The cambial response of Scots pine trees to girdling and water stress

Marek Fajstavr[1,4*], Kyriaki Giagli[1], Hanuš Vavrčík[1], Vladimír Gryc[1], Petr Horáček[4], Josef Urban[2,3]

[1]Department of Wood Science and Technology, Faculty of Forestry and Wood Technology, Mendel University in Brno, Zemědělská 3, 61300 Brno, Czech Republic

[2]Department of Forest Botany, Dendrology and Geobiocenology, Faculty of Forestry and Wood Technology, Mendel University in Brno, Zemědělská 3, 61300 Brno, Czech Republic

[3]Siberian Federal University, Svobodnyj Prospect 79, Krasnoyarsk, 660041 Krasnoyarsk, Russia

[4]Department of xylogenesis and biomass allocation, Domain of environmental effects on terrestrial ecosystems, Czechglobe - Global Change Research Institute, The Czech Academy of Sciences, Belidla 4a, 60300 Brno, Czech Republic.

*Corresponding author: e-mail: fajstavr.marek@seznam.cz, tel.: +420 739 313 583

ABSTRACT

We monitored six healthy dominant trees and six girdled Scots pine trees for two successive growing seasons (2014 and 2015) to investigate the seasonal dynamics, cambial activity, and morphology of the new xylem and phloem cells formed under environmental stress when girdling was applied during the dormant period (15 January 2014). Microcore (1.8 mm) samples were collected weekly using a Trephor tool above and below the girdling area, and weather data were measured on site. Drought stress in combination with girdling reduced the total number of differentiation days cell formation. In 2014, no significant differences in tracheid dimensions were observed between the girdled area and the control trees, while in 2015, the control trees showed significantly smaller cell wall thickness and radial dimensions of the latewood tracheids (LW) compared to 2014 and girdled trees had no occurrence of LW. Under stressful heat waves and prolonged periods of no precipitation, the trees tended to reduce the number of tracheids that were formed and exhibited smaller radial dimensions (narrower tree rings) to increase their hydraulic efficiency. Trees responded to limited water

availability by forming intra-annual density fluctuations (IADFs *L)* in the zone of the LW to overcome stressful conditions. Although xylem cell differentiation was affected by stressful conditions, no significant variability in phloem cell dimensions was observed. Thus, the phloem tissue was less sensitive to exogenous factors.

***Keywords*:** Cell wall thickness, phloem, radial dimension, *Pinus sylvestris* L., tracheid, xylem, differentiation.

INTRODUCTION

The xylem formation process is described by five successive differentiation stages, including cell division (embryonic stage), cell enlargement (volume growth), cell wall thickening, lignification (maturation), and programmed cell death (Rossi *et al.* 2014). This process is a complex result of various interacting factors, i.e., water availability, temperature, nutrients, hormones, and genetic predisposition (Hölttä *et al.* 2010; Zhang *et al.* 2014; Fischer *et al.* 2019). For instance, the cell enlargement stage is affected by the process when several vacuoles cumulate into one large central vacuole whose uptake of water becomes intensive. The enlarging tracheids reach their final radial diameter during this stage, and therefore, the amount of water supply is a regulating factor for the size of the radial cell dimension (Wodzicki 1971; Kozlowski *et al.* 1991; Plomion *et al.* 2001). On the other hand, the final thickness of the cell wall of tracheids is known to be related to the air temperature, assimilate transport and rate of carbon allocation during the secondary cell wall thickening stage (sink activity) (Larson 1967; Körner 2015; Fonti & Babushkina 2016; Castagneri *et al.* 2017). Generally, thin-walled earlywood (EW) tracheids with large radial dimensions require longer enlargement stages. In contrast, the thick-walled latewood (LW) tracheids with small radial dimensions remain in the secondary cell wall thickening and lignification stage longer, which predisposes them to their final dimensions. Cartenì *et al.* (2018) explained that at the

beginning of the growing season, low sugar availability in the cambium causes slow wall deposition, resulting in a longer enlargement time; hence, EW (large cells with thin walls) is formed. High sugar availability during late summer/early autumn forms the LW (narrower cells with thick cell walls).

In the case of phloem, the annual increment is formed by early (EP) and late phloem (LP) sieve cells. The cambial zone on the phloem side always divides less compared to xylem, explaining the considerable disproportion existing between phloem and xylem tissues (Plomion *et al.* 2001). Gričar & Čufar (2008) described the determination of the growth ring boundaries, reporting that the tangential walls of the first formed EP sieve cells adjacent to the previous year's crushed sieve cells have a slightly convex shape. As in xylem, the conductive capacity of phloem cells is affected not only by their anatomical structure (conduit size and number) but also by their sieve pore size and frequency along the pathway. Nevertheless, their development is primarily influenced by endogenous factors and less dependent on environmental factors compared to xylem cell growth (Gričar & Čufar 2008; Mullendore *et al.* 2010).

Scots pine species have no typical collapsed phloem sieve cells from the previous year, which is common in many other coniferous species; thus, the boundary between newly formed and old growth sieve cells is detectable by the increased number of newly forming EP cells (Panshin & De Zeeuw 1980; Larson 1994; Kozlowski & Pallardy 1997; Gričar *et al.* 2016; Fajstavr *et al.* 2017). Furthermore, Gričar *et al.* (2016) found that trees with indistinguishable growth ring boundaries of phloem (i.e., *Pinus halepensis* Mill. and partly *Pinus sylvestris* L.) grow particularly well in the Mediterranean area. It was confirmed that stable phloem formation patterns and structures could be found only in trees of similar age, position in stands, vigour and vitality, which represents trees growing in similar environments, while LP is more variable than EP (Gričar *et al.* 2014b; Gričar *et al.* 2015).

75 The drought events that have often occurred in temperate forests in recent decades are
76 currently considered to be a global issue that can potentially affect the forest cover over large
77 territories in a significant way (Christensen 2007). Intermittencies of the cambial activity
78 during the growing season (caused by fluctuations in the temperature and tree water
79 availability) are responsible for the deviations from the "normal" succession of the EW and
80 LW zones. However, the limited photosynthesis caused by defoliation (biotic or abiotic
81 origin) could also be another influencing factor (De Micco *et al.* 2016). The occurrence of
82 intra-annual density fluctuations (IADFs) has been studied mostly in Mediterranean
83 ecosystems, but IADFs have also been observed in other environments (e.g., temperate,
84 boreal, and tropical ecosystems) affected by different environmental conditions (De Micco *et*
85 *al.* 2016). A lack of water supply hinders the physiological functions of trees, causing dieback
86 and mortality. Changes in carbon dynamics closely related to wood anatomical features have
87 been observed (Fonti *et al.* 2010; McDowell 2011; Pellizzari *et al.* 2016). Drought-stressed
88 conifer species in non-carbon-limited conditions usually form thick cell walls (DeSoto *et al.*
89 2011; Bryukhanova & Fonti 2013; Liang *et al.* 2013), while Scots pine trees undergoing
90 drought reduce the carbon costs of their water conducting system by decreasing the number
91 and cell wall thickness of tracheids and proportionally increasing the lumen diameter
92 (Eilmann *et al.* 2009). Hence, the analysis of xylem cell dimensions can provide useful
93 information on tree responses to extreme drought stress (Fonti *et al.* 2010; Pellizari *et al.*
94 2016).
95 Knowledge of tree survival mechanisms and the underlying processes leading to death
96 due to stress factors is a useful tool for physiologists, ecologists and foresters. One of the most
97 commonly used methods for artificially inducing stress is the girdling of the stem (Stone
98 1974; Wilson 1998). The method is mainly used in the fields of horticulture and landscaping
99 (reproduction control, fructification increase) for deciduous and evergreen tree species (Lewis

100 & McCarty 1973; Day & DeJong 1999; Rivas *et al.* 2008). Girdling consists of the removal of
101 a complete strip of bark (phloem and cambium) from around the entire circumference of the
102 stem at breast height (1.30 m), interrupting the supply of plant hormones and photosynthesis
103 products into the root system (Jordan & Habib 1996; Tombesi *et al.* 2014). Girdled trees often
104 also suffer from a significant reduction in the water supply from the root system (Noel 1970;
105 Taylor 2002; Domec & Pruyn 2008). This fatally affects the dynamics of cambial activity,
106 tree-ring width (number of cells), the proportion of LW within the tree ring, and eventually
107 wood density (Wilson & Gartner 2002; Domec & Pruyn 2008; Sellin *et al.* 2013). Fajstavr *et*
108 *al.* (2017) found that below the stem girdling area, typical thick-wall LW tracheids were never
109 formed, while above the stem girdling area, cell formation and tissue differentiation
110 continued, until the end of the growing season, at a less vigorous rate compared with the
111 control trees.
112 The ability of a tree to adapt to the environment can be assessed by analysing xylem
113 and phloem formation processes, especially under stressful conditions (Denn & Dodd 1981;
114 Sass & Eckstein 1995). When the girdling method was used to disrupt assimilation flow in the
115 mid-growing season (July 15), both cambial activity and cell formation stopped two weeks
116 after the applied treatment (Fajstavr *et al.* 2017). Following this research, we investigated the
117 impact of girdling stress on cambial activity and cell formation when applied before the
118 upcoming growing season (January 15). Under this prism, the basic hypothesis of this study
119 was established; girdling applied during the dormant period (January 15) will modify the
120 reactivation of cambial activity and cell formation in comparison with healthy trees (2014)
121 while promoting adaptation to drought stress. The analysis was focused on the (i) seasonal
122 dynamics of the cambial activity, (ii) timings of xylem and phloem formation, and (iii)
123 evaluation of the morphometric traits of the tracheids and sieve cells in the xylem and phloem

increment formed in 2014 and 2015, when healthy and girdled Scots pine trees were growing under a stressful environment (precipitation deficit combined with heat waves).

MATERIALS AND METHODS

Site characteristics

Twelve healthy dominant Scots pines (*Pinus sylvestris* L.) trees growing in the territory of the "Training Forest Enterprise Masaryk Forest Křtiny", in Soběšice, Brno (49°15′39′′ N, 16°36′20′′ E, 404 m a. s. l., Czech Republic) were monitored. The forest in Soběšice consists mostly of planted Scots pine trees (70%) mixed with European larch and deciduous tree species. The soil type is categorized as mesotrophic Cambisol. The selected Scots pine trees were approximately 80 years old and 25 m tall on average, while the stem diameter at breast height was found to be 36 ± 7 cm. Six of the selected trees were used as control trees, while the remaining six trees were girdled. The girdling was performed before the onset of the growing season of the first studied year (January 15, 2014). The trees were girdled at breast height (h = 1.30 m) by removing a wide strip of bark (7–10 cm) including the phloem and cambial zone around the entire circumference of the stem.

Weather data

Average daily air temperature, daily precipitation totals, average daily soil water potential and the daily sum of the effective temperature were calculated. Air temperature (Minikin TRH, EMS Brno, Czech Republic), precipitation totals (MetOne Instruments, Grants Pass, Oregon, USA) and soil water potential (GB-2, Delmhorst Inc., Towaco, NJ, USA attached to an SP3 data logger, EMS Brno, Czech Republic) data were obtained directly on site. The soil water potential above -1.1 MPa (technical limit of the device) was measured at depths of 15 cm, 50 cm, and 90 cm in two repetitions. Data were acquired every 10 minutes. Additionally, the sum of effective temperatures (Σ_{ET}) was estimated each day (Figs. 2c & d).

148 This cumulative value was obtained as the sum of all temperatures exceeding the set threshold
149 - the active zero (≥ 5 °C) (Fajstavr *et al.* 2017). The acquired weather data were compared
150 with the long-term average values (Climate Research Unit Time Series, CRU TS3.23; via
151 http://climexp.knmi.nl).

152 ***Sampling***

153 Microcores with a thickness of 1.8 mm were collected at weekly intervals (from mid-
154 March until mid-November) by using the Trephor increment borer (Rossi *et al.* 2006). From
155 the control trees, microcores were obtained circumferentially starting from breast height. The
156 distance between individual sampling points was more than 2 cm to avoid wound tissue
157 during sampling (Fajstavr *et al.* 2017). In the case of the girdled trees, we took samples from
158 two different areas - from the area above the girdling (AGA) and the area below the girdling
159 (BGA), keeping the minimum distance of 20 cm from the removed strip.

160 ***Sample preparation***

161 Microcores were immersed in FAA (90 ml of 70% ethanol, 5 ml of acetic acid
162 solution, 5 ml of 36–38% formaldehyde) immediately after sampling. One week later, the
163 samples were rinsed in water and stored in ethanol (70%). Thereafter, the microcores were
164 dehydrated in an ethanol series (70%, 90%, 95%, and 100%) and embedded in paraffin using
165 a tissue processor (Leica TP1020). Transverse sections were cut (8–12 μm thick) with a rotary
166 microtome and then dried in a laboratory oven (70 °C for 20 minutes). The microsections
167 were first rid of the paraffin content (Bio Clear, Bio Optica, Milano, Italy) and then
168 selectively stained by safranin (0.04%) and Astra blue (0.15%) dyes (van der Werf *et al.*
169 2007) to distinguish the lignin and cellulose contents. Finally, permanent sections were
170 prepared using Euparal mounting medium (Waldeck, Münster, Germany) (Gričar *et al.* 2014;
171 Fajstavr *et al.* 2017).

172 ***Measurements and data processing***

173 A light microscope (Leica DMLS, including a polarisation filter) with an attached
174 digital camera (Leica DFC 280) was used to analyse the cambial activity and the process of
175 xylem and phloem cell formation. During both growing seasons, the numbers of cells in the
176 cambial zone (CC, Fig. 3), tracheids in the phase of cell enlargement (PC; Fig. 3c), secondary
177 cell wall thickening (SW; Fig. 3c), mature tracheids (MT; Fig. 3c), sieve cells in the early
178 phloem (EP; Fig. 3b; Figs. 3c & e) and late phloem formation (LP; Figs. 3c & e) were counted
179 in three files within the weekly interval (Deslauriers *et al.* 2008; Fajstavr *et al.* 2017). The
180 border between the PC and SW phases was distinguished by glistening secondary cell walls
181 under a polarisation filter, and the EP and LP sieve cells were determined by the layer of axial
182 parenchyma (red filled cell lumens as dyed by safranin solution) (Fig. 3e).

183 The types of the cells were determined according to the following rules. The dormant
184 CCs were identified by their narrow radial dimensions (flattened rectangular shaped cells) and
185 thin, nonlignified primary cell walls (blue-stained). The newly formed EP sieve cells and
186 enlarging xylem cells were distinguished by their radial dimensions that were two times larger
187 than the those of the flattened cambial cells. The fully formed xylem cells were determined by
188 their thick, lignified cell walls (red-stained) and empty lumen. The phloem sieve cells were
189 distinguished by their thin, nonlignified, blue-stained cell walls with a round to irregular
190 shape. The cell walls of the sieve cells were slightly thicker than those of the CC. The EP
191 sieve cells were larger than the LP considering their radial dimensions (Gričar *et al.* 2016).

192 The marginal zone between the old and newly formed phloem increment was detected
193 and counted by the increasing number of newly formed large cells in comparison with the
194 measured increment of the previous year (Fajstavr *et al.* 2017). Following Gričar & Čufar
195 (2008), we determined the first phloem cells by their slightly rounded thin tangential walls
196 and differentiated phloem cells into EP and LP sieve cells, which were separated by an axial

197 parenchyma band. The beginning of cambial activity was defined when the CC gradually
198 began to divide (metabolically active) and hence increase in number (Fig. 3b) (Prislan *et al.*
199 2016; Fajstavr *et al.* 2017).

200 Four IADFs types were monitored and classified in line with the literature (Campelo *et*
201 *al.* 2007; De Micco *et al.* 2016): EW (E and E+) and LW (L and L+). Type "E" occurs in the
202 first half of the growing season as a zone of LW-like tracheids in the EW, type "E+" occurs at
203 the end of the EW as transition tracheids between real EW and LW, type "L" is formed during
204 the second half of the growing season as EW-like tracheids in the LW, and type "L+" occurs
205 between the LW and EW of the next growing season as a zone of EW-like tracheids with
206 narrower lumen and thicker cell walls than real EW tracheids and is similar to the transition
207 zone between EW and LW tracheids (De Micco *et al.* 2016).

208 The morphological parameters of cells were analysed in the last formed tree ring,
209 where three radial files of xylem and phloem cells were selected (Deslauriers *et al.* 2008). The
210 morphological parameters of cells (cell wall thickness, radial dimension of the cell lumen,
211 total radial dimension of cells, total number of formed cells) were measured by ImageJ
212 software (Abramoff *et al.* 2005).

213 Mork's criterion was used to distinguish EW from LW (Denne 1988). According to
214 Mork (1928), when the double cell wall thickness is larger than the cell lumen diameter, the
215 cell is recorded as LW (Fig. 1). To calculate the total radial dimension of one cell, we used
216 equation 1:

217 $$R = ½ (2CW) + L + ½ (2CW)$$ Equation 1

218 where R is the total cell radial dimension, $2CW$ *is* the thickness of a double cell wall, and L is
219 the cell lumen width.

The relative position (RP) of each tracheid within a tree ring was calculated by equation 2:

$$RP = X_n/N \quad \text{Equation 2}$$

where X_n is the rank of the cell in the tree ring (increment) and N is the total number of cells.

In due course, we investigated the differences between control and girdled trees (only in the AGA). In the BGA, cambium did not activate during any of the examined years. Hence, the analysis was performed between two groups (AGA and control trees). The differences between years (2014 and 2015) and samples in the listed xylem and phloem anatomical variables were determined using individual one-way repeated measures analysis of variance (ANOVA, α=0.05).

The Kruskal–Wallis test (α=0.05) was used for testing (p-values), assuming that the samples had equal mean values since the dataset did not fit ANOVA's assumptions. The Kruskal–Wallis test was performed in R software.

The dynamics of xylem radial growth were modelled using the Gompertz function (Rossi *et al.* 2003). The model (1st derivatives) estimated the daily number of cells formed per year, as described by equation 3:

$$y = A\mathrm{e}^{-\mathrm{e}(\beta-\kappa t)} \quad \text{Equation 3}$$

where y is the cumulative value of the number of cells in one week, t is the day of the year, A is the upper asymptote of the maximum number of cells, β is the parameter location along the x-axis, and κ is the inflection point on the curve representing the maximum daily increment of cells.

Wodzicki's algorithm was used to analyse the timings of the differentiation stages per individual tracheid (Wodzicki 1971). Three values were used: a) the starting date of the PC

stage, b) the duration of the PC stage, and c) the time that a given cell spent at the SW and MT stages together, up to the stage of a fully matured cell wall. This model was used in the context of all sample trees, and averages for all trees were evaluated in this way. The analysis was used for the comparison between cells in particular portions (quarters) of fully formed annual rings of individual analysed trees.

RESULTS

Weather conditions

The examined years 2014 and 2015 were both found to be drier than the long-term average values, mostly due to low precipitation during the spring of both years (Table 1). The year 2015 was drier than 2014 in all seasons. The lack of precipitation in 2015 was combined with high average daytime temperatures (during summer months). This finding was confirmed by the sum of effective temperatures as well as the soil water potential (Table 1 & Fig. 2).

Cambial activity and cell formation

Significant differences between the AGA and control trees were observed during the onset ($p = 0.006$) as well as the end ($p = 0.013$) of cambial activity in 2014 (Table 2). The onset of cambial activity in the AGA ($\Sigma_{ET} = 57.9$ °C) was recorded one week earlier (March 21, DOY 80 ± 3) than in the control trees ($\Sigma_{ET} = 77.1$ °C), which was 65 days after girdling was performed (65 dAPG). The rest of the monitored differentiation stages had nearly identical courses for both groups (Table 2). The first xylem cells at the PC stage were formed during the first week of April ($\Sigma_{ET} = 107.9$ °C) in both groups (78 dAPG), and the first fully lignified tracheids were observed one month later (112 dAPG) in the first week of May 2014 ($\Sigma_{ET} = 309.9$ °C). This observation coincided with the intensive period of cambial activity, i.e., the cambial zone consisted of more than 8 cells during May (Fig. 5a). In mid-June (150

267 dAPG), a sudden reduction in the number of cambial cells was recorded after 16 days of no
268 precipitation and notably increased average daily temperatures (Figs. 2a & b). In due course,
269 when the precipitation totals increased again (early August), we noticed that cambium was
270 reactivated once again (Fig. 5a). This phenomenon affected all differentiation stages (Figs. 5c
271 & e), initiating the formation of the IADFs.

272 Regarding phloemogenesis, a significant difference ($p = 0.039$) was detected between
273 the two sample groups during the time of EP formation (Table 2), which was reflected in the
274 difference in the total duration of phloem cell formation ($p = 0.046$).

275 In 2015, no significant difference (Table 2) was found at the beginning of cambial
276 activity ($\Sigma_{ET} = 29.3$ °C) between the examined groups, since the activity occurred in the same
277 week (435 dAPG). However, a significant difference was found in the timing of
278 differentiation stages, excluding the beginning of the PC stage (Table 2). In the first week of
279 April 2015, the sum of effective temperatures was only 30.7 °C, which was approximately 70
280 °C less than that in the previous year (Fig. 2d & Table 2). The first fully lignified tracheids
281 appeared in the control trees on May 19, 2015 (DOY 139 ± 6, $\Sigma_{ET} = 323.6$ °C), i.e., which was
282 more than a week earlier than in the AGA. The number of cells in the cambium zone (9–11)
283 was the highest in both tree groups during May, but due to drought stress (Figs. 2b & d), the
284 cambial activity of the control trees finished in the first half of August, which was over one
285 month later than in the AGA case (540 dAPG).

286 The drought stress in 2015 also influenced the length of phloem formation in the
287 AGA, which significantly differed from that in the control trees. Namely, the phloem
288 formation lasted until only the first half of June ($p = 0.003$) in the AGA, significantly
289 affecting the LP formation time ($p = 0.009$) and hence shortening the duration of
290 phloemogenesis compared to that in the control trees ($p = 0.011$, Table 2).

291 ***Growth ring formation dynamics***

292 The Gompertz function model showed that the total annual radial increment of xylem
293 and phloem in 2014 did not differ significantly between the AGA and control trees, but only
294 the increment of the AGA was of high intensity (Table 3; Figs. 5q & s).
295 In 2015, there was a significant reduction in the number of xylem cells in the total
296 increment of both the AGA (by approximately 10 tracheids) and the control trees (by
297 approximately 20 tracheids) compared to the number of cells in 2014 (Table 3; Figs. 4c & d;
298 Fig. 5r). The daily cell production rate was nearly the same in both sample groups this year.
299 Nevertheless, only half of the xylem cells were formed in the AGA compared with that in the
300 control trees. Additionally, the total duration of xylem cell production was 50 days shorter in
301 the AGA according to the Gompertz function.
302 At the same time, the final number of cells in the phloem growth ring, as well as the
303 rate of phloem cell production, was twice as high compared to that in the AGA (2015).
304 Interestingly, the day when the maximum increment occurred coincided in both studied
305 groups (DOY 108, Table 3). The number of days it took to form all phloem cells was only 5
306 days higher for the control trees.

307 ***Kinetics/timing of tracheid development***

308 In 2014, although the first 50% of tracheids formed in both examined groups followed
309 a similar course of differentiation, the formation of the following half of tracheids was
310 significantly different in the AGA (Table 4; Figs. 6a & b). First, their tracheids differed
311 significantly both at the PC stage ($p = 0.002$) and throughout the duration of differentiation
312 (PC + SW, $p = 0.012$) in the third quarter of the tracheids that were formed. Furthermore, a
313 significant difference was observed mainly in the last quarter of tracheids that were formed
314 (Fig. 6b & Table 4) at the PC stage ($p = 0.000$) and SW stage ($p = 0.000$). The total
315 differentiation time of the last quarter of tracheids was more than twice as fast in the AGA as
316 that in the control trees ($p = 0.003$).

All tracheids formed in the control trees remained at the SW stage longer than at the PC stage, while in the AGA, the ratio was balanced (Table 4; Figs. 6c & d). Dividing the tracheid formation into quarters, we noticed that the first quarter of tracheids that were formed by both groups had initially almost the same rate of differentiation (Table 4), but the remaining tracheids differed between the two groups. In the second quarter, the differences were observed in 25–50% of the tracheids, since the AGA spent a significantly more days (p = 0.000) at the PC stage and half as many days at the SW stage (p = 0.000) compared to the control trees. In the third quarter of the tracheids that were formed, the AGA differed significantly at the SW stage (p = 0.000), resulting in reduced overall differentiation time (PC + SW) by up to 10 days (p = 0.002) in comparison with the control trees (Fig. 6d & Table 4). Finally, the last quarter of the tracheids that were formed by the AGA completed with almost one week longer total differentiation time than that in the control trees (Fig. 6d; p = 0.005). The last quarter of tracheids in the control trees spent three times longer at the SW stage at the expense of the PC stage (Fig. 6c).

Morphometric traits of cells

In 2014, the analysed morphometric parameters of tracheids depicted no notable differences between AGA and control trees (Table 5). The tracheid double cell wall thickness increased depending on the relative position of the tracheid in a tree ring (Fig. 7a). According to the tracheid radial dimension curve, the greatest dimensions were observed in the first half of the growing season, significantly decreased due to the IADFs appearance and then increased again to the normal level (Fig. 7c). In 2014, the first differences between the tracheid dimensions of AGA and the control trees began to occur in the last 25% of LW that formed in AGA, which remained more than twice as short during the maturation (SW + MT) stage (see Figs. 6a & b). Student's t-distribution revealed that the considerably shorter time affected the formation of significantly thinner cell walls of these tracheids (p = 0.002; Fig.

342 4b). However, the tracheid radial dimensions did not differ between AGA and the control
343 trees ($p = 0.159$).
344 In contrast to xylem cells, the morphometric parameters of sieve cells of phloem
345 manifested very low variability in the relative position of the annual increment (Figs. 7e & g).
346 In 2015, significant differences were noted compared to 2014 as well as between the
347 two groups (Figs. 7b & d; Tables 5 & 7). The dimensions of EW of control trees manifested
348 hardly any changes in comparison with the previous year; on the other hand, we observed a
349 significant reduction in all traits of LW (Table 5). The tracheid double cell wall dimensions of
350 control trees in 2015 manifested significantly lower values in the second half of the growing
351 season (0.6–0.8 relative position in a tree ring), and in particular, there was not such a
352 considerable variance among these values as in 2014 (Figs. 7a & b). However, a significant
353 reduction in the dimensions of both types of tracheids was recorded for the AGA (Figs. 4b &
354 d). Namely, the radial dimensions of their EW and LW were reduced by over 10 μm
355 compared to those in 2014. A significant reduction was observed in the double cell wall
356 thickness, especially in LW, in all AGA samples (Table 5). The dimensions of the double cell
357 walls of AGA exhibited negligible variance in 2015, which was caused by the minimum
358 representation of LW (Fig. 4d). Fig. 7d shows that the radial dimensions did not exhibit a
359 fluctuating trend as in 2014. A significant difference in cell traits within the comparison
360 between AGA and control trees in 2015 was proven for EW (Table 7) in terms of the total
361 radial dimensions ($F = 146.69$; $p = 0.000$), radial dimension of lumen ($F = 123.20$; $p = 0.000$)
362 and double cell wall thickness ($F = 84.76$; $p = 0.000$). No significant differences were
363 observed for LW in 2015 (Table 7).
364 In line with 2014, the morphometric traits of sieve cells of phloem showed a very low
365 variability in the RP of the annual increment again in 2015 (Figs. 7e & g). However,

366 significant differences were observed when the two groups of sample trees were compared as
367 well as 2014 and 2015, in particular for the radial dimensions of LP (Tables 6, 7).

368 DISCUSSION

369 ***Drought-stressed cambial phenology and IADFs formation***

370 The rate and duration of cambial derivative production affect the annual radial increment in
371 trees. Cambial cell division directly affects the transport efficiency of water, mineral nutrients
372 and photoassimilates, which are responsible for the formation of new xylem and phloem
373 elements (Plomion *et al.* 2001; Sorce *et al.* 2013). Thus, the newly formed cells are driven by
374 the dynamics of cambial phenology (Kozlowski *et al.* 1991; Larson 1994). Cambium cells and
375 their divisional activity are influenced by changes in temperature (Kozlowski *et al.* 1991;
376 Deslauriers & Morin 2005). In *Pinus sylvestris* L., temperature is found to influence cell
377 production and differentiation at the earliest stage of formation (Wodzicki 1971).

378 In 2014, the timings of the xylem and phloem did not significantly differ, even though
379 the cambial activity began and ceased earlier in the AGA than in the control trees. In contrast,
380 in 2015, the finding was reversed, i.e., the onset of cambial activity and PC stage coincided in
381 both groups, but all following stages were initiated with a significant delay in the AGA
382 compared to that in the control trees. Additionally, phloem cell formation ceased significantly
383 early in the AGA.

384 Since the precipitation amount in the III–V period (from March to May) did not differ
385 much between the two years (Table 1), the air temperature was the parameter that presumably
386 caused a delay of the PC stage (over 14 days on average) in comparison to the previous year.
387 This effect was manifested in the delay of all differentiation stages, especially in the AGA.
388 Nevertheless, the reactivation, duration, and cessation of cambial divisions are influenced by
389 factors other than air temperature and precipitation, since different physiological processes
390 may independently act in different phases of cell division and differentiation (Rossi *et al.*

2003; Thibeault-Martel *et al.* 2008; Lupi *et al.* 2010). The effect of plant hormones (IAA - indole-3-acetic acid) and carbohydrate allocation can play key roles during the reactivation of cambial cells and initiation of cell formation (Savidge & Wareing 1982; Uggla *et al.* 1998; Sundberg *et al.* 2000, Sorce *et al.* 2013), especially by increasing carbohydrate accumulation above girdling during the absence of a root sink below girdling (Wilson 1968; Little *et al.* 1990; Sundberg *et al.* 1994; Domec & Pruyn 2008). This factor probably affected the cambial reactivations in the AGA. The physiological changes that were observed (e.g., cell wall maturation) were probably affected by the increasing impact of drought stress in the rest of the growing season in 2015.

The drought stress - alternation of precipitation episodes and long-lasting precipitation absence combined with heat waves caused a fast cambial temperature response (latency and subsequent reactivation), resulting in IADFs in all monitored years and both types of sample trees (Figs. 4a–c). Reduced cambial divisions (less than 7 cambial cells in the cambial zone) were observed when the average air temperature exceeded 25 °C and the soil water potential values dropped below -1 MPa. In 2014, the number of cambial cells began to increase in the last week of July related to precipitation abundance after the water deficit during June. The thresholds of air temperature and soil water potential corresponded with the findings of Antonova & Stasova (1993, 1997) and Myers & Talsma (1992), respectively. Myers & Talsma (1992) investigated the threshold regarding the wilting point (-1.5 MPa). Within the *Pinus* genus, the EW-like tracheids in the LW zone (IADFs *L*) are described as a reaction of favourable moisture conditions after the summer precipitation episode (Campelo *et al.* 2007; Novak *et al.* 2013; Carvalho *et al.* 2015), which matches our findings. According to Balzano *et al.* (2018), the EW-like tracheids in the LW zone (named L-IADFs) were formed in autumn when precipitation was abundant, particularly when the precipitation in October was more than double that in September. They also noted a double pause in cell production leading to L-

IADFs as well. This effect strongly correlated with the double peaks of cambial production, which coincides with our results (Fig. 5a & Figs. 5c–f).

According to the classification of Campelo *et al.* (2007), the IADFs of control trees matched the "IADFs *L*" (Fig. 4a), and AGA seemed to be more similar to the "L^{+}" at the end of 2014 (Fig. 4b), but the causality of tracheid differentiation is questionable. The effect (thin-walled LW tracheids) potentially originated from carbon starvation in response to a lack of carbohydrate supply (Fig. 3g). In contrast with the control trees, AGA exhibited no response to precipitation episodes in mid-August 2015 (Figs. 5d & f; Figs. 2b & d); thus, the AGA did not reactivate their cambial activity, and the increment of typical thick-walled LW was not observed in 2015 (Fig. 4d). Nevertheless, the IADFs of control trees matched the IADFs L^{+} definition (Figs. 4c & e) rather than the significantly earlier cessation of cambial activity in mid-August 2015 (Fig. 5b & Table 2).

The process triggering IADFs formation is linked with increased cell enlargement due to high turgor pressure resulting from high water availability after an abundant precipitation episode in autumn (Sperry *et al.* 2006; De Micco *et al.* 2016; Pacheco *et al.* 2016; Balzano *et al.* 2018). The occurrence of IADFs is an adjustment response to the balancing between hydraulic efficiency and hydraulic safety of water transport (De Micco *et al.* 2008, 2009) and thereby indicates a more adaptive capacity to cope with changing water availability typical of Mediterranean climates (Balzano *et al.* 2018).

Differentiation timings of individual tracheids related to their dimensions

Cuny *et al.* (2014) and Carvalho *et al.* (2015) stated that the tracheid's radial dimension is strongly affected by the rate of cambial cell production. Furthermore, the more intensive cell division involves narrower tracheid formation associated with a decrease in the period spent in the cell enlargement stage. Under the increased rate of cambial division, the newly divided cells pushed the previous cells out of the enlargement zone towards the cells in

441 the wall thickening stage (Carvalho *et al.* 2015). This process is influenced by the width of the
442 auxin radial gradient, which determines the width of the cell enlargement zone (Uggla *et al.*
443 1996) and thus is affected by the time spent in the stage (Carvalho *et al.* 2015). Therefore, the
444 lumen diameter is not only determined by the soil water supply during the accumulation of
445 vacuoles (Kozlowski *et al.* 1991) but also influenced by the auxin gradient and the time spent
446 in the cell enlargement stage (Carvalho *et al.* 2015). In relation to the annual ring width,
447 Rathgeber *et al.* (2011) estimated that 75% of tree-ring width variability is attributable to the
448 rate of cell production, and only 25% is attributable to its duration.
449 In Soběšice, while the control trees differed only in their LW dimensions between
450 2014 and 2015, in the AGA, all tracheid dimensions were considerably reduced between the
451 two observed years. At the beginning of 2015, the first 25% of formed tracheids remained in
452 both the enlargement and maturation (SW + MT) stages during the same period. Nevertheless,
453 the remaining 50% of the formed tracheids remained for a shorter time (SW + MT). The
454 IADFs formation changed the course of tracheid differentiation by decreasing the duration of
455 the enlargement stage. The variation in the radial dimensions of tracheids depends on seasonal
456 changes in the rate of growth during the cell enlargement stage (Wodzicki 1971). This
457 probably occurred in our case during the IADFs *L* formation after the summer precipitation
458 episode.

459 ***The causality of drought stress and tracheid dimensions***

460 Usually, stressed trees prefer the development of roots over aboveground growth
461 (Dewar *et al.* 1994). The adaptation processes combined with reduced sink activity (Körner
462 2003) might also adjust the cell wall thickening process (Martin-Benito *et al.* 2013).
463 Therefore, the cell wall thickness is closely related to xylem carbon costs (Fonti *et al.* 2010),
464 which form half of the plant biomass (Körner 2015). Cuny *et al.* (2014) refuted the long-
465 lasting assumption that the increasing thickness of tracheids along the annual increment is

466 driven by increased biomass fixation during the SW thickening process. Their findings
467 revealed that the amount of material used for cell wall formation remained almost constant
468 over the entire radial increment of formed tracheids. Additionally, they stated that cell size
469 affects the changes in cell wall thickness. Therefore, the time spent in the cell enlargement
470 stage contributes to 75% of the changes in cell size (Cuny *et al.* 2014). However, in Soběšice
471 (2014), a significantly smaller cell wall thickness in the last 25% of formed tracheids of AGA
472 was observed despite the radial dimension not differing from that in the control trees. The
473 significant differences in tracheid dimensions found in the following year (2015) were
474 probably caused by other stressful factors (e.g., IADFs, water deficit) and adaptation
475 processes. During drought stress, trees respond by adjusting their carbon allocation to form
476 reserves (Wiley & Helliker 2012).
477 In this study, it was not easy to clarify the influencing factors that had a drought-
478 triggered effect on xylem cell differentiation related to tracheid dimensions. Even other
479 authors revealed contrasting findings (Pellizzari *et al.* 2016). Bryukhanova &Fonti (2013)
480 shared a statement with Liang *et al.* (2013) that a drought-reduced tracheid lumen leads to a
481 decline in stem hydraulic conductivity. In contrast, Eilmann *et al.* (2009) found that drought-
482 stressed pine trees significantly increased the radial dimension of tracheids as a result of
483 adaptation to a reduced conducting area (reduced number of tracheids). Hacke *et al.* (2001)
484 reported that a mechanism exists that decreases the lumen diameter of the tracheid to increase
485 hydraulic safety. According to De Micco *et al.* (2008, 2009), IADFs formation is a balancing
486 process between hydraulic efficiency and hydraulic safety. This parallel reduction in the
487 number of tracheids and their cell wall thickness (mainly in water-conductive EW) seems to
488 be forced by the limited carbon availability rather than the increased demand for hydraulic
489 safety (Fonti & Babushkina 2016). However, Scots pine differs (Eilmann *et al.* 2009) from

other drought-stressed coniferous plants, which form thicker cell walls to increase hydraulic safety (DeSoto *et al.* 2011; Liang *et al.* 2013).

During the two examined growing seasons (2014 and 2015), it was found that the cambial stress response altered the course of xylem formation. Furthermore, the cambial activity was significantly shortened in the AGA, and the IADFs formation influenced the duration of xylem cell differentiation, significantly affecting the final tracheid dimensions compared to those in the control trees. Although our primary hypothesis was confirmed, many interactive relationships affected xylem cell morphology during drought stress. Hence, it is difficult to determine whether cambial divisions directly drive cell dimensions.

CONCLUSIONS

In this study, considerable responses of cambial divisions to drought stress were found. These responses resulted in EW-like tracheid formation in the LW zone (IADFs *L*). Moreover, IADFs *L* formation affected the course of differentiated timings of individual tracheids, which was reflected by significant differences in cell morphology. Namely, the xylem cells reflected the drought stress responses by changing their dimensions.

In 2014, no significant differences in tracheid dimensions were observed between AGA and the control trees. The differences were obvious in 2015 when the control trees presented significantly smaller cell wall thickness and radial dimensions of the LW compared to those in 2014. Furthermore, the tracheid dimensions in the AGA were significantly smaller than those in both the control trees and the previous examined year. The phloem response to drought stress was also depicted by the reduction in the number of sieve cells, as well as the duration and cessation time of their annual formation. However, the phloem cells showed a rather more homogenous structure (low variability of dimensions) compared to the xylem cells. Thus, the phloem tissue seemed to be less sensitive to exogenous factors.

514 Conclusively, Scots pine trees undergoing drought stress tended to reduce the number
515 of formed tracheids (narrower tree ring) and relatively decreased their radial dimensions to
516 increase hydraulic efficiency. During the combination of stressful heat waves and prolonged
517 periods of precipitation absence, this process could compensate for the adaptability to water
518 deficits by forming the IADFs *L* in the zone of the LW. The formation of IADFs *L* could
519 support the function of the hydraulic conductive zone to overcome the stressful conditions. In
520 the case of girdled trees, the anatomical changes potentially originated from Eilman's
521 assertion as one of the dieback processes, but this needs to be elucidated by further research.

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774 Table 1. Meteorological data recorded during the growing seasons of 2014 and 2015 compared with
775 long-term data. *T* – average air temperature, *P* – precipitation amount. III-V: March-May period, VI-VIII:
776 June-August period, IX-XI: September-November period. $\sum ET$: the sum of effective temperature at the
777 end of May ($\sum ET_1$), August ($\sum ET_2$) and November ($\sum ET_3$).

Observed period	Annual *T* (°C)	Annual *P* (mm)	*P* III-V (mm)	*P* VI-VIII (mm)	*P* IX-XI (mm)	$\sum ET_1$ (°C)	$\sum ET_2$ (°C)	$\sum ET_3$ (°C)
Long term data	8.1	601.0	132.5	204.5	121.0	491.1	1759	2225
2014	11.3	562.6	98.2	202.4	200.0	520.9	1712	2240
2015	10.0	405.6	92.0	147.0	104.2	411.1	1828	2316

Observed period	Warmest month *T* (°C)	Coldest month *T* (°C)	Highest *P* (mm)	Lowest *P* (mm)	III-V *T* (°C)	VI-VIII *T* (°C)	IX-XI *T* (°C)
Long term data	Jul (18.1)	Jan (-2.7)	Jul (82.0)	Feb (27.5)	9.5	18.8	9.3
2014	Jul (20.1)	Jan (0.7)	Sep (119.6)	Feb (14.4)	10.5	17.9	10.9
2015	Aug (22.4)	Jan (0.7)	Aug (69.6)	Apr (6.2)	8.8	20.4	9.8

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Table 2. Cambial activity and differentiation of xylem and phloem cells timings in the AGA (girdled) and control trees (2014–2015). P-values for the Kruskal-Wallis test (H_0: Medians of all groups are equal if $p < \alpha$ (0.05) => rejecting H_0). DOY: day of year, Control: control trees, AGA: above girdling area, $\sum ET$: sum of effective temperature, K─W test: results (*p*-values) of Kruskal–Wallis test for xylem and phloem cells timings, CA: onset of cambial activity, CCA: cessation of CA, PC: onset of cell enlargement, CPC: cessation of PC, SW: onset of secondary wall thickening, CSW: cessation of SW, MT: occurrence of the first matured tracheids. EP: onset of early phloem formation, LP: onset of late phloem formation and cessation of EP, CLP: cessation of LP. Bold – statistically significant difference (the result of Kruskal-Walis test, $\alpha = 0.05$).

	2014					2015				
Parameters (DOY)	Control	$\sum ET$ (°C)	AGA	$\sum ET$ (°C)	K─W test	Control	$\sum ET$ (°C)	AGA	$\sum ET$ (°C)	K─W test
CA	87 ± 3	77.0	80 ± 3	57.9	**0.006**	83 ± 4	16.8	86 ± 5	29.3	0.206
PC	94 ± 3	125.3	92 ± 3	107.9	0.176	108 ± 7	89.4	110 ± 4	95.8	0.601
SW	115 ± 3	224.7	115 ± 3	224.7	1.000	122 ± 4	175.2	132 ± 4	261.2	**0.007**
MT	127 ± 7	309.9	126 ± 6	302.5	0.794	139 ± 6	323.6	147 ± 5	375.4	**0.037**
CCA	277 ± 14	2025.2	261 ± 6	1906.7	**0.013**	226 ± 7	1574.2	190 ± 8	942.7	**0.003**
CPC	268 ± 15	1959.9	259 ± 7	1885.3	0.209	197 ± 11	1038.7	185 ± 6	853.5	**0.042**
CSW	293 ± 13	2147.4	287 ± 11	2109.6	0.417	281 ± 12	2166.3	208 ± 6	1239.0	**0.003**
CA duration (days)	190 ± 14	-	181 ± 5	-	0.132	144 ± 6	-	104 ± 8	-	**0.003**
PC duration (days)	174 ± 12	-	167 ± 8	-	0.36	89 ± 11	-	76 ± 5	-	**0.02**
SW duration (days)	177 ± 11	-	172 ± 13	-	0.417	159 ± 13	-	76 ± 8	-	**0.004**
EP	88 ± 4	82.7	87 ± 3	77.0	0.523	92 ± 8	30.7	96 ± 4	30.7	0.195
LP	129 ± 8	327.3	123 ± 6	290.9	0.111	128 ± 5	226.6	128 ± 6	226.6	0.665
CLP	181 ± 14	890.1	174 ± 4	808.3	0.082	175 ± 5	708.2	158 ± 4	519.8	**0.003**
EP duration (days)	47 ± 10	-	36 ± 5	-	**0.039**	36 ± 5	-	32 ± 4	-	0.434
LP duration (days)	51 ± 14	-	50 ± 7	-	0.655	47 ± 10	-	29 ± 8	-	**0.009**
Total duration (days)	98 ± 13	-	86 ± 4	-	**0.046**	83 ± 12	-	62 ± 6	-	**0.011**

791 Table 3. Dynamics of xylem and phloem formation from parameters of the Gompertz function during

792 2014 and 2015 in the AGA (girdled) and control trees.

793

	2014		2015	
Parameters	Control	AGA	Control	AGA
The final number of xylem cells	38.60	34.16	28.33	14.00
Daily cell rate of xylem	0.19	0.25	0.25	0.22
Maximal daily cell rate of xylem	0.28	0.36	0.36	0.32
Day of maximal daily cell rate of xylem (DOY)	142.344	130.11	132.23	131.51
Duration of xylem formation (days)	202.74	137.68	114.28	64.12
The final number of phloem cells	11.60	11.06	8.92	3.81
Daily cell rate of phloem	0.15	0.17	0.12	0.05
Maximal daily cell rate of phloem	0.22	0.18	0.17	0.08
Day of maximal daily cell rate of phloem (DOY)	103.69	111.33	108.32	108.08
Duration of phloem formation (days)	77.11	63.67	76.31	71.50

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795 Table 4. Development of differentiated tracheids according to the Wodzicki algorithm in the AGA
796 (girdled) and control trees. P-values for one-way repeated measures of ANOVA (α=0.05). (H_0:
797 Medians of all groups are equal, if $p < \alpha$ (0.05) => rejecting H_0). PC – stage of cell enlargement, SW –
798 stage of secondary wall thickening, MT – stage of tracheid maturation. Control: average values of
799 control trees, AGA: average values of girdled trees from the above girdling area, ANOVA: results (*p*-
800 values) of ANOVA test. Bold – statistically significant difference between Control and AGA (α =
801 0.05).

		Differentiating xylem						PC + SW + MT duration (days)		
	Formed	PC duration (days)			SW + MT duration (days)					
Year	tracheids	Control	AGA	Anova	Control	AGA	Anova	Control	AGA	Anova
2014	0—25%	15.4 ± 2.7	14.6 ± 4.4	0.678	17.7 ± 2.9	17.1 ± 4.3	0.763	33.0 ± 1.8	31.7 ± 1.6	0.137
	25—50 %	16.1 ± 1.0	14.7 ± 2.1	0.078	15.2 ± 1.9	15.5 ± 2.0	0.774	31.3 ± 2.5	30.2 ± 2.3	0.438
	50—75 %	7.6 ± 1.4	10.6 ± 2.0	**0.002**	12.7 ± 2.5	12.6 ± 1.9	0.889	20.4 ± 2.6	23.2 ± 2.5	**0.012**
	75—100 %	10.5 ± 1.3	4.9 ± 2.5	**0.000**	17.3 ± 2.8	8.6 ± 1.3	**0.000**	27.8 ± 2.9	13.4 ± 3.0	**0.003**
2015	0—25%	11.0 ± 1.6	13.2 ± 2.8	0.121	17.9 ± 2.1	16.0 ± 2.2	0.188	28.8 ± 0.7	29.2 ± 1.4	0.576
	25—50 %	9.1 ± 0.6	13.2 ± 1.5	**0.000**	18.6 ± 0.7	12.2 ± 1.9	**0.000**	27.7 ± 0.1	25.4 ± 3.1	0.054
	50—75 %	10.0 ± 2.2	7.6 ± 1.1	0.074	15.0 ± 1.7	8.1 ± 1.4	**0.000**	25.0 ± 3.9	15.8 ± 2.0	**0.002**
	75—100 %	2.9 ± 2.2	5.6 ± 2.7	0.104	9.4 ± 0.8	13.0 ± 5.3	0.103	12.3 ± 2.7	18.5 ± 2.6	**0.005**

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Table 5. Descriptive statistics of morphometric parameters in the AGA (girdled) and control trees for xylem tracheids (2014–2015). C: control trees, AGA: above girdling area, EW – earlywood tracheids, LW – latewood tracheids, RD – radial dimension, LD – lumen diameter, 2CW – double cell-wall thickness. AVG: average value, SD: standard deviation, N: number of measured items. Bold – statistically significant difference between C and AGA ($\alpha = 0.05$). Units – μm.

2014	EW						LW					
	RD		LD		2CW		RD		LD		2CW	
	C	AGA	C	AGA	C	AGA	C	AGA	C	AGA	C	AGA
AVG	37.5	36.0	32.4	31.1	5.0	4.9	21.3	20.9	13.0	13.9	8.2	**6.4**
SD	9.1	10.8	9.4	10.8	1.0	1.0	7.5	6.6	6.9	6.9	2.9	2.3
N	279	401	279	401	279	401	267	326	267	326	267	326
2015												
AVG	37.2	**25.8**	32.8	**22.2**	4.4	**3.6**	14.5	**10.7**	8.9	**6.1**	5.4	**4.3**
SD	8.8	7.9	9.0	7.8	1.0	0.8	3.9	1.0	3.5	0.9	1.6	0.1
N	320	279	320	279	320	279	117	4	117	4	117	4

Table 6. Descriptive statistics of morphometric parameters in the AGA (girdled) and control trees for phloem cells (2014–2015). C: control trees, AGA: above girdling area, EP – earlyphloem, LP – latephloem, RD – radial dimension, LD – lumen diameter, 2CW – double cell-wall thickness. AVG: average value, SD: standard deviation, N: number of measured items. Bold – statistically significant difference between C and AGA ($\alpha = 0.05$). Units – µm.

2014	EP						LP					
	RD		LD		2CW		RD		LD		2CW	
	C	AGA	C	AGA	C	AGA	C	AGA	C	AGA	C	AGA
AVG	20.3	19.4	18.7	17.9	1.5	1.4	23.4	21.2	21.9	19.8	1.5	1.4
SD	5.0	5.1	5.1	5.2	0.3	0.2	4.5	4.8	4.4	4.8	0.4	0.3
N	214	127	214	127	214	127	50	55	50	55	50	55
2015												
AVG	21.4	**18.9**	19.9	**17.5**	1.5	**1.3**	21.6	**19.6**	20	**18.2**	1.5	**1.3**
SD	5.3	4.9	5.4	4.9	0.3	0.3	5.5	6.0	5.5	5.9	0.3	0.3
N	118	78	118	78	118	78	42	18	42	18	42	18

817 Table 7. Summary of one-way repeated measures ANOVA of morphometric parameters in six control
818 and six girdled (AGA) trees (2014–2015). P-values for ANOVA test (H_0: Medians of all groups are
819 equal, if $p < \alpha$ (0.05) => rejecting H_0). C: control trees, AGA: above girdling area. Bold – statistically
820 significant difference ($\alpha = 0.05$).

	Type (C/AGA)		Year (2014/2015)		Type*Year	
Anatomical variable	*F*	*p*	*F*	*p*	*F*	*p*
Number of tracheids	0.11	0.738	**34.356**	**0.000**	**6.800**	**0.011**
Number of earlywood tracheids	0.71	0.403	3.239	0.077	**4.744**	**0.033**
Number of latewood tracheids	0.24	0.624	**17.965**	**0.000**	0.931	0.339
The radial dimension of earlywood tracheids	**146.69**	**0.000**	**97.387**	**0.000**	**86.034**	**0.000**
The radial dimension of latewood tracheids	1.50	0.221	**24.926**	**0.000**	0.920	0.338
The radial dimension of the lumen in earlywood tracheids	**123.20**	**0.000**	**63.214**	**0.000**	**76.070**	**0.000**
The radial dimension of the lumen in latewood tracheids	0.30	0.585	**12.936**	**0.000**	1.290	0.256
Double cell-wall thickness of earlywood tracheids	**84.76**	**0.000**	**285.840**	**0.000**	**49.567**	**0.000**
Double cell-wall thickness of latewood tracheids	0.92	0.339	**10.578**	**0.001**	0.042	0.837
Number of sieve cells	3.29	0.074	**14.120**	**0.000**	**41.975**	**0.000**
Number of early sieve cells	0.56	0.458	1.162	0.285	**34.653**	**0.000**
Number of late sieve cells	**7.09**	**0.010**	**39.798**	**0.000**	**16.528**	**0.000**
The radial dimension of early sieve cells	**22.01**	**0.000**	0.397	0.529	0.076	0.784
The radial dimension of late sieve cells	**5.86**	**0.017**	**3.956**	**0.048**	0.027	0.869
The radial dimension of the lumen in early sieve cells	**18.99**	**0.000**	0.182	0.670	0.129	0.720
The radial dimension of the lumen in late sieve cells	**5.34**	**0.022**	**4.206**	**0.042**	0.043	0.835
Double cell-wall thickness of early sieve cells	**28.65**	**0.000**	**4.521**	**0.034**	0.362	0.548
Double cell-wall thickness of late sieve cells	**5.34**	**0.022**	**4.206**	**0.042**	0.043	0.835

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Figure 1. Scheme of morphometric cellular parameters measurements. EW: earlywood zone, LW: latewood zone, CW: cell-wall, 2CW: double cell-wall thickness, L: lumen radial dimension, R: an entire radial dimension of tracheid. Scale bar: 50 μm.

Figure 2. Weather data and soil water potential recorded during the growing seasons of 2014 and 2015. a & b ─ daily precipitation amount (bars) and average daily air temperature (solid line). c & d ─ average daily soil water potential (solid line) and the sum of effective temperature (dashed line). DOY ─ the day of the year (70: March 11, 100: April 10, 130: May 10, 160: June 9, 190: July 9, 220: August 8, 250: September 7, 280: October 7, 310: November 6).

Figure 3. The xylem and phloem cell formation and structure of fully formed tracheids during one growing season. a – phase of dormancy, b – activated cambial zone and occurrence of the new phloem cells, c – all differentiation phases of newly forming xylem and phloem cells in the last week of June 2014, d – distinguished glistening secondary cell-walls under polarized filter, e – formation of intra-annual density fluctuation (IADFs) after reactivated cambial zone (caused by precipitation episode) in mid-September 2014 and fully formed phloem annual ring, f & g – occurrence of the earlywood-like tracheids in latewood zone in both of defined types (IADFs *L* and IADFs L^{+}). AP – axial parenchyma, CC – cambial cells, CP – collapsed phloem cells of previous years, EP – early phloem cells, LW – latewood tracheids, LP – late phloem cells, MT – fully matured xylem cells (tracheids), PC – phase of cell enlargement, pLP – late phloem cells of previous year, PZ – phloem zone, SW – phase of secondary cell-wall thickening. IADFs – intra-annual density fluctuation with earlywood-like tracheids in latewood zone of two types (IADFs *L* and IADFs L^{+}) according to Campelo *et al.* (2007). The displayed scale bars: a – 50 μm; b, c, d, e, f, g – 100 μm.

Figure 4. Fully formed annual rings and IADFs structure of control and girdled trees during the growing seasons 2014 and 2015. a – formed annual ring of control tree with "IADFs *L*" in 2014, b –

formed annual ring of girdled tree with "IADFs *L*"and "IADFs L^+" in 2014, c – formed annual ring of control tree with "IADFs L^+" in 2015, d – annual ring of girdled tree with no occurrence of latewood zone in 2015, e & f – frequency of IADFs formed annual rings of the last five years period (2011–2015) in control (E) and girdled (F) trees. CC – cambial cells, EW – earlywood zone, LW – latewood zone, PZ – phloem zone. IADFs – intra-annual density fluctuation with earlywood-like tracheids in latewood zone of two types (IADFs *L* and IADFs L^+) according to Campelo *et al.* (2007). The displayed scale bars: a, b, c, d – 100 μm; e & f– 400 μm.

Figure 5. Dynamics of cambial activity, xylem and phloem cells' development fitted to Gompertz function during the growing seasons of 2014 and 2015. The number of cells within the cambial zone (a, b), in the PC phase (c, d), in the SW phase (e, f), in the MT phase (g, h), total cell number within fully formed annual increment (i, j). The number of cells in the EP phase (k, l), in the LP phase (m, n), total cell number within fully formed annual increment (o, p). Total cell increment according to Gompertz function and weekly cell rate of xylem and phloem cells (q, r, s, t). Control trees – solid line with black circles, AGA (above girdling area) – solid line with white circles. Bell shaped curves – weekly cell rate, S-shaped curves – Gompertz function. DOY – the day of the year (70: March 11, 100: April 10, 130: May 10, 160: June 9, 190: July 9, 220: August 8, 250: September 7, 280: October 7, 310: November 6). Vertical solid lines – standard errors. Vertical dashed lines – the month of the year. M, A, M, J, J, A, S, O, N – months in order.

Figure 6. Development of differentiated tracheids during the years 2014 and 2015 according to Wodzicki algorithm in the AGA (girdled) and control trees. Empty stripes – phase of cell enlargement (PC), grey stripes – phase of secondary cell-wall thickening and maturation (SW + MT), (n) – tracheids order. a – differentiated tracheids of control trees during 2014, b – differentiated tracheids of AGA during 2014, c – differentiated tracheids of control trees during 2015, d – differentiated tracheids of AGA during 2015.

875 Figure 7. Xylem and phloem cells' dimensions measured in 2014 and 2015. Double cell-wall thickness
876 of tracheid (a & b), the radial dimension of tracheid (c & d), the double cell-wall thickness of sieve
877 cell (e & f) and radial dimension of sieve cell (g & h). Control trees – black curve (line) and black
878 circles, AGA (above girdling area) – gray curve (line) and white circles.