Xylogenesis of Scots Pine in an uneven-aged stand of the Minusinsk Depression (Southern Siberia)

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Studies on tree-ring formation allow assessing the impact and timing of environmental factors on growth at intra-seasonal resolution. This information is relevant to understand plant acclimatization to current and expected climate changes. Still little is known on how tree age can affect the duration and intensity of annual ring formation. In this study we investigate tree-ring formation of Scots pine (*Pinus sylvestris* L.) of different age (two classes of 30 and 95 years) from a forest-steppe zone in Southern Siberia. The main tasks were 1) to identify the timing of cambial activity by distinguishing the phases of tracheids division, enlargement, wall thickening and maturation; and 2) to compare the anatomical structure of the tracheids forming the annual rings of the differently aged trees. Observations were performed on stem tissue sampling at weekly resolution from April to September 2014. The results showed different duration of the phases of xylem formation between the groups by up to 1-2 weeks, and that young trees formed a slightly narrower ring width. The tracheids size of the formed cells (i.e. the results of the enlargement phase) was not differed among the groups, whereas the dynamics of the cell-wall thickness showed significant differences. The obtained data can provide references to calibrate processed-based models linking environment to wood formation. In particular, this data allows to benchmarking time-explicit simulated measures of annual ring increment and cell anatomical structures to observation in mature trees growing under natural conditions.

Keywords: *Pinus sylvestris*, xylem, cambial activity, radial tree-growth, wood anatomical features.

Особенности ксилогенеза сосны обыкновенной в разновозрастном древостое Минусинской котловины (Южная Сибирь)

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Исследования сезонного формирования ксилемы годичных колец деревьев позволяют оценивать влияние факторов среды на рост древесных растений с высоким временным разрешением и скорость их адаптации к текущим и ожидаемым изменениям климата. Все еще недостаточно изученным является вопрос, как возраст деревьев может влиять на скорость их роста и продолжительность ксилогенеза. В связи с этим целью нашего исследования было установить особенности сезонного формирования годичных колец сосны обыкновенной (*Pinus sylvestris* L.), произрастающей в зоне лесостепи Южной Сибири, для деревьев двух возрастных групп (30 и 95 лет), и получить данные о продолжительности камбиальной активности, растяжении трахеид, утолщении их клеточной стенки и появлении зрелых трахеид в кольце, а также сопоставить анатомическую структуру образованных годичных колец у деревьев разного возраста. Отбор сезонных образцов проводился еженедельно в течение 2014 года с апреля по сентябрь. Результаты исследования показали, что длительность различных фаз формирования ксилемы у этих групп отличалась на 1-2 недели, при этом в течение сезона роста у молодых деревьев сформировалось более узкое кольцо с меньшим числом клеток, по сравнению со старыми деревьями. Радиальные размеры трахеид полностью сформированного кольца у двух групп деревьев оставались практически одинаковыми, тогда как динамика толщины клеточной стенки показала значимые различия. Полученные данные могут быть использованы в дальнейшем как входящие параметры имитационных моделей роста древесных растений и позволят получить биологически обусловленную согласованность между измеренной и имитационной величиной годичного прироста и анатомической структуры ксилемы.

Introduction

The effect of short-term climatic fluctuations (including weather anomalies) on the tree-ring growth and productivity of tree species might be assessed by xylogenesis observations performed a weekly resolution over the growing season (Lobzhanidze, 1961; Vaganov, Shashkin, 2000; Antonova, Stasova, 1997; Vieira et al., 2009; Cuny et al., 2015; Rathgeber et al., 2011). Such observation provides time-explicit ecophysiological information of the process of growth allowing us to associate physiological processes (photosynthesis, transpiration, accumulation and redistribution of carbohydrates, etc.) with structural and functional changes in xylem and to consider the wide range of factors influenced plant growth with high temporal resolution (Rinne et al., 2015; Steppe et al., 2015; Churakova et al., 2016; Fonti et al., 2018). This information is widely required to understand current and to predict expected multi-parameter tree-growth respons to climate change.

To correctly interpret the environmental variability of growth responses to environmental changes, it is also important to investigate what is the impact of ontogenetic effect on the dynamic of tree-ring formation. For example, still little is known about the effect of age on the rate and duration of tree growth (different phases of xylem formation). Previous rare studies have observed that under equal environmental conditions young trees have an earlier onset and a longer duration of each phase of xylogenesis in comparison with the old trees (Rossi et al., 2008). For this reasons young trees usually form a higher amount of cells during the vegetation period, they are more sensitive to drought, and more easily form intra-annual density fluctuation (Rossi et al., 2008; Kishchenko, 2014; Campelo et al., 2015; Vieira et al., 2018; Popkova et al., 2018). It might be assumed that the discrepancy in the timing of the different phases of xylem formation causes variability of the climate signal recorded by different parameters of tree ring as the ring width, the cell anatomical structure, and the intra-annual wood density (Connor, Lanner, 1990; Vaganov, Shashkin, 2000).

The aim of this study was to estimate the differences in the timing and rate of intra-annual xylem formation of young (30 years) and old (95 years) Scots pine trees, growing in forest-steppe zone of Southern Siberia (Russia). By quantifying the timing of cambial activity, we also assessed the onset and cessation of different phases of tracheid formation as the division, enlargement, wall thickening and maturation and estimate impact of these processes on the anatomical parameters of tracheids forming the tree rings. Analysis was performed by comparing between two groups of trees of different age.

Material and Methods

The studied area is located in the forest-steppe zone of the Southern Siberia, close to the city Minusinsk (53°39′ N, 91°35′ E, 319 m a.s.l.). The selected uneven-age *Pinus sylvestris* L. stand belongs to the pine forest band of the Minusinsk Depression (Fig. 1). The stand understory was covered by 30% of shrubs (*Caragana arborescens* Lam., *Cotoneaster melanocarpus* Fisch. ex Blytt., *Rosa acicularis* Lindl.), 40-50 % by grasses (*Iris ruthenica* Ker.-Gawl., *Phleum phleoides* (L.) H. Karst., *Poa pratensis* L., *Pulsatilla flavescens* (Zucc.) Juz., *Thalictrum minus* L.). The climate at the area is continental with an annual air temperature of 1.2 ^{o}C and the annual precipitation sum of 343 mm (data are retrieved from the Minusinsk meteorological station of the Russian Research Institute of Hydrometeorological Information for the period 1936-2015, (https://meteo.ru)).

To monitor tree stem radial growth, wood cores 5-mm in diameter were collected perpendicular to the stem axis at breast height from 20 dominant and co-dominant pine trees with regular growth and equally distributed among two age groups. Annual tree-ring width (TRW) was measured on each core using a LINTAB measuring table combined with the program TSAP (Rinntech, Heidelberg, Germany) with 0.01 mm precision (Rinn, 1996). Obtained time-series were visually cross-dated and dating quality was verified using the program COFECHA (Holmes, 2001). Five trees per age group (30 and 95 years old, respectively) were chosen for further xylogenesis observations.

Stem wood samples of a length of about 1 cm (including two–three annual rings over the last growth years) were taken from the five young and five old selected trees on a weekly basis from the beginning of April to mid-September in 2014 using a Hagloff increment borer (Sweden). Samples were taken following a spiral around the trunk at 5 cm increments starting at a stem height of 1.3 m. After collection, samples were immediately placed into cylindrical containers with glycerin-alcohol-water fixing fluid at a 1:1:1 ratio. In total, seasonal wood samples were collected 20 times over the whole vegetation period.

For each sample a 15 μm-thin microsection was obtained using a sledge microtome Thermo Scientific Microm HM 450. Sections were stained into 2% astra-blue and 1% safranin solution to increase contrast of lignified and unlignified tissues. The size of different zones of rings and the number and size of cells were measured using an image analysis system (Carl Zeiss, Jena, Germany) and AxioVision SE64 Rel. 4.9.1 software package, which make it possible to determine the parameters of cell structures in different parts of an annual ring in a semiautomatic regime. Three tracheid radial rows (from the internal to external tree-ring boundary) were measured for each annual ring and the values were then averaged.

We used the following scheme to assign the cells into developmental zones: (1) cambial zone; (2) cell extension zone; (3) zone of primary cell wall lignification; and (4) mature tracheid zone. The use of a polarization filter was used to discriminate between cell in extension and cell initiating the deposition of the secondary wall.

Tracheid anatomical parameters were measured for the last collected samples, which included all mature tracheids formed during the 2014 growing season. Five radial rows of cells with the largest cross sectional area were selected. The radial lumen diameter (LD) and double cell-wall thickness (2CWT) were measured and then used to calculate the radial size of tracheids ($D = LD + 2CWT$). According to Mork's index (Denne, 1989), the ratio between the width of the double cell wall and lumen diameter was used to assign the tracheid to early- (EW, 2CWT < LD) and late wood (LW, 2CWT > LD) of each ring. Due to different number of cells within annual ring of young and old trees, cell number (cell-wall thickness and cell radial diameter) were standardized to a common number (15) to compare the same ring sectors between two groups (Vaganov, 1990; Babushkina et al., 2019). The significance of differences of anatomical parameters between the two groups was estimated by t-test.

Results

The cambial zone of the studied trees included five to seven cells during their dormant period (in spring and autumn) (Fig. 2A). The beginning of the cambial activity, as observed on the xylem side, occurred simultaneously in both the groups on April 11 (101 day of the year (DOY)). The duration of the cambial activity lasts 110 days and on average there was 10 dividing cells within the zone. The maximum activity appeared two weeks earlier for young trees than for old trees (May 18 and June 2, correspondingly) simultaneously to the first appearance of first cells in the enlargement zone (Fig. 2B). At the end of July, the cambial activity terminated. Cell enlargement of young trees ended two weeks earlier than for the old one.

The emergence of cells in the cell-wall thickening and primary lignification zone were recorded for young trees in May 8 (128 DOY) and for old trees in May 16 (136 DOY), reaching the maximum in August 7 and 28 for young and old trees, respectively. At the last sampling day (September 18) two young trees out of five did not finished cell-wall lignification, whereas all old trees had already completed xylem formation.

The appearance of mature tracheids with the fully formed secondary cell wall in pine earlywood was simultaneous for both age group and was observed at the beginning of June. Maximum cell amount (up to 60) was counted for trees of the old group. However, the percentage of the mature tracheids in relation to the total cell number within tree ring was higher for the young trees, and reached 80% already at the beginning of August (Fig. 3).

Mean tree-ring width of young and old trees for the common period from 1985 to 2014 was 2.34 and 1.41 mm, respectively. Age dynamics of young trees was similar to the one of the old trees when they were of the same age (Fig. 4A). Similarly, the range of tree-ring width is also comparable between the groups at similar age (Fig. 4B). Periods of 1962-1965 and 1975-1977 were characterized by dry conditions (Kostyakova et al., 2018), which significantly affected the old trees (Fig. 4A). The absence of fire scars suggest that the selected trees were not affected by forest fires, despite it is common that pine forest from the Minusinsk Depression burn in years characterized by low amount of spring precipitation following a low snow period.

In 2014 tree-ring width of young and old trees was 1.52 and 1.76 mm ($p = 0.399$), with the mean cell number of 46 and 52, respectively. The intra-annual variability of the cell radial diameter of young and old trees showed similar pattern with 45 μm sized tracheids in the first part of the ring, and a regular decreased until the ring border (Fig. 5A). The cell-wall thickness have statistically significant differences between the groups, which are better pronounced in the standardized tracheidograms (Fig 5B, C). Cell-wall thickness is higher for the young trees at the second part of earlywood and transition zones (position 8-12 of the tracheidogram, $p < 0.10$), with the maximum CWT up to 6.2 μm of young trees in comparison with 5.5 μm of old trees. It is also important to notice that the cell-wall thickening occurred when the differences between the integral growth rate of the two age groups was maximized (Fig. 3).

Discussion

Based on the climatic data, the year of 2014 for the studied territory was one of the favorable among the last decades (Tychkov et al., 2019). The observed xylogenesis dynamics of the Scots pine corresponded to the earlier described observation for other conifer species in the boreal zone (Rossi et al., 2008; Bryukhanova et al., 2013). Maximum cell number in the enlargement zone reflected the highest level of the cambial activity and corresponded to the longest sunshine duration and maximal rate of photosynthesis and production of assimilates (Vaganov et al., 2006). The non-significant differences of the number of cells in the cambial zone of young and old trees resulted in similar tree rings width and number of tracheids produced. Observed delay of the cell enlargement and cell-wall thickening of old trees was reflected in only differences of the cell-wall thickness and not in the cell radial diameter. Based on the obtained results, maximal cell number in the wall thickening and mature cells zones was observed in August for the young trees, and in September for the old trees. Considering that cell-wall thickening can take place for up to 45-50 days for the old pine trees (Vaganov, Shashkin, 2000), it is possible to assume that the kinetic of cell-wall thickening not only started later, but also had a lower rate. To identify if this difference is the result of a lack of assimilates for

biosynthesis of the cell wall, or from the competition for assimilates among tracheid differentiation processes (Carteni et al., 2018), it still requires more detailed measurements and targeted experiments. However, these differences in the physiological processes (photosynthesis, transpiration, discrimination of carbon isotopes), as well as in the accumulation and redistribution of assimilates during the growing season affecting the stem growth rate and productivity of both studied groups, may also be related to their age (Meinzer et al., 2011). Indeed, as noted earlier, young trees are characterized by intensive growth, whereas mature and old trees may have more conservative growth strategy (Vieira et al., 2009). In addition, the direct influence of exogenous factors, in particular solar radiation, on the growth of Scots pine and the anatomical structure of its annual rings can be modified by the canopy position of the tree (Kishchenko, 2015). Due to the fact, that under natural (non-experimental) conditions it is rather difficult to separate the influence of the tree size from its age on stem growth, this might be the next step of a complex xylogenesis study of even- and uneven-aged forests.

It is important to notice that a smaller cell-wall thickness means less carbon accumulation in the stem of woody plants. Consequently, more active kinetics of seasonal growth of the young trees in the studied region also imply a greater carbon mass accumulation per unit of wood volume. This reinforces the proposals for the formation of young stands as potential reserves of carbon dioxide from the atmosphere (Zhou et al., 2015).

Conclusion

The data obtained could not confirm the unambiguous effect of tree age on the timing and duration of various phases of wood formation at the studied site. Considering the differences of the xylogenesis between the trees of different age classes, it is necessary to take into account the vigor, the social status, the possible level of competition and other factors that might limit growth. It is also necessary to notice that the long-term changes in annual radial growth of this species and the duration of the juvenile period play an important role. Despite the similar duration the growing season (160 days) of both young and old trees, the delay in some developmental phases of older trees compared to the young ones suggests potential time shift in the recorded climatic signal by the numerous tree-ring parameters. This assumption can be confirmed in the future by the dendroclimatic analysis. The data on the onset and duration of the cambial activity, the dynamics of seasonal cell production, their extension, and cell-wall thickening in young and old pine trees can be used in the future as input parameters for the VS-model (Vaganov, Shashkin, 2000). This will allows obtaining biologically-supported observation to benchmark simulated value of the annual growth and derived xylem anatomical structure (observed and modeled tracheidogram).

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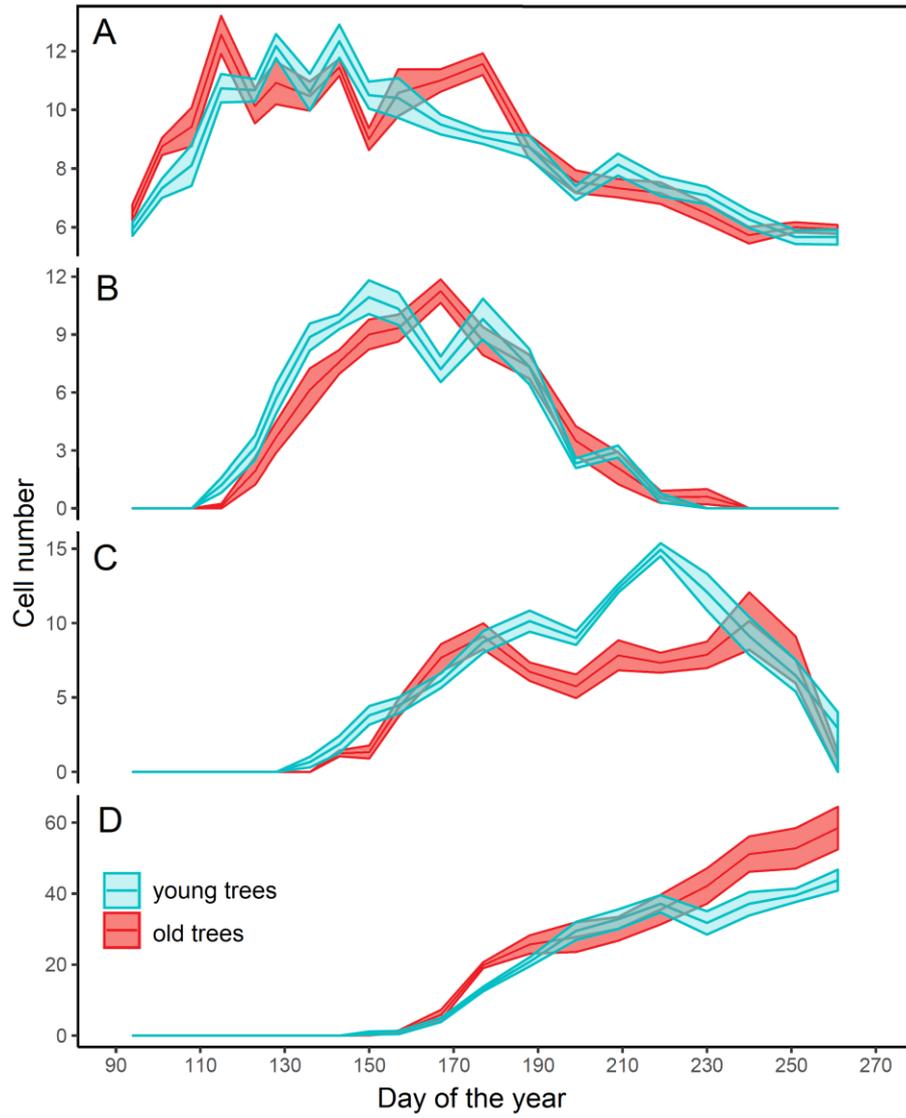
Fig. 1. Photo of the studied stand.

Fig. 2. Mean cell number (± standard error) of the different zones of the developing tree ring: A – cambial zone, B – zone of enlarging cells, C – cell-wall thickening zone, D – zone of the mature cells. Blue is young trees, red is old trees.
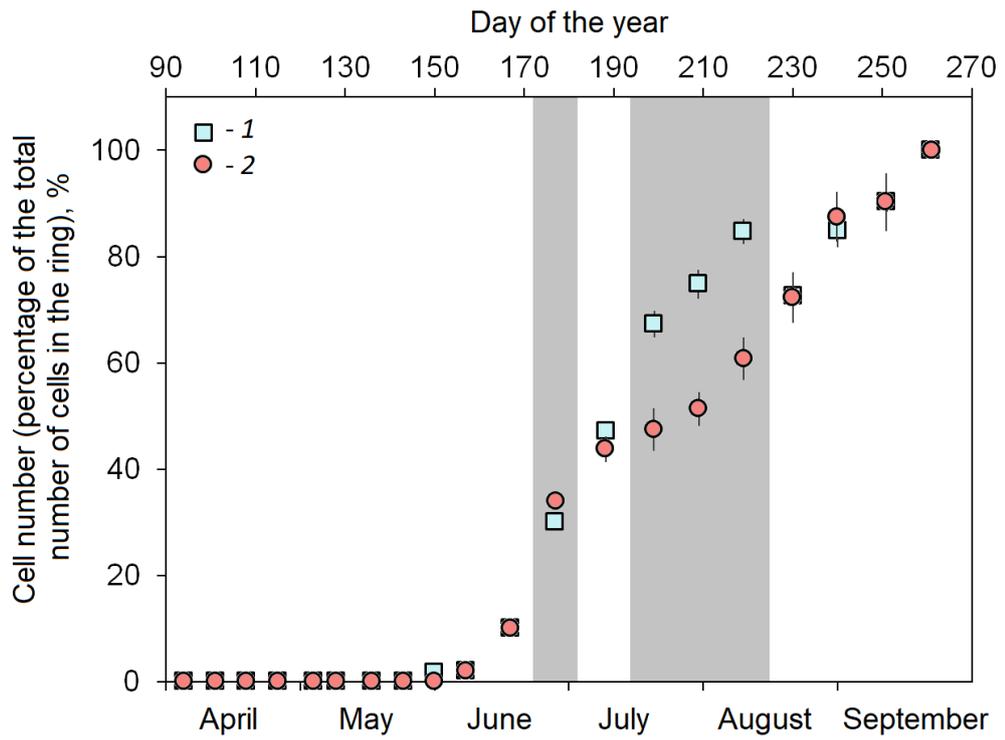
Fig. 3. Dynamics of cell number (± standard error) within tree rings of 2014: *1* − young trees, *2* − old trees. Gray area shows statistically significant differences ($p < 0.05$) between the trees of two age classes.
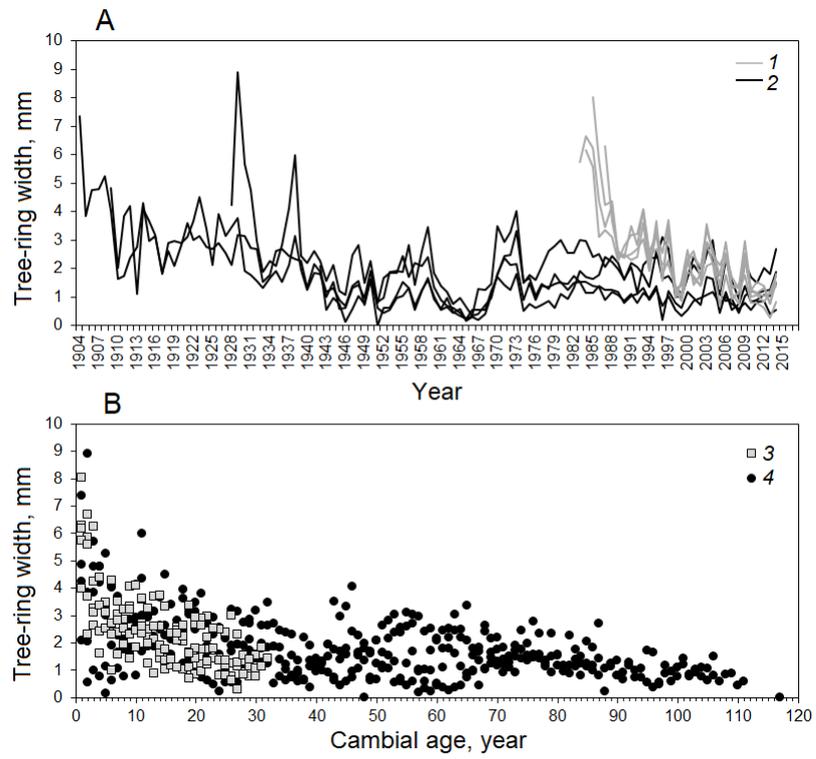
Fig. 4. *A* − individual tree-ring width chronologies of studied trees, *B* − tree radial growth according to the cambial age. *1, 3* − young trees, *2, 4* − old trees.
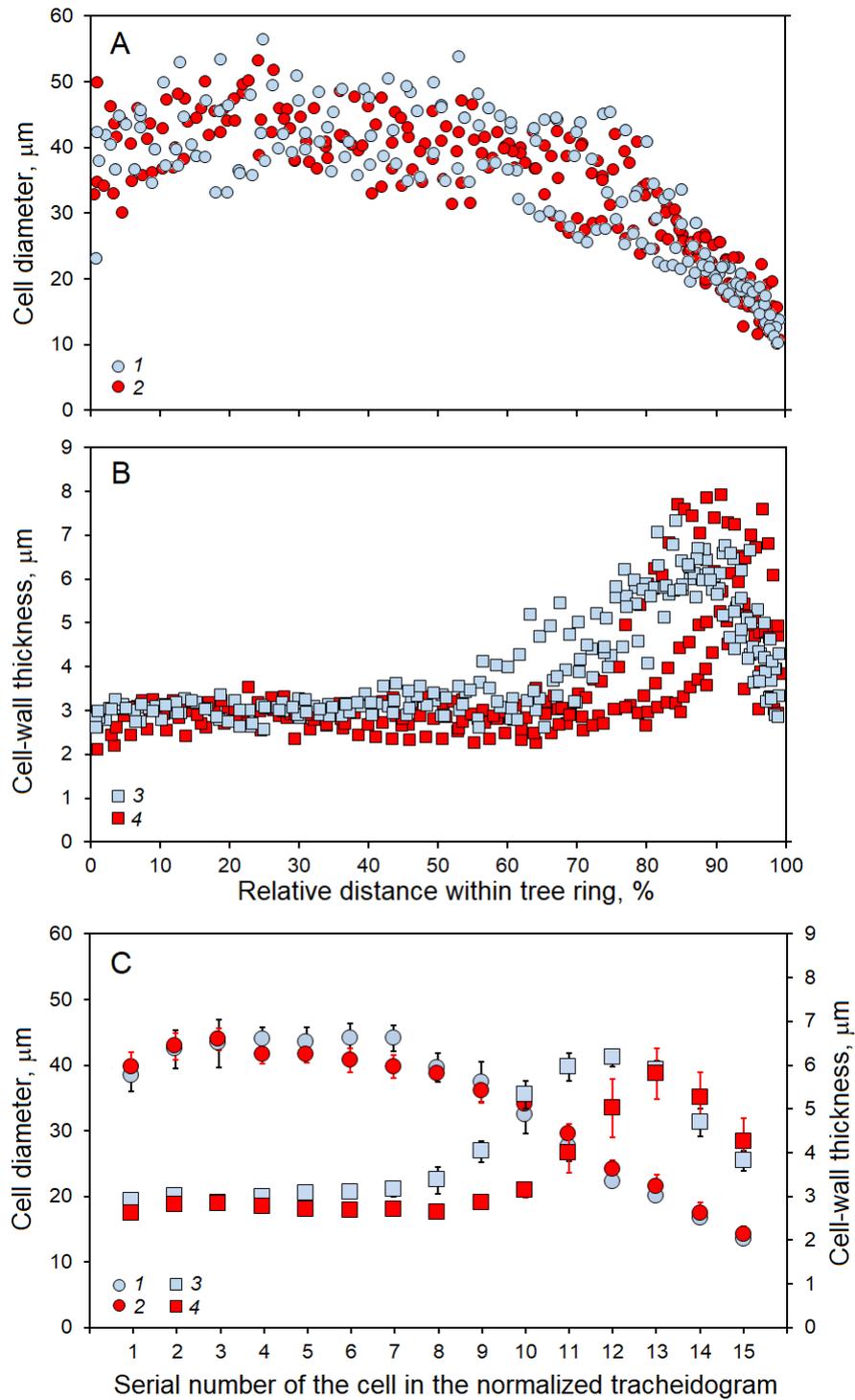
Fig. 5. Cell radial diameter (*A*) and cell-wall thickness (*B*) within pine tree rings, formed in 2014, and (*C*) standardized tracheidogram of both anatomical parameters: *1, 3* − young trees, *2, 4* − old trees. Vertical thin lines indicate the standard error.