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ORIGINAL ARTICLE

Wood structure of *Populus alba* formed in a shortened annual cycle system

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Abstract Wood formation of trees that grow along the seasons has an annual rhythm. Due to this rhythm, physiological research on the mechanism of wood formation has been difficult to conduct in a typical experimental room. In the present study, we observed the wood tissue formation in a shortened annual cycle system, which was developed for poplar trees grown in a growth chamber with dormant and non-dormant cycles. Poplar trees were grown in this system by repeating the cycle three times. The resulting wood tissue consisted of three growth rings and very similar structures were observed around the ring boundary of the wood in a field-grown stem. This result suggests that the shortened annual cycle system can be adopted as a model for physiological, cell biological and molecular research of wood and annual ring formation.

Keywords Annual cycle · Annual ring · Wood formation · Dormancy · Poplar (*Populus alba*)

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Introduction

Wood formation has an annual rhythm in the temperate region and the structures within the annual ring are synchronized to the seasons. Typically, large vessels of ring-porous wood are formed in spring, and thick-walled tracheids in conifer wood are formed during late summer to autumn. Annual ring is an important aspect of the physical properties, influences texture of wood species and appearance, and is significant in the field of wood research. On the contrary, annual rhythm of the field-grown trees restricts researchers to obtain only one chance a year to apply experimental treatments to the stem that forms specific types of cells or structures, and/or harvesting such stem. Researchers have to accomplish all these activities within a short period or prolong their experiments over multiple years.

Although there are many physiological studies conducted on trees in controlled day length and/or temperature setting using growth chambers [1–18], there is no description about wood formation. Most studies focused on buds and/or shoots and their dormant status [1–14]. Only two reports focused on tree stem grown in a growth chamber with changing culture conditions. One report described the amount of nuclear DNA in cambial cells of *Abies balsamea* [15], while the other focused on gene expression of bark storage protein in poplar [16]. Both studies did not refer to wood formation inside the stem. All those studies aimed to understand the response to changes in day length and/or temperature. As such, the growth condition was changed only once; the conditions did not vary when growing trees.

We recently established a shortened annual cycle system for poplar trees using growth chambers [19]. Poplar is widely adopted as a model angiosperm tree [20], and the

author continually maintains a subculture of poplar in the growth room for the past 15 years, and produces transgenic plants for experimental use [21–27]. In this shortened annual system for poplar, we mimicked leaf color change, defoliation, dormancy, bud breaking and growing within a period of 4–5 months. Furthermore, phosphate retranslocation from leaves to stem during winter condition occurred similar to that seen in the field-grown poplar trees [19], and a heavily branched architecture was formed [28]. In the present study, we grew poplar trees using this system for three cycles, and the resulting wood tissue was compared to that of wood grown in a field, to verify whether this shortened annual cycle system has the potential to serve as a model for investigating wood formation with an annual rhythm.

Materials and methods

Plant materials

All the individuals of poplar (*Populus alba* L.) in this study were ramets of the same clone, including both in the growth room and in the field.

Cuttings of 3–5 cm length with 1–3 leaves were obtained from subcultured poplar trees approximately 20–40 cm tall, which were grown in a growth room (closed, 14-h light, 24–28 °C). The cuttings were placed in pots (7.5 cm diameter, 6.5 cm depth) containing a mix of vermiculate:red clay ball soil (2:1). The potted cuttings were incubated with water only, until rooting. After the confirmation of new shoot growth from the axillary bud, the potted cuttings were placed in plastic containers (16 cm × 11 cm, 4.5 cm depth) one by one, and cultured with 2000-fold-diluted Hyponex fertilizer (N:P:K = 6:10:5, HYPONeX Japan, Osaka, Japan) placed at 0.5–2 cm depth. Four individual poplar trees were cultured for three cycles of the shortened annual cycle system described below.

As for the field-grown tree, a 3-year-old branch that grew upright from the basal part of a tree (12 years old in 2016, 10 m approx. height and 30 cm diameter at breast height) was obtained. The tree was planted at the Uji campus of Kyoto University. This branch was used for the microscopic observations of wood tissue.

Shortened annual cycle system

The culture conditions are presented in Table 1 [19]. This system contained three stages: Stage 1 (long day, high temperature) was carried out in the growth room as described above, Stages 2 (short day, middle temperature), and 3 (short day and low temperature) were carried out in a plant growth chamber (LH-410PFD-SP, NK System, Osaka, Japan). Duration of Stages 1 and 2 was set at 1 month each, and the duration of Stage 3 was determined based on visual assessment of the leaves, such as if they turned completely yellow and easily detached from the stem due to formation of the abscission layer. Therefore, Stage 3 had a slightly extended period (2–3 months) compared to Stages 1 and 2. The time span of culturing for three cycles in this system was approximately 15 months, including new root and shoot formation of the cuttings.

Four other cuttings prepared at the same time as the experimental samples were used as a control which was subjected to a fixed growth condition identical to Stage 1 of the shortened annual cycle system.

To assess the thickening growth of stems of trees, the stem diameter of the newly growing shoot of the cuttings at approximately 2 cm height from the basal point of the bud was measured at the end of each stage. Similar measurements were recorded at the same time for the control tree samples as well.

Microscopy

After completing the third cycle of culture using the shortened annual cycle system, the stem samples were cut and preserved in FAA fixative (5% formalin, 5% acetic acid, and 40% ethanol) until use. Transverse sections of 25-μm thickness were prepared using a sliding microtome and stained with a mixed solution of 1% Safranin and 1% Astra blue. After dehydrating the sections with an ethanol series, the sections were soaked in xylene twice prior to mounting on glass slides with Biolite (Okenshoji, Tokyo, Japan). These sections were observed under a light microscope (BX50, Olympus, Tokyo, Japan) along with those from field-grown trees and control trees cultured under fixed conditions.

Table 1 Culture conditions used in the shortened annual cycle

Stages	Temperature (°C)	Day/night (h)	Period (month)	Mimicked season
1	24–28	14/10	1	Spring/summer
2	15	8/16	1	Autumn
3	5	8/16	2–3	Winter

The period of Stages 1 and 2 were set for 1 month, but for Stage 3 was determined by the leaf condition: easily removed when it was touched. As a result, the period for the last stage had a range of 2–3 months

Results and discussion

All the newly grown shoots of the cuttings that were cultured in the shortened annual cycle system for three cycles displayed leaf senescence during each Stage 3 and bud breaking at the beginning of each Stage 1 during the cycle. The stem diameter was measured at the end of each Stage and is shown in Fig. 1. In the first cycle, the diameter of all the trees (1–4) increased the most in Stage 1, slightly in Stage 2, and did not change in Stage 3. Subsequently, only tree 4 failed to grow, although it showed new leaf formation after every dormant phase. The other trees (1–3) continued to grow in both the second and third cycles similar to that in the first cycle. However, in Stage 2 of the second cycle, the trees showed more growth than in the same stage of the other cycles. The control trees, which grew only in Stage 1, displayed linear growth during the first 4–5 months. Later, their growth rate decreased, but the stem diameter remained unchanged.

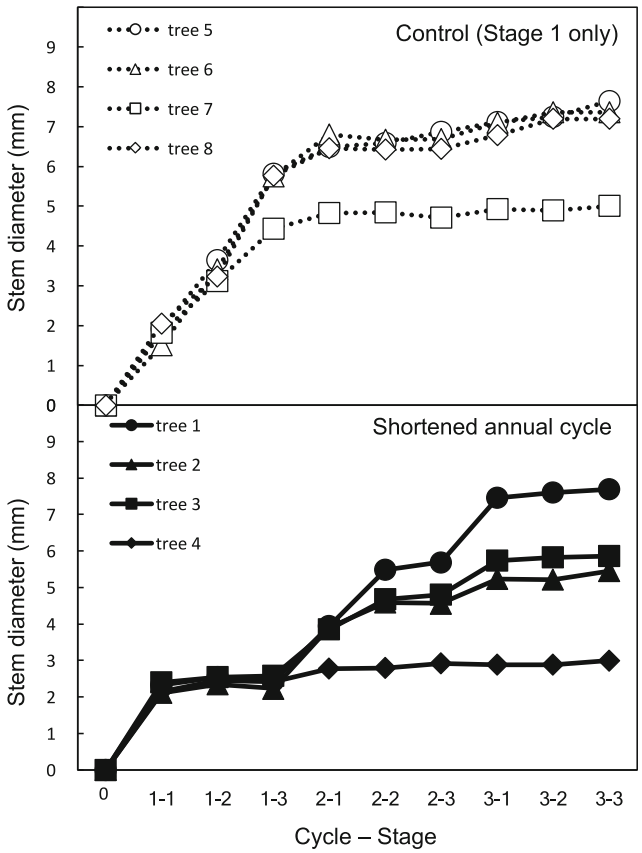

Fig. 1 Thickening growth under shortened annual cycle conditions. The diameter of the trees under the shortened annual cycle was measured at the end of each stage. Those of the control trees were also measured at the same time as above

Transverse sections of the stem after the third culture cycle are shown in Fig. 2. There were three growth rings with two boundaries in trees 1–3, and two rings were found in tree 4. Such distinct rings were not found in the stems of control trees (5–8, Fig. 3). Magnified micrographs highlighting areas around the ring boundary are shown in Fig. 3. All trees grown under the shortened annual cycle system had structures very similar to those of field-grown trees. Terminal parenchyma with a smaller radial diameter on the ring boundary, and axial parenchyma cells, which had inclusion well stained with Astra blue, were more visible in the early wood than in the late wood. Additionally, the vessel diameter in the early wood was found to be almost twice of that observed in the late wood. A micrograph of control (tree 5) showed a tangential cell line with small radial diameter (arrow in Fig. 3), but there was no difference in the vessel diameter and parenchyma cell rate between cells above and below this line. Within a growth ring (Fig. 4), the trees grown under the shortened annual cycle system showed a sudden decrease in vessel size, in the last quarter of the ring, whereas the field-grown trees showed a gradual decrease in vessel size during ring formation. This would likely be caused by the stepwise

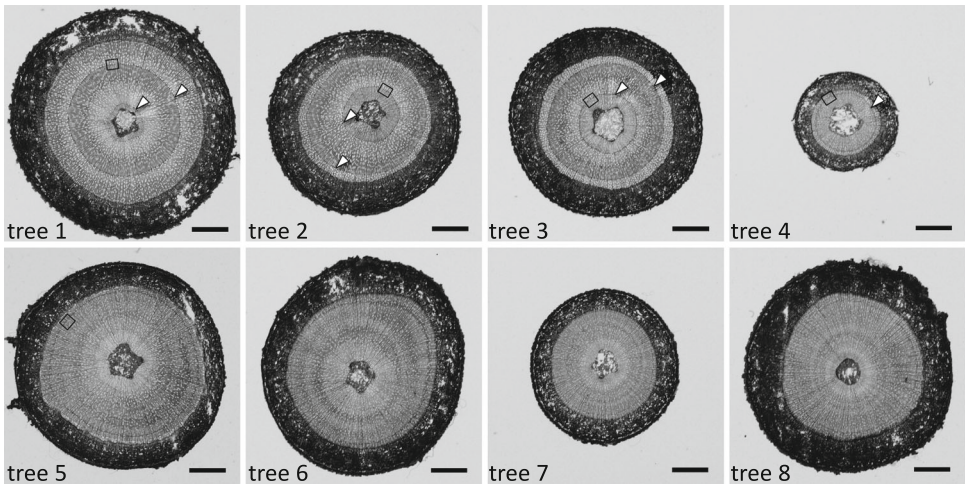

Fig. 2 Transverse sections of poplar stem grown in the shortened annual cycle (trees 1–4) and in the control (trees 5–8). Arrowheads indicate the ring boundaries, and rectangles show the magnified area in Fig. 3. Bar = 1 mm

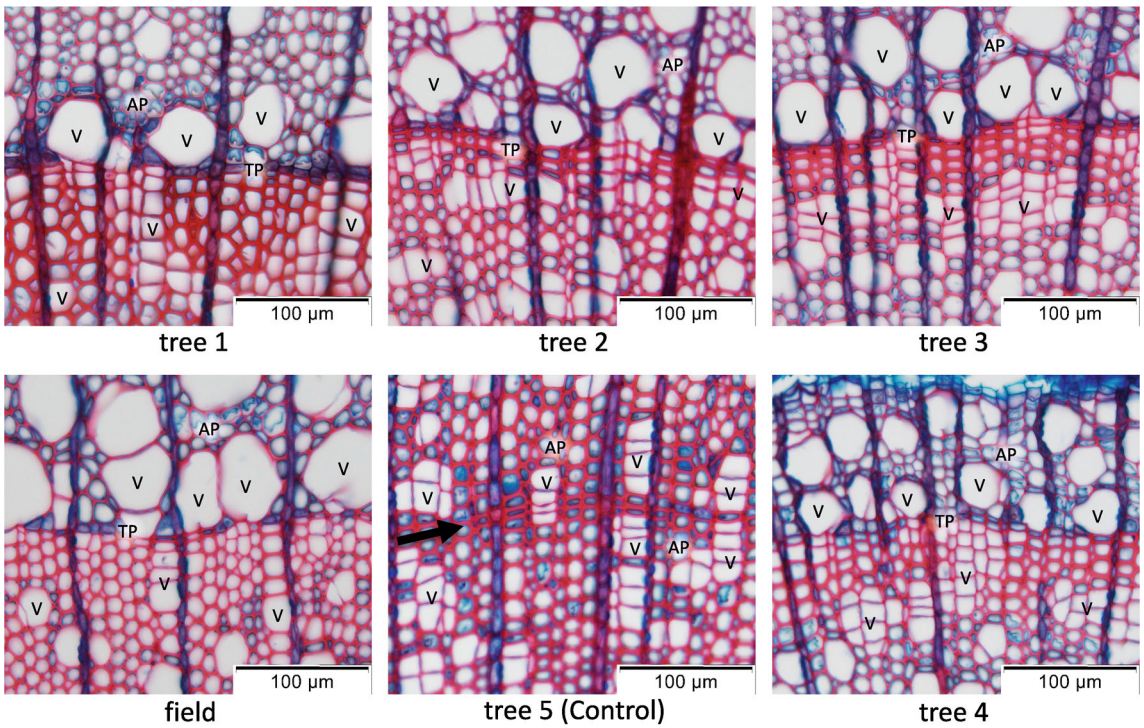

Fig. 3 Micrographs of annual ring boundaries. *V* vessel, *TP* terminal parenchyma, *AP* axial parenchyma. Only tree 5 did not form early wood; dense tissue appearance was observed on both sides of the boundary-like cell line (arrow)

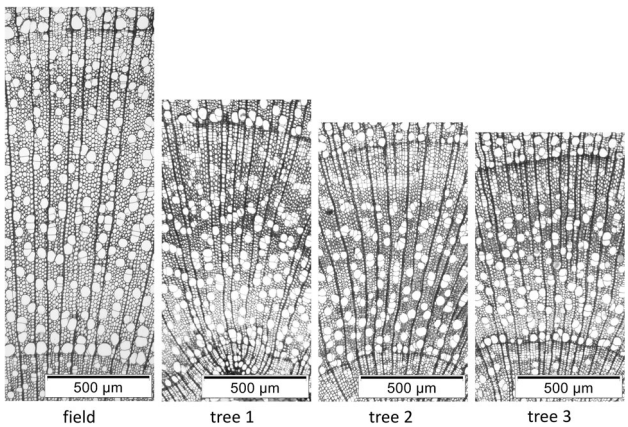

Fig. 4 Vessel size transition within an annual ring. The annual rings shown here were: field; the second year of 3-year-old field-grown branch, trees 1–3; the second cycle of the shortened annual cycle system

change in temperature and/or day length in the shortened culture system.

These results suggest that the shortened annual cycle system is a potential model for the physiological, cell biological and molecular studies of wood formation with an annual rhythm, at least between the dormant and dormancy-breaking stages. To simulate more precise formation of late wood, improvements can be attempted, specifically in Stage 2. This shortened annual cycle system could also become a useful model system for field studies such as investigating the timing between bud breaking and cambial activity [29–32], cambial reaction to partial heating [33–35] and partial cooling [36] of tree stem. In fact, using a similar system, it was reported that the roots alter their functions depending on the seasons [17, 18]. This shortened culture system would condense the duration of research study if used as a model to test various hypotheses prior to conducting elaborate field experiments. In addition, it provides an easy control and the ability to manipulate the growth conditions.

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References

1. Keskitalo J, Bergquist G, Gardeström P, Jansson S (2005) A cellular timetable of autumn senescence. Plant Physiol 139:1635–1648
2. van der Schoot C, Rinne PLH (2011) Dormancy cycling at the shoot apical meristem: transitioning between self-organization and self-arrest. Plant Sci 180:120–131
3. Rinne P, Saarelainen A, Junttila O (1994) Growth cessation and bud dormancy in relation to ABA level in seedling and coppice shoots of *Betula pubescens* as affected by a short photoperiod, water stress and chilling. Physiol Plant 90:451–458

4. Ruttink T, Arend M, Morreel K, Storme V, Rombauts S, Fromm J, Bhalerao RP, Boerjan W, Rohdea A (2007) A molecular timetable for apical bud formation and dormancy induction in poplar. Plant Cell 19:2370–2390
5. Rinne PLH, Paul LK, Vahala J, Ruonala R, Kangasjärvi J, van der Schoot C (2015) Long and short photoperiod buds in hybrid aspen share structural development and expression patterns of marker genes. J Exp Bot 66:6745–6760
6. Howe GT, Gardner G, Hackett WP, Furnier GR (1996) Phytochrome control of short-day-induced bud set in black cottonwood. Physiol Plant 97:95–103
7. Wake CMF, Fennell A (2000) Morphological, physiological and dormancy responses of three *Vitis* genotypes to short photoperiod. Physiol Plant 109:203–210
8. Pagter M, Liu F, Jensen CR, Petersen KK (2008) Effects of chilling temperatures and short photoperiod on PSII function, sugar concentrations and xylem sap ABA concentrations in two *Hydrangea* species. Plant Sci 175:547–555
9. Welling A, Kaikuranta P, Rinne P (1997) Photoperiodic induction of dormancy and freezing tolerance in *Betula pubescens*. Involvement of ABA and dehydrins. Physiol Plant 100:119–125
10. Welling A, Moritz T, Palva ET, Junttila O (2002) Independent activation of cold acclimation by low temperature and short photoperiod in hybrid aspen. Plant Physiol 129:1633–1641
11. Heide OM (1974) Growth and dormancy in Norway spruce ecotype (*Picea abies*) I. Interaction of photoperiod and temperature. Phyiol Plant 30:1–12
12. Olsen JE, Junttila O, Moritz T (1997) Long-day induced bud break in *Salix pentandra* is associated with transiently elevated levels of GA_1 and gradual increase in indole-3-acetic acid. Plant Cell Physiol 38:536–540
13. Azeez A, Miskolczi P, Tylewicz S, Bhalerao RPA (2014) Tree ortholog of APETALA1 mediates photoperiodic control of seasonal growth. Curr Biol 24:717–724
14. Böhlenius H, Huang T, Charbonnel-Cambaa L, Brunner A, Jansson S, Strauss S, Nilsson O (2006) CO/FT regulatory module controls timing of flowering and seasonal growth cessation in trees. Science 312:1040–1043
15. Meilerowicz EJ, Riding RT, Little CHA (1992) Periodicity of cambial activity in *Abies balsamea*. II. Effects of temperature and photoperiod on the size of the nuclear genome in fusiform cambial cells. Physiol Plant 85:526–530
16. Zhu B, Coleman GD (2001) Phytochrome-mediated photoperiod perception, shoot growth, glutamine, calcium, and protein phosphorylation influence the activity of the poplar bark storage protein gene promoter (bspA). Plant Physiol 126:342–351
17. Noda Y, Furukawa J, Aohara T, Nihei N, Hirose A, Tanoi K, Nakanishi TM, Satoh S (2016) Short day length-induced decrease of cesium uptake without altering potassium uptake manner in poplar. Sci Rep 6:38360
18. Aohara T, Mizuno H, Kiyomichi D, Abe Y, Matsuki K, Sagawa K, Mori H, Iwai H, Furukawa J, Satoh S (2016) Identification of a xylem sap germin-like protein and its expression under short-day and non-freezing low temperature conditions in poplar root. Plant Biotech 33:123–127
19. Kurita Y, Baba K, Ohnishi M, Anegawa A, Shichijo C, Kosuge K, Fukaki H, Mimura T (2014) Establishment of a shortened annual cycle system; a tool for the analysis of annual retranslocation of phosphorus in the deciduous woody plant (*Populus alba* L.). J Plant Res 127:545–551
20. Chaffey N (2002) Why is there so little research into the cell biology of the secondary vascular system of trees? New Phytol 153:213–223
21. Kaku T, Baba K, Tanigchi T, Kurita M, Konagaya K, Ishii K, Kondo T, Serada S, Iizuka H, Kaida R, Taji T, Sakata Y, Hayashi T (2012) Analyses of leaves from open field-grown transgenic poplars overexpressing xyloglucanase. J Wood Sci 58:281–289
22. Park YW, Baba K, Furuta Y, Kojiro K, Yoshida M, Hayashi T (2010) Characterization of poplar overexpressing xylanase. Wood Res J 1:50–55
23. Kaida R, Kaku T, Baba K, Sri H, Enny S, Hayashi T (2009) Enhancement of saccharification by overexpression of poplar cellulase in sengon. J Wood Sci 55:435–440
24. Kaku T, Serada S, Baba K, Tanaka F, Hayashi T (2009) Proteomic analysis of the G-layer in poplar tension wood. J Wood Sci 55:250–257
25. Baba K, Park YW, Kaku T, Kaida R, Takeuchi M, Yoshida M, Hosoo Y, Ojio Y, Okuyama T, Taniguchi T, Ohmiya Y, Kondo T, Shani Z, Shoseyov O, Awano T, Serada S, Norioka N, Norioka S, Hayashi T (2009) Xyloglucan for generating tensile stress to bend tree stem. Mol Plant 2:893–903
26. Kaida R, Kaku T, Baba K, Oyadomari M, Watanabe T, Nisida K, Kanaya T, Shani Z, Shoseyov O, Hayash T (2009) Loosening xyloglucan accelerates the enzymatic degradation of cellulose in wood. Mol Plant 2:904–909
27. Park YW, Baba K, Furuta Y, Iida I, Sameshima K, Arai M, Hayashi T (2004) Enhancement of growth and cellulose accumulation by overexpression of xyloglucanase in poplar. FEBS Lett 564:183–187
28. Baba K, Kurita Y, Mimura T (2017) Architectural morphogenesis of poplar grown in a shortened annual cycle system. Sustain Humanosphere 13 (**in print**)
29. Kudo K, Yasue K, Hosoo Y, Funada R (2015) Relationship between formation of earlywood vessels and leaf phenology in two ring-porous hardwoods, *Quercus serrata* and *Robinia pseudoacacia*, in early spring. J Wood Sci 61:455–464
30. Takahashi S, Okada N, Nobuchi T (2013) Relationship between the timing of vessel formation and leaf phenology in ten ring-porous and diffuse-porous deciduous tree species. Ecol Res 28:615–624
31. Takahashi S, Okada N, Nobuchi T (2014) Relationship between vessel porosity and leaf emergence pattern in ring- and diffuse-porous deciduous trees in a temperate hardwood forest. Botany 93:31–39
32. Kitin P, Funada R (2016) Earlywood vessels in ring-porous trees become functional for water transport after bud burst and before the maturation of the current-year leaves. IAWA J 37:315–331
33. Kudo K, Nabeshima E, Begum S, Yamagishi Y, Nakaba S, Oribe Y, Yasue K, Funada R (2014) The effects of localized heating and disbudding on cambial reactivation and formation of earlywood vessels in seedlings of the deciduous ring-porous hardwood, *Quercus serrata*. Ann Bot 113:1021–1027
34. Oribe Y, Kubo T (1997) Effect of heat on cambial reactivation during winter dormancy in evergreen and deciduous conifers. Tree Physiol 17:81–87
35. Begum S, Nakaba S, Yamagishi Y, Oribe Y, Funada R (2013) Regulation of cambial activity in relation to environmental conditions: understanding the role of temperature in wood formation of trees. Physiol Plant 147:46–54
36. Begum S, Kudo K, Matsuoka Y, Nakaba S, Yamagishi Y, Nabeshima E, Rahman MH, Nugroho WD, Oribe Y, Jin H-O, Funada R (2016) Localized cooling of stems induces latewood formation and cambial dormancy during seasons of active cambium in conifers. Ann Bot 117:465–477