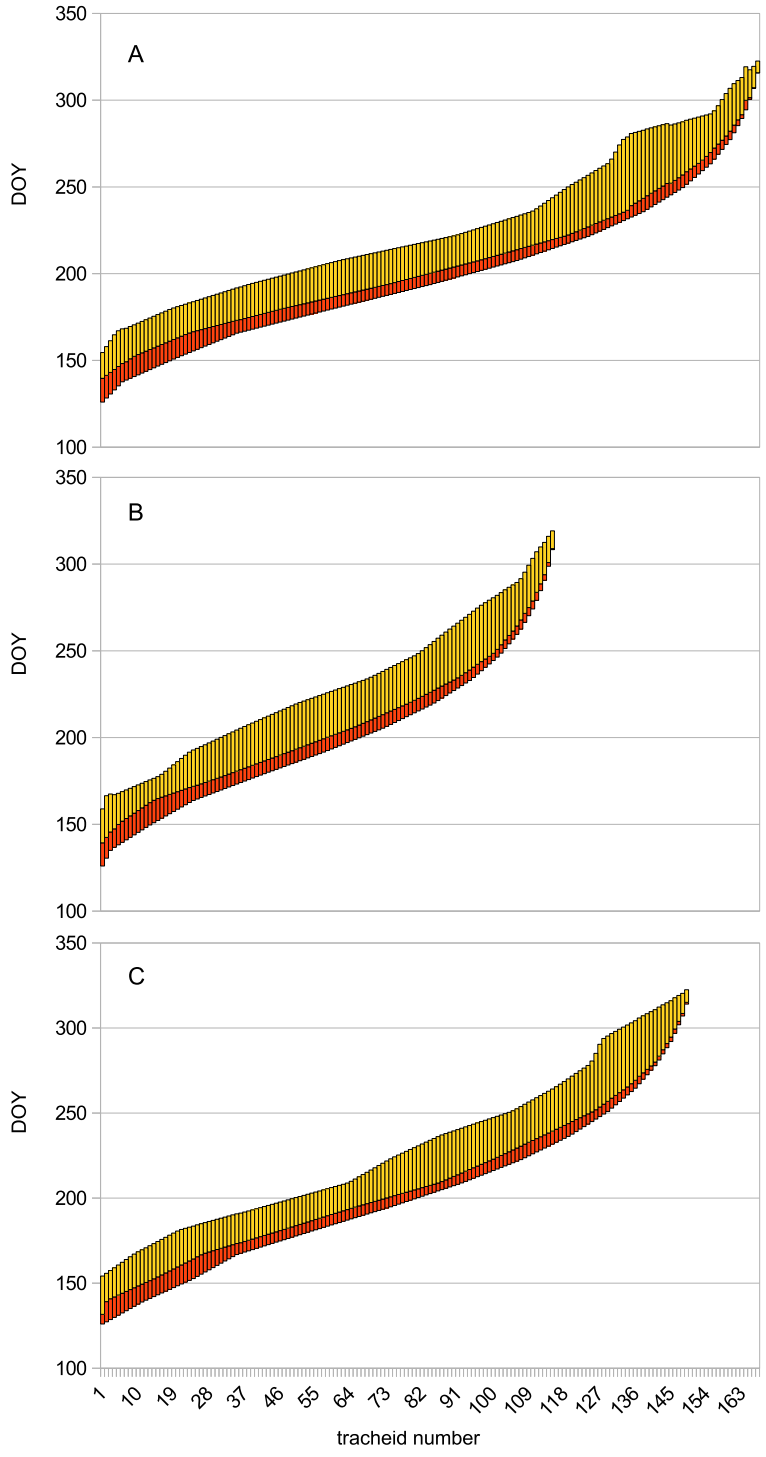

Fig. 5. Duration of radial cell PC and SW phases per tracheid for 14-year-old Norway spruce trees growing at the treated plots examined (plot C, heavy thinning; plot A, mild thinning) and the untreated plot (plot B,: control).

Table 5. Duration (days) of the PC and SW phase per tracheid in the plots examined.

Phase	Plot	Min	Max	Median	Mean	SD	Var. coef. (%)
PC	A	0.10	13.68	6.48	7.01	2.34	33.3
	B	0.58	13.32	7.43	7.67	2.42	31.5
	C	1.01	12.25	6.17	6.85	2.45	35.8
SW	A	6.74	42.30	19.43	22.26	6.47	29.1
	B	10.28	32.52	24.97	23.90	5.05	21.1
	C	7.42	38.64	21.10	22.52	6.06	26.9

PC, cell enlargement phase; SW, secondary wall formation phase; plot C, heavy thinning; plot A, mild thinning; plot B, untreated control.

Table 6. Kruskal–Wallis and *post hoc* Dunn's tests for the duration of the PC and SW phase per tracheid of the plots examined.

	PC phase per tracheid		SW phase per tracheid	
Plots	A	B	A	B
B	0.0003*	–	0.0000*	–
C	0.1565	0.0000*	0.2735	0.0005*

PC, cell enlargement phase; SW, secondary wall formation phase; plot C, heavy thinning; plot A, mild thinning; plot B, untreated control.

Focusing on the formation of the produced tracheids, our analysis of the duration of the PC and SW per tracheid suggests that the thinning can affect both the rate and the duration of the PC and SW phases in the tracheid formation process, possibly regardless of the total duration of the growing season. This demonstrated certain flexibility during the formation of the tracheids within the growing season, most likely affected by the weather conditions but the relationship between the intensity of the thinning and its impact on tree growth under different weather conditions, which seems to moderate the tree growth response of Norway spruce accordingly, remains undefined.

Thinning frequently results in increased radial increment and amount of produced tracheids, potentially due to the modification of several interactive factors, such as an increase in soil moisture content and nutrients availability, or even the photosynthetic capacity of the canopy through the increment of the foliar mass of the crown (Aussenac 2000; Blanco *et al.* 2005; Primicia *et al.* 2013). Deslauriers & Morin (2005) reported that the cell production rate was largely dependent on minimum air and soil temperature during most of the cell production period in the balsam fir stems. When tree competition was reduced by thinning, *Pinus nigra* increased the sensitivity of radial growth to air temperature, while unthinned plots responded more to precipitation (Martín-Benito *et al.* 2010). In *Pinus radiata*, limiting conditions diminish growing duration and consequently total stem growth more in unthinned stands than in thinned stands (Drew *et al.* 2018). Olivar *et al.* (2014) reported that the radial growth in Aleppo pine was mainly controlled by soil water availability during the growing season but also differed by crown class, assessing that the removal of 30 and 45% of the initial basal area produced a growth release that did not take place under mild thinning treatments. In the Aleppo pine plantation in Spain, forest management confirmed its value for improving the effects of water limitations on individual tree growth, suggesting that this can contribute to understanding how modifying stand density will differentially affect stem diameter growth responses to short-term climatic fluctuations (Olivar *et al.* 2014). Lemay *et al.* (2017) concluded that thinning causes an increase in the cell production rate, as well as the total number of cells produced in both stem and roots, without changing the timing of wood formation phenological phases, a condition which could potentially induce frost damage during spring or autumn. Nevertheless, previous researchers suggest that thinning is less effective on dry sites if it is not intense enough because of the stronger inter-tree competition for water. Hence, drier sites cannot support stands sufficiently (Cotillas *et al.* 2009; Linares

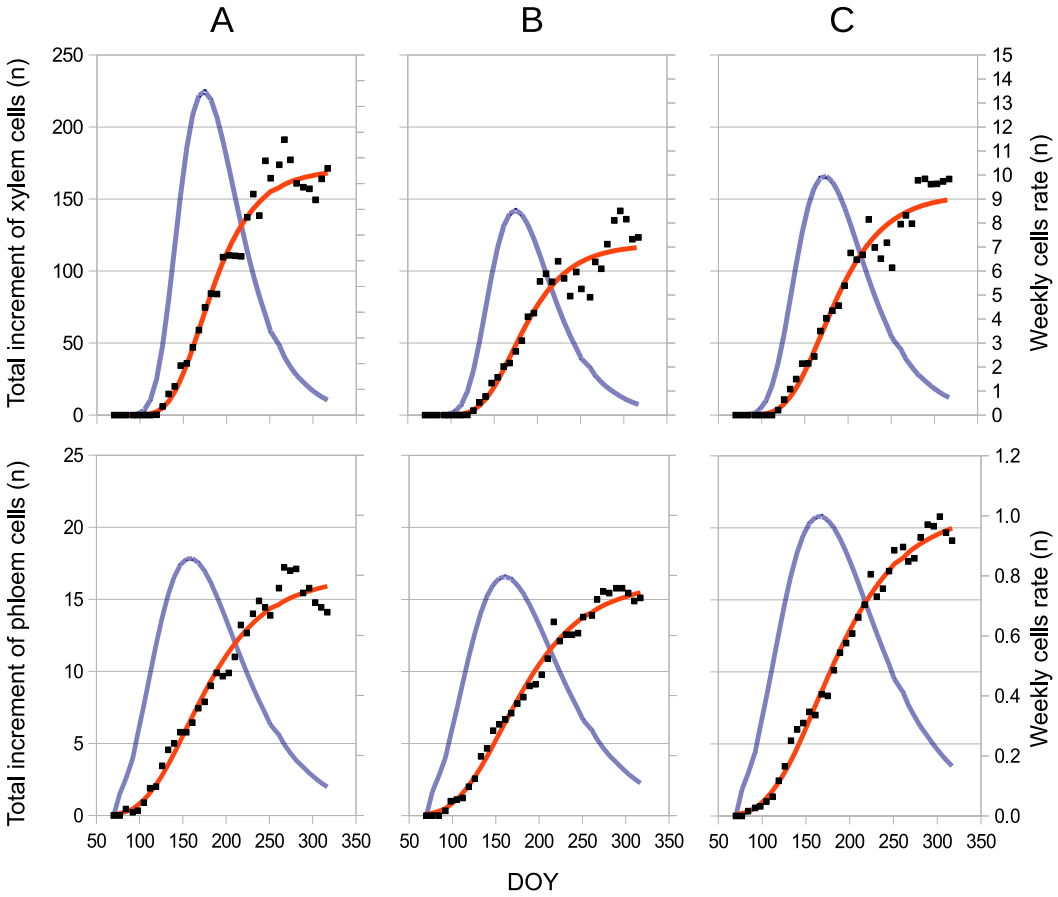

Fig. 6. Rate of total increment of the xylem and phloem produced cells at the treated plots examined (plot C, heavy thinning; plot A, mild thinning) and the untreated plot (plot B, control).

et al. 2009; Moreno & Cubera 2008; Olivar *et al.* 2014). Primicia *et al.* (2013) also assessed that the radial increment and xylogenesis (tracheid production) were mainly affected by tree water status (air and soil humidity, throughfall) differences caused by treatments and justifiable variabilities in tree size and tree-to-tree competition.

The reduction of stand density through thinning seems to improve the overall resistance of individual trees to drought stress, and the extent of this differential response changes within the same species along climatic gradients (Misson *et al.* 2003; Moreno & Cubera 2008; Gea-Izquierdo *et al.* 2009; Olivar *et al.* 2014). Based on that and supported by our findings, thinning can potentially assist the vulnerable Norway spruce to survive extreme events including drought. However, sparse stands should not be concluded to be universally desirable (Gea-Izquierdo *et al.* 2009). Hynynen (1993) mentioned that the corresponding stem number in a Norway spruce stand is ca. 1400 trees/ha, which seems to render heavy thinning in our plot C too intensive, while our plot A looks optimum considering our findings and taking into account that trees examined in this research were only 14-year-old stands. In line with Olivar *et al.* (2014), forest managers should maintain sufficient stand density to address the effects of extreme weather events, also regarding regeneration and/or soil protection parameters. Overall, according to Gea-Izquierdo *et al.* (2009) and Olivar *et al.* (2014), the effect of different silvicultural treatments and stand densities on tree growth response to climate is probably a key factor in influencing forest preservation, especially when drought events occur.

Additionally, it is important to investigate the impact of the thinning treatment on tree growth in the long term. Primicia *et al.* (2013) reported that nine years after thinning, a higher seasonal radial increment and a greater number

of tracheids were produced by the Scots pine canopy under the unthinned silvicultural regime, while no differences between canopy types were observed in the thinned regime. Hence, the type of canopy may modulate sensitivity responses to climate and should be considered for further investigation in our treated plots A and C to detect the effect more years after their thinning treatment.

Conclusions

The preliminary results of this study showed that the thinning treatment can strongly affect the xylogenesis and phloemogenesis of the Norway spruce trees in terms of the number of produced xylem and phloem cells, as well as the rate of the total radial increment of the stem. On the other hand, thinning shows no significant influence on the timings of the phenological phases of the xylem and phloem formation. The findings provide us with the framework to extend and deepen this research in several directions. The level of intensity in the thinning approach needs to be considered to achieve optimum results in the total production of wood. Further investigation is needed for the future behavior of the canopy density in the years after the thinning application. Finally, the interlinkage between forest management treatments, tree growth, and microclimate responses needs to be elucidated to assess the future survival of the trees under the ongoing climate change effect.

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