Research paper

Age dependence of xylogenesis and its climatic sensitivity in Smith fir on the south-eastern Tibetan Plateau

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An age effect on growth trends and climate/growth relationships of trees can possibly be discovered by analysing the seasonal dynamics of xylem development. The aims of this study, therefore, were to compare xylem formation of young (43 ± 4 years) and old (162 ± 26 years) Smith fir (*Abies georgei* var. *smithii* (Viguie & Gaussen) W. C. Cheng & L. K. Fu) trees in the Sygera Mountains, south-eastern Tibetan Plateau and, to identify the association between wood formation and climate. The seasonal radial growth dynamics of young and old trees was monitored on microcores collected at weekly intervals during two growing seasons. Transverse sections through phloem, cambium and outermost xylem of 9–12 μm thickness were observed with a light microscope under bright field and polarized light to follow the cambial activity and differentiation of the developing xylem. Young trees were characterized by an earlier onset of xylogenesis, a longer growing season and a higher growth rate, resulting in a higher number of xylem cells. Both young and old trees responded fast to changes of the minimum air temperature, confirming that this factor was dominant by controlling Smith fir growth on the south-eastern Tibetan Plateau.

Keywords: *Abies georgei* var. *smithii*, age dependence, dendroclimatology, minimum temperature, south-eastern Tibetan Plateau, tree ring, weekly wood formation, xylogenesis.

Introduction

Tree-ring chronologies are considered suitable tools for reconstructing past climate (Fritts 1976, Schweingruber 1996, Hughes et al. 2011). Their reliability is based on the assumption that the processes linking climate with tree-ring growth remain unaltered over time (Hutton 1788). However, several recent studies have demonstrated that the relationships between growth and climate differ with tree age (e.g., Eckstein and Krause 1989, Carrer and Urbinati 2004, Linderholm and Linderholm 2004, Mencuccini et al. 2005, Yu et al. 2008, De Luis et al. 2009, Rozas et al. 2009, Vieira et al. 2009, Wang et al. 2009), but such evidence was not generally confirmed (Esper et al. 2008). As tree rings are the most widely distributed and best replicated proxies for reconstructing climate, it is necessary to evaluate the age dependence of growth responses to climate and to confirm the assumption that environment affects tree-ring formation independently of tree age.

An increasing number of studies on the temporal dynamics of cambial activity have been conducted with various tree species worldwide during the past three decades (e.g., Eckstein 1983, Sass et al. 1995, Deslauriers and Morin 2005, Gričar et al. 2005, Rossi et al. 2006, Camarero et al. 2010), enhancing our understanding of wood formation and its association with climate. It has also been shown that both intra- and inter-annual growth–climate associations may provide more climatic information than those derived from either of them (Čufar et al. 2008, 2011, Mäkinen et al. 2008, Oberhuber and Gruber 2010, Seo et al. 2011).

On the Tibetan Plateau, dendroclimatic investigations have considerably increased in number over the last decade (see a brief review by Liang et al. 2012), but basic knowledge on the temporal dynamics of xylem development is still limited. A main limitation is retrieving climatic signals from tree rings from a physiological point of view. Smith fir (*Abies georgei* var. *smithii* (Viguie & Gaussen) W. C. Cheng & L. K. Fu) is one of the dominant tree species with a strong dendroclimatic potential on the south-eastern Tibetan Plateau (Liang et al. 2009). However, information on its intra-annual wood formation dynamics and age-related xylogenesis is lacking. Important questions remain whether the xylem in young trees is able to record useful climatic signals, and whether this information remains stable during the lifespan of the trees.

The aims of the present study, therefore, were to compare the timing and duration of different phases of wood formation and their relationships with climate between two age classes to investigate the age-dependent dynamics of xylem formation in Smith fir. Based on the recent findings (e.g., McMillan et al. 2008, Rozas et al. 2009, Copenheaver et al. 2011, Haavik et al. 2011), we challenged the hypotheses that cambial phenology changes with age and that age increases the sensitivity of trees to climate.

Materials and methods

Study area and tree species

The study was conducted on the eastern side of the Sygera Mts. on the south-eastern Tibetan Plateau, China (Figure 1). The climate is semi-humid, with abundant monsoon rainfall during summer. According to the meteorological records in Nyingchi (Linzhi, 29°34′N, 94°28′E, 3000 m a.s.l.) for the period 1960–2009, mean annual precipitation is 673 mm, 72% of which falls during the monsoon season from June to September (Liang et al. 2010). The annual mean temperature is ~8.8°C, with snow cover usually persisting from November until May.

Smith fir (*A. georgei* var. *smithii*) grows along an altitudinal gradient from 3550 to 4400 m a.s.l. (Liang et al. 2011*a*). The study site is located at 3850 m a.s.l. (Figure 1) on a south-east facing slope with an angle of 15° and characterized by acid brown forest soils with a thickness of >0.5 m. The forest canopy coverage is around 0.6.

Sampling design and determination of wood formation

Two age classes were defined and five trees per class were selected on the same site in April 2007, resulting in young and old trees with a mean age of 43 ± 4 and 162 ± 26 years, and diameter at breast height of 11 ± 2 and 54 ± 9 cm, respectively. Only healthy and undamaged trees were selected, while trees with polycormic stems, partially dead crowns or obvious reaction wood were avoided.

Wood formation was weekly monitored from May to October in 2007 and 2008. Microcores (15 mm long, 2 mm in diameter) were sampled around the stem at breast height using Trephor (Rossi et al. 2006) and fixed in formalin–ethanol–acetic acid solution. In the laboratory, the microcores were rinsed with water, dehydrated in a graded series of ethanol and embedded in paraffin. Transverse sections, 9–12 μm thick, were cut with a Leica RM 2245 rotary microtome (Leica
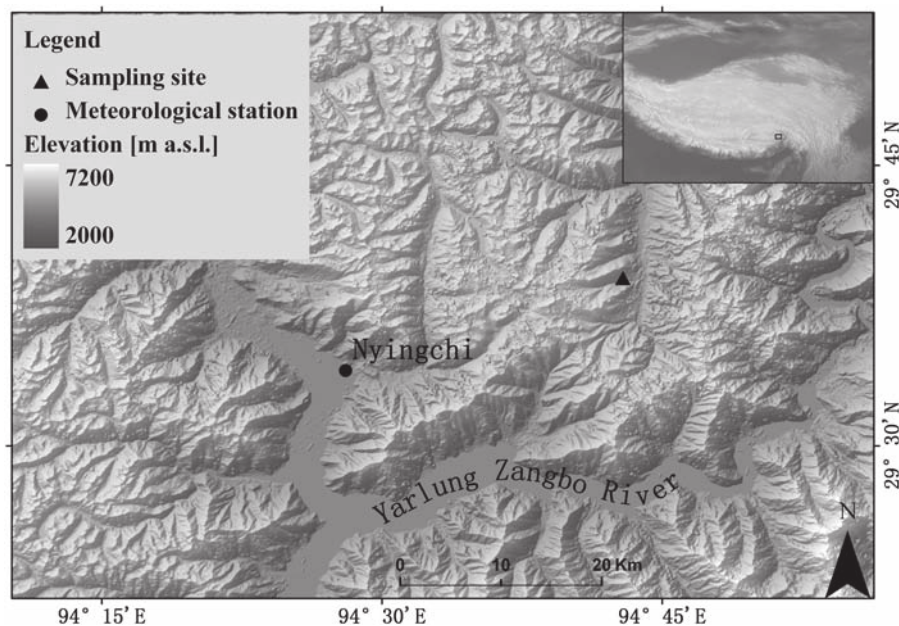

Figure 1. Map showing the location of the forest sampling site and of the meteorological station at Nyingchi; inset (upper right corner) shows the location of the study area on the Tibetan Plateau.

Microsystems, Wetzlar, Germany), using Feather N35H knives (Osaka, Japan), and stained with 3% safranin (Merck, Darmstadt, Germany) and 0.5% astra blue (Sigma-Aldrich, Steinheim, Germany), both in 95% ethanol. The sections were observed with a Nikon Eclipse 800 light microscope under bright field and polarized light to follow the cambial activity and to differentiate the developing xylem.

In spring, when at least one cell was observed in the enlarging phase, xylem formation was generally considered to have begun (Rossi et al. 2006). For most microcore cross-section slides, three radial files were randomly selected for observations, in few cases we chose the files in which xylem differentiation phases could be easily identified. Considering a weekly sampling interval in our study, an occurrence of 1–2 enlarging xylem cells along any of the checked three radial files was a threshold to identify the onset of wood formation. The phases of xylem differentiation (Figure 2) were defined as follows: cells in the phase of enlarging (EM) and of wall thickening and lignification (WT) and mature cells (MC) (Gričar et al. 2005, Rossi et al. 2006, Oladi et al. 2011). In a cross section, cambial cells were characterized by thin cell walls and small radial diameters. During cell enlargement, the tracheids contained protoplast and had thin primary walls but their radial diameter was at least twice the size of cambial cells (Rossi et al. 2006). Measurements in five young and five old Smith fir trees showed that the radial diameters of the first mature earlywood tracheids were 31.80 ± 1.05 and $30.65 \pm 1.75\,\mu m$ in 2007, and 25.54 ± 1.34 and $29.01 \pm 3.32\,\mu m$ in 2008, respectively. The dimensions of tracheids showed no significant age-dependent differences (analyses of variance (ANOVA), $P > 0.05$). Thus, tree age should have no effect on identifying the first enlarging cells according to the above definition. Observations under polarized light enabled us to discriminate between enlarging and cell-wall-thickening tracheids. When all walls of the tracheids were lignified and the protoplast was lost, cells were mature and wood formation was considered complete (Rossi et al. 2006). The numbers of tracheids in each of the developmental stages were counted along three radial files. We should keep in mind that they can be different along the three checked radial files. The mean rate of xylem-cell production was estimated as a ratio between the final number of cells and the duration of xylogenesis.

Statistical analyses

Air temperature and precipitation have been recorded daily by a meteorological station (linear distance ~50 km from the study site) in Nyingchi, which suitably represented variations in temperature and precipitation of the study area (Liang et al. 2011b), and Pearson's correlations with xylem-cell production (including cells in enlarging and wall-thickening phase and mature cells) were calculated. To verify the presence of time-lag effects, cross-correlations were used. The daily weather data were averaged for various 7-day periods, where the first one corresponded to the period between two subsequent sampling dates (referred to as P0), and then to the periods which were successively shifted backwards by 1–10 days before the sampling date (referred to as P1 to P10). Herein, weekly minimum and maximum temperatures represented the lowest and the highest temperature of the average 7-day period (Deslauriers and Morin 2005).

Timing and duration of xylogenesis, the final number of xylem cells and the rate of cell production were compared between the age classes and study years using ANOVA (SAS Institute Inc., Cary, NC, USA). Before, the data were checked for normal distribution and uniformity of variance using the Shapiro–Wilk and Bartlett's test, respectively (Zar 1999).
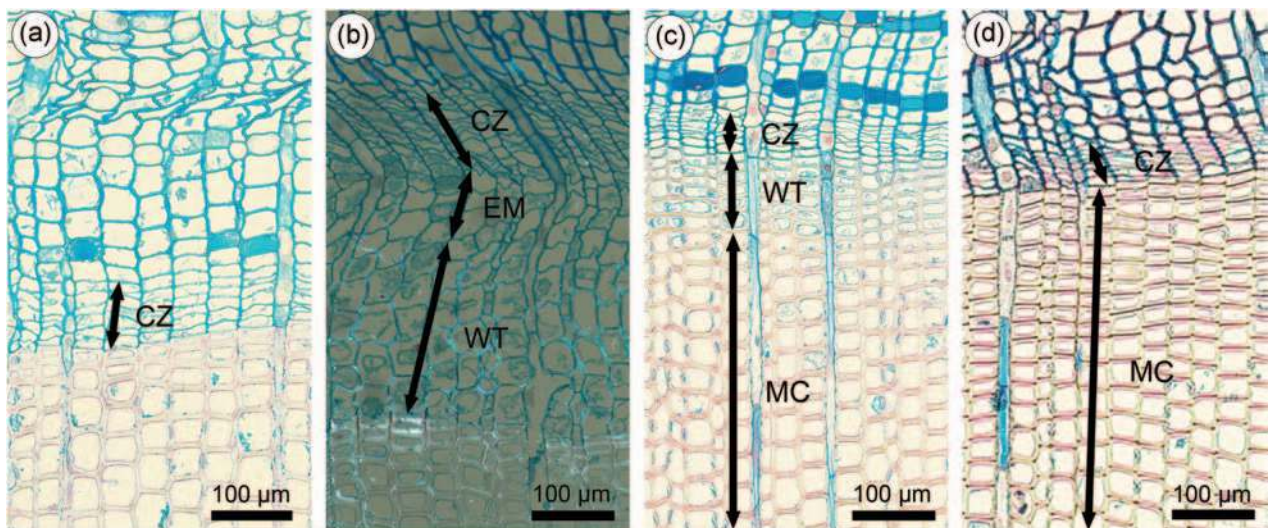
Figure 2. Phases of xylem formation in Smith fir: (a) cambial cells (CZ) on 21 April, (b) enlarging (EM) and wall-thickening (WT) cells, under polarized light, on 16 June, (c) cell-wall-thickening (WT) and mature cells (MC) on 19 August, (d) mature cells on 30 September.

Results

Xylem differentiation

The timing of cell differentiation differed between young and old trees in both years. In the first samples collected on 24 May 2007, eight and two layers of enlarging (EM) cells were detected in young and old trees, respectively. Because of too high snow level, the sampling area could not be accessed and therefore we missed the onset of wood formation in 2007, but higher number of newly formed tracheids in young trees suggested that wood formation started earlier in young than in old trees (Figure 3). In 2008, cell enlargement started in young trees on 10 May and in old trees on 31 May (Figure 3; Table 1) (ANOVA, $P < 0.001$); in both cases, we observed 1–2 EM cells. Heavy snowfall between 10 and 31 May prevented us from sampling, but small number of EM cells and no cells in the phase of wall thickening indicated that cell production in old trees started at the end of May.

In 2007, the highest numbers of enlarging cells in both age classes were observed from the end of May to mid-June, while
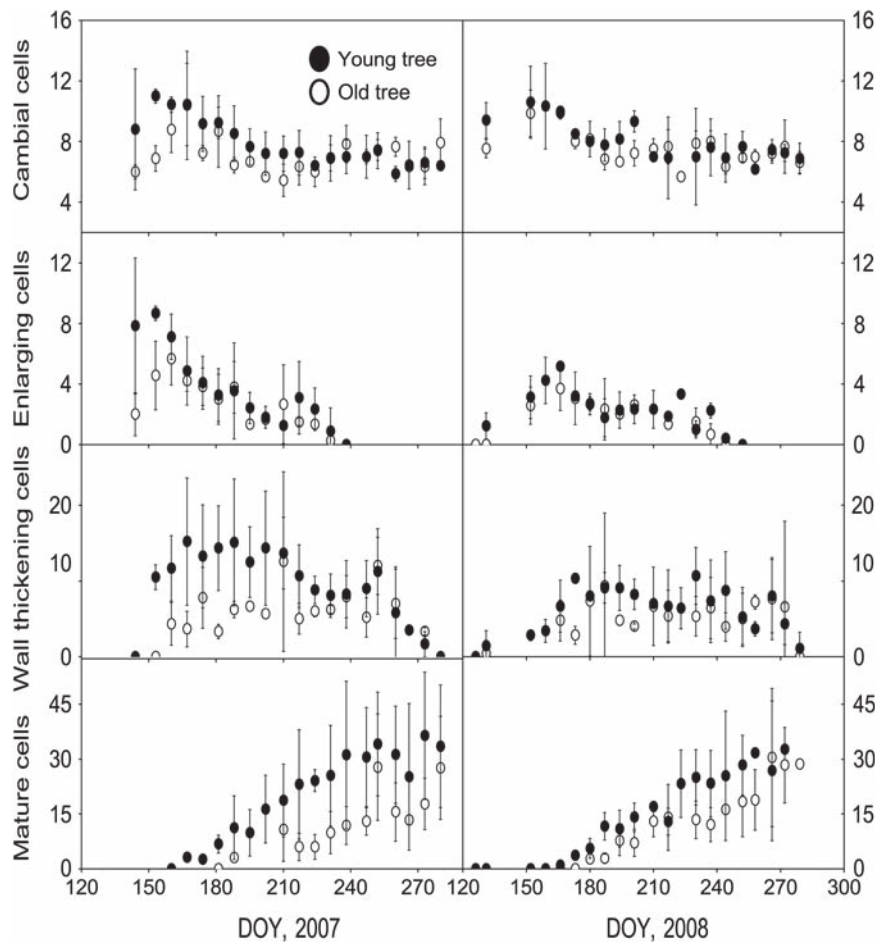

Figure 3. Number of cells in different stages of xylem differentiation in young and old trees along days of the year (DOY) in 2007 and 2008; error bars indicate ± SD for five trees per sampling date.

Table 1. Timing and duration of xylem differentiation and cell production in young and old Smith fir trees in 2007 and 2008 (n = 5 trees per age group); onset and end of xylem formation phase are given in days of the year.

Age group	Enlargement			Wall thickening				Xylem differentiation		
	Onset	End		Onset		End		Duration (days)	Xylem cells (n)	
	2008	2007	2008	2007	2008	2007	2008	2008	2007	2008
Young	131 ± 0	235 ± 4	243 ± 3	153 ± 0	159 ± 0	273 ± 7	279 ± 0	148 ± 0	47 ± 8	49 ± 2
Old	152 ± 0	232 ± 3	241 ± 4	165 ± 7	166 ± 0	272 ± 8	272 ± 5	119 ± 3	21 ± 5	21 ± 1

in 2008 the highest values were observed in mid-June. The end of cell enlargement lasted from late August to early September in both young and old trees (Figure 3; Table 1). No significant difference in the date of termination of cell enlargement was detected between the two age classes in 2007 and 2008 (ANOVA, $P > 0.05$).

In both years, wall thickening in young trees started in early June, ~1–2 weeks earlier than in old trees. In 2007, the maximum number of cells in the stage of wall thickening was observed at the end of June, with 10–14 and 6–7 cell layers in young and old trees, respectively. In 2008, the maximum number of wall-thickening cells was observed during July, with 9–13 and 7–9 layers in young and in old trees, respectively. Cell wall thickening and lignification ended between late September and early October. No significant difference in the date of cessation of this differentiation phase was found between the two age classes (ANOVA, $P > 0.05$) and years (Figure 3; Table 1). In 2008, the duration of wood formation lasted ~5 and 4 months in young and old trees, respectively.

The period when there was no cambial division lasted from August 2007 through April 2008; during dormancy the cambium consisted of 6–8 cells in both age classes. At the top of the growing seasons, between the end of May and mid-June, the cambial zone reached a maximum number of cells, around 10 cell layers.

Significant differences were found in the number of xylem cells and in the rate of cell production between young and old trees (ANOVA, $P < 0.001$). Young trees produced up to 50% more cells than old trees (Figure 4a, c and d; Table 1). The cell-production rate in young trees was also higher by 54 and 47% in 2007 and 2008, respectively (Figure 4b). An earlier onset of cell differentiation, and consequently a longer growing season and a higher rate of cell production in young trees led to a higher number of xylem cells along the radial files than in old trees in both study years.

Relationship between xylogenesis and climate

Throughout the two growing seasons, the number of xylem cells produced (Figure 4) up to the sampling date in comparison with the situation 1 week earlier was significantly and positively correlated with the minimum air temperature with a time lag in both age groups (Figure 5). The temporal pattern of the correlations was rather similar in both age classes. Minimum temperature during the week when the cells were produced
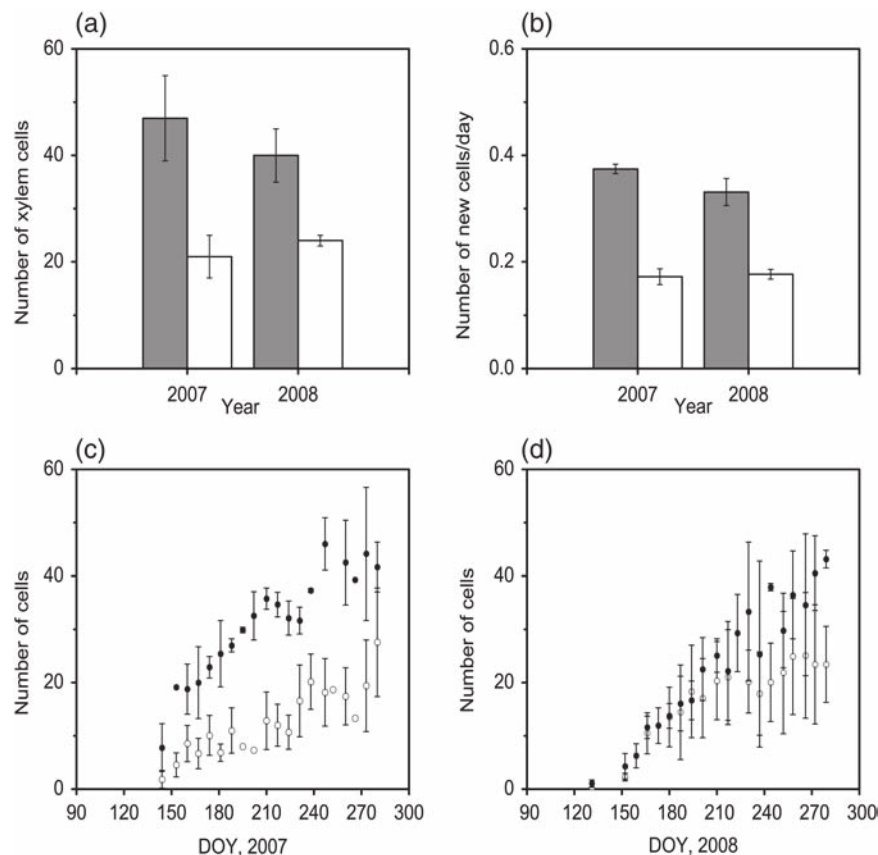

Figure 4. Xylem growth in young (closed circles and black bars) and old (open circles and open bars) trees in 2007 and 2008: (a) number of xylem cells; (b) average cell production (number of cells/day) in young and old trees; (c, d) increasing number of produced xylem cells (including enlarging, wall-thickening and mature xylem cells) vs. time in 2007 and 2008; error bars indicate ±SD for five trees in (a,b) and for five trees per sampling date in (c,d).
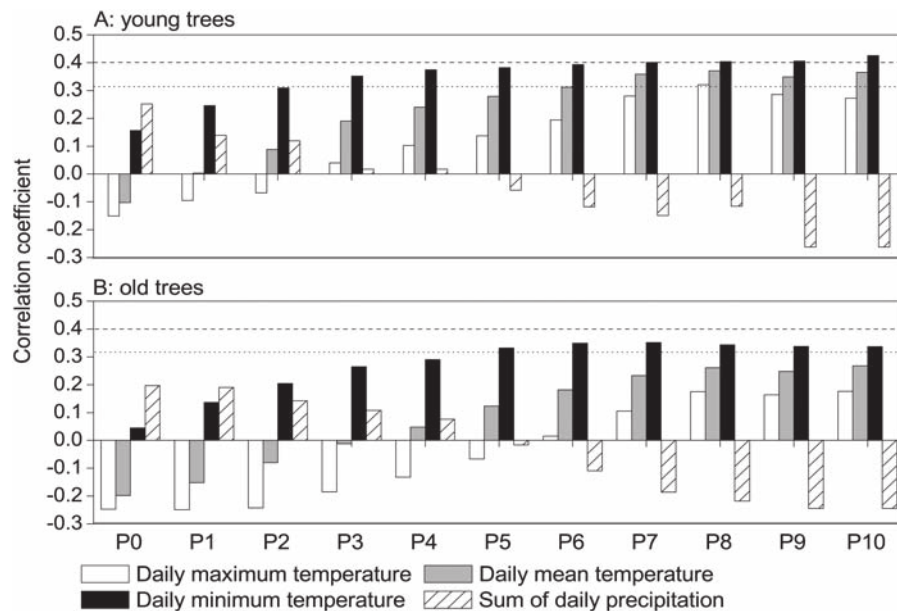
Figure 5. Cross-correlation between mean number of xylem cells produced each week and climatic variables for a time-lag period from 0 to 10 days. P0 represents the weekly climatic mean for the exact period between two sampling dates. P1 to P10 represent the 7-day period shifted backwards from 1 to 10 days before the sampling date. Dotted and dashed horizontal lines show the 95 and 99% confidence limits, respectively.

had no significant influence on cell production. However, we observed significant correlations between cell production and temperatures with time lags. In old trees, positive correlations were found with minimum temperatures at time lags ≥ 5 days ($r = 0.33$, $P < 0.05$), whereas in young trees the correlations were significant with time lags >3 days ($r = 0.35$, $P < 0.05$). Cell production was not significantly correlated with maximum temperature, except at P8 in young trees. Correlations between cell production and mean temperature were significant in case of time lags from P7 to P10; they can be explained by an effect of minimum temperature. However, no significant correlation was found between cell production and precipitation from P0 to P10.

Discussion

Age-dependent xylogenesis

Our results supported the hypothesis that xylogenesis differed with tree age. Although high snow levels in May made it difficult to reach the sampling area, we could show that the onset of cell production and differentiation (cell enlargement and wall thickening) occurred earlier in young trees, as also reported by Deslauriers et al. (2003) and Rossi et al. (2008a, 2008b). However, in contrast to Rossi et al. (2008a, 2008b), no differences were observed for the end of cell differentiation between the two age classes. As reported by several authors (e.g., Thibeault-Martel et al. 2008, Gričar et al. 2009, Lupi et al. 2010, Rathgeber et al. 2011), we also observed that the higher the amount of xylem produced, the longer was the period of

wood formation. In young trees, xylem differentiation started earlier and, as a result, more xylem cells were formed than in old ones, notwithstanding the fact that wood formation in both age classes ended at the same time.

Timing and duration of wood formation are closely related to the resumption of xylogenesis (Rossi et al. 2012). The onset of cell production is affected by several external and internal factors, and is related to age (genetic control) or size (physiological control) of the trees. In fact, the supposed age-related timing of xylogenesis, as found by Rossi et al. (2008a, 2008b), was detected in the observed trees of both ages and sizes. Thus, the older trees analysed by Rossi et al. (2008a, 2008b) exhibited also larger diameters and thicker barks, which could negatively influence the spring dynamics of warming of the stems. Rathgeber et al. (2011) avoided the problem of age by analysing even-aged trees, and found that cambial activity started earlier and ended later in dominant trees than in intermediate and suppressed ones; consequently, they deduced that cell differentiation could be affected by tree height and crown area. In our study, however, xylem differentiation started earlier in young and less high trees, which had smaller crowns than the old trees. Xylem development is regulated by auxin, which is produced in activated buds and exported basipetally along the stem (Uggla et al. 1998, Aloni 2001). According to this hypothesis, one possible explanation for the earlier onset of xylogenesis in young trees is that the base of the stem is closer to the crown—and hence to the source of auxin—than in old trees (Rossi et al. 2008a). However, no robust pattern for the progression of the onset of cambial activity was observed between

stem and roots, nor along the stem (Savidge 2000, Thibeault-Martel et al. 2008, Anfodillo et al. 2012). As observed by Sundberg et al. (2000), auxin concentration was similar between dormant and active cambia, indicating that auxin transport from young shoots may not be a prerequisite for cambium reactivation (Rossi et al. 2009). The gene expression in meristems may be fundamentally altered as the trees get older and larger (Woo et al. 1994, Day et al. 2002). As a consequence, tree age could influence the seasonal reorganization of the xylem transcriptome (Li et al. 2010), genetic control of cell anatomical traits (Lenz et al. 2010) and tree physiology (Vanninen and Mäkelä 2000, Li et al. 2009). The actual knowledge of age-related xylem differentiation therefore still remains fragmentary and could be related to possible complex interactions among gene expression, hormonal signals and environmental factors.

Influence of climatic factors on wood formation

Throughout the two growing seasons, minimum temperature positively influenced wood formation in both young and old trees. This is in agreement with results from high latitudes and altitudes (Deslauriers and Morin 2005, Rossi et al. 2008b, Moser et al. 2010, Zhu et al. 2011) and also with previous dendroecological and dendroclimatological studies in Smith fir in the Sygera Mts. (Liang et al. 2010). Cambium is a carbon sink (Deslauriers et al. 2009) and xylem formation is a complex process involving cell divisions and differentiation for several weeks during the year, which requires a continuous supply of sucrose. Rossi et al. (2008b) reported that there is evidence for a temperature threshold above which tree growth occurs, although the effects of minimum and maximum temperatures were not disentangled. In our work, the key factor limiting the production and differentiation of xylem cells of high-elevation Smith fir was minimum temperature, while no significant results were observed with maximum temperature. Comparing the daily growth responses of conifers to maximum and minimum temperatures, stronger correlations were observed with the latter (Antonova and Stasova 1993, Deslauriers and Morin 2005, Xiong et al. 2007, Rossi et al. 2008b, Boulouf Lugo et al. 2012). Moreover, the cell-doubling time remains quite constant at temperatures of 10 – 25 °C, but triplicates when temperatures fall from 10 to 5 °C (Körner 2003), which could correspond to the situation at our site. As suggested by Hosoo et al. (2002), cell growth is assumed to occur mainly during night, when temperature is low. There is increasing evidence that minimum rather than maximum temperature plays the most important role in constraining growth by acting on tissue formation in cold environments, such as at alpine timberlines across the Tibetan Plateau (e.g., Bräuning and Mantwill 2004, Liang et al. 2009, Zhu et al. 2011).

Both young and old Smith firs are sensitive to climate during xylem formation, which rejects the initial hypothesis that old trees were more sensitive to climate than young trees. Compared with young trees, old trees have a higher hydraulic resistance and lower photosynthetic rates, which might potentially increase the climatic sensitivity reflected in their growth (Carrer and Urbinati 2004, Mencuccini et al. 2005, Wang et al. 2009, Mérian and Lebourgeois 2011). However, in our case, both young and old Smith firs significantly responded to temperature. This is in agreement with conclusions by Shi et al. (2011), who demonstrated that tree-ring $\delta^{18}O$ series from young Linzhi spruce trees (*Picea likiangensis* var. *linzhiensis* W. C. Cheng & L. K. Fu) in south-eastern Tibet record similar climatic signals as old ones. Due to a higher cell-production rate, young trees might respond faster to changes in minimum temperature. A more direct response of young trees was found by Begum et al. (2010), who observed new cell plates in the cambium of 55-year-old *Cryptomeria japonica* (L.f.) D. Don. after 4 weeks and of 80-year-old trees after 5 weeks of artificial heating. This indicated that young trees were more ready to respond to changes in temperature, and helped to explain their early onset of xylogenesis in spring. This work will help one to understand better the recent studies based on tree-ring width and climate relationships which report that the growth of young trees is also climatically sensitive (McMillan et al. 2008, Rozas et al. 2009, Copenheaver et al. 2011, Haavik et al. 2011).

Conclusions

Despite an increasing number of dendroecological and dendroclimatological studies in the recent decades, this is to date the first study on intra-annual xylem development of high-elevation forests on the Tibetan Plateau. The results represent an important step towards a better understanding of xylem growth and factors affecting tree-ring formation. The duration of wood formation was shorter in old than young trees. Both young and old trees were sensitive and responded rapidly to changes in minimum air temperature. This investigation demonstrated that minimum rather than maximum temperature was the dominant climatic regulator for Smith fir growth in the Sygera Mts. However, long-term monitoring of age-dependent xylem development of Smith fir at various elevations in the Sygera Mts. will be necessary to confirm these preliminary results.

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Conflict of interest

None declared.

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References

Aloni R (2001) Foliar and axial aspects of vascular differentiation: hypotheses and evidence. J Plant Growth Regul 20:22–34.

Anfodillo T, Deslauriers A, Menardi R, Tedoldi L, Petit G, Rossi S (2012) Widening of xylem conduits in a conifer tree depends on the longer time of cell expansion downwards along the stem. J Exp Bot 63:837–845.

Antonova GF, Stasova VV (1993) Effects of environmental factors on wood formation in Scots pine stems. Trees 7:214–219.

Begum S, Nakaba S, Oribe Y, Kubo T, Funada R (2010) Cambial sensitivity to rising temperatures by natural condition and artificial heating from late winter to early spring in the evergreen conifer *Cryptomeria japonica*. Trees 24:43–52.

Boulouf Lugo J, Deslauriers A, Rossi S (2012) Duration of xylogenesis in black spruce lengthened between 1950 and 2010. Ann Bot 110:1099–1108.

Bräuning A, Mantwill B (2004) Summer temperature and summer monsoon history on the Tibetan Plateau during the last 400 years recorded by tree rings. Geophys Res Lett 31:L24205.

Camarero JJ, Olano JM, Parras A (2010) Plastic bimodal xylogenesis in conifers from continental Mediterranean climates. New Phytol 185:471–480.

Carrer M, Urbinati C (2004) Age-dependent tree-ring growth responses to climate in *Larix decidua* and *Pinus cembra*. Ecology 85:730–740.

Copenheaver CA, Crawford CJ, Fearer TM (2011) Age-specific responses to climate identified in the growth of *Quercus alba*. Trees 25:647–653.

Čufar K, Prislan P, De Luis M, Gričar J (2008) Tree-ring variation, wood formation and phenology of beech (*Fagus sylvatica*) from a representative site in Slovenia, SE Central Europe. Trees 22:749–758.

Čufar K, Cherubini M, Gričar J, Prislan P, Spina S, Romagnoli M (2011) Xylem and phloem formation in chestnut (*Castanea sativa* Mill.) during the 2008 growing season. Dendrochronologia 29:127–134.

Day ME, Greenwood MS, Diaz-Sala C (2002) Age- and size-related trends in woody plant shoot development: regulatory pathways and evidence for genetic control. Tree Physiol 22:507–513.

De Luis M, Novak K, Čufar K, Raventós J (2009) Size mediated climate-growth relationships in *Pinus halepensis* and *Pinus pinea*. Trees 23:1065–1073.

Deslauriers A, Morin H (2005) Intra-annual tracheid production in balsam fir stems and the effect of meteorological variables. Trees 19:402–408.

Deslauriers A, Morin H, Begin Y (2003) Cellular phenology of annual ring formation of *Abies balsamea* in the Quebec boreal forest (Canada). Can J For Res 33:190–200.

Deslauriers A, Giovannelli A, Rossi S, Castro G, Fragnelli G, Traversi L (2009) Intra-annual cambial activity and carbon availability in stem of poplar. Tree Physiol 29:1223–1235.

Eckstein D (1983) Biological basis of dendrochronology. Mitt Bundesforsch 141:11–20.

Eckstein D, Krause C (1989) Dendroecological studies on spruce trees to monitor environmental changes around Hamburg. IAWA Bull 10:175–182.

Esper J, Niederer R, Bebi P, Frank D (2008) Climate signal age effects—evidence from young and old trees in the Swiss Engadin. For Ecol Manag 255:3783–3789.

Fritts HC (1976) Tree rings and climate. Academic Press, New York.

Gričar J, Čufar K, Oven P, Schmitt U (2005) Differentiation of terminal latewood tracheids in silver fir trees during autumn. Ann Bot 95:959–965.

Gričar J, Krže L, Čufar K (2009) Number of cells in xylem, phloem and dormant cambium in silver fir (*Abies alba*), in trees of different vitality. IAWA J 30:121–133.

Haavik LJ, Stahle DW, Stephen FM (2011) Temporal aspects of *Quercus rubra* decline and relationship to climate in the Ozark and Ouachita Mountains, Arkansas. Can J For Res 41:773–781.

Hosoo Y, Yoshida M, Imai T, Okuyama T (2002) Diurnal difference in the amount of immunogold-labeled glucomannans detected with field emission scanning electron microscopy at the innermost surface of developing secondary walls of differentiating conifer tracheids. Planta 215:1006–1012.

Hughes MK, Swetnam TW, Diaz HF (2011) Dendroclimatology: progress and prospect. Springer, Dordrecht.

Hutton J (1788) Theory of the earth; or an investigation of the laws observable in the composition, dissolution, and restoration of land upon the globe. Trans R Soc Edinburgh 1:209–304.

Körner K (2003) Carbon limitation in trees. J Ecol 91:4–17.

Lenz P, Cloutier A, Mackay J, Beaulieu J (2010) Genetic control of wood properties in *Picea glauca*—an analysis of trends with cambial age. Can J For Res 40:703–715.

Li C, Wu C, Duan B, Korpelainen H, Luukkanen O (2009) Age-related nutrient content and carbon isotope composition in the leaves and branches of *Quercus aquifolioides* along an altitudinal gradient. Trees 23:1109–1121.

Li XG, Wu HX, Southerton SG (2010) Seasonal reorganization of the xylem transcriptome at different tree ages reveals novel insights into wood formation in *Pinus radiata*. New Phytol 187:764–776.

Liang EY, Shao XM, Xu Y (2009) Tree-ring evidence of recent abnormal warming on the southeast Tibetan Plateau. Theor Appl Climatol 98:9–18.

Liang EY, Wang YF, Xu Y, Liu B, Shao XM (2010) Growth variation in *Abies georgei* var. *smithii* along altitudinal gradients in the Sygera Mountains, southeastern Tibetan Plateau. Trees 24:363–373.

Liang EY, Wang YF, Eckstein D, Luo TX (2011a) Little change in the fir tree-line position on the southeastern Tibetan Plateau after 200 years of warming. New Phytol 190:760–769.

Liang EY, Liu B, Zhu LP, Yin ZY (2011b) A short note on linkage of climatic records between a river valley and the upper timberline in the Sygera Mountains, southeastern Tibetan Plateau. Glob Planet Change 77:97–102.

Liang EY, Lu XM, Ren P, Li XX, Zhu LP, Eckstein D (2012) Annual increments of juniper dwarf shrubs above the tree line on the central Tibetan Plateau: a useful climatic proxy. Ann Bot 109:721–728.

Linderholm H, Linderholm K (2004) Age-dependent climate sensitivity of *Pinus sylvestris* L. in the central Scandinavian Mountains. Boreal Environ Res 9:307–317.

Lupi C, Morin H, Deslauriers A, Rossi S (2010) Xylem phenology and wood production: resolving the chicken-or-egg dilemma. Plant Cell Environ 33:1721–1730.

Mäkinen H, Seo JW, Nöjd P, Schmitt U, Jalkanen R (2008) Seasonal dynamics of wood formation: a comparison between pinning, microcoring and dendrometer measurements. Eur J For Res 127:235–245.

McMillan AMS, Winston GC, Goulden ML (2008) Age-dependent response of boreal forest to temperature and rainfall variability. Glob Change Biol 14:1904–1916.

Mencuccini M, Martínez-Vilalta J, Vanderklein D, Hamid HA, Korakaki E, Lee S, Michiels B (2005) Size-mediated ageing reduces vigour in trees. Ecol Lett 8:1183–1190.

Mérian P, Lebourgeois F (2011) Size-mediated climate-growth relationships in temperate forests: a multi-species analysis. For Ecol Manag 261:1382–1391.

Moser L, Fonti P, Büntgen U, Franzen J, Esper J, Luterbacher J, Frank D (2010) Timing and duration of European larch growing season along altitudinal gradients in the Swiss Alps. Tree Physiol 30:225–233.

Oberhuber W, Gruber A (2010) Climatic influences on intra-annual stem radial increment of *Pinus sylvestris* (L.) exposed to drought. Trees 24:887–898.

Oladi R, Pourtahmasi K, Eckstein D, Bräuning A (2011) Seasonal dynamics of wood formation in Oriental beech (*Fagus orientalis* Lipsky) along an altitudinal gradient in the Hyrcanian forest, Iran. Trees 25:425–433.

Rathgeber CBK, Rossi S, Bontemps JD (2011) Cambial activity related to tree size in a mature silver-fir plantation. Ann Bot 108:429–438.

Rossi S, Deslauriers A, Anfodillo T (2006) Assessment of cambial activity and xylogenesis by microsampling tree species: an example at the Alpine timberline. IAWA J 27:383–394.

Rossi S, Deslauriers A, Anfodillo T, Carrer M (2008a) Age-dependent xylogenesis in timberline conifers. New Phytol 177:199–208.

Rossi S, Deslauriers A, Gričar J, Seo J-W, Rathgeber CBK, Anfodillo T, Morin H, Levanic T, Oven P, Jalkanen R (2008b) Critical temperatures for xylogenesis in conifers of cold climates. Glob Ecol Biogeogr 17:696–707.

Rossi S, Rathgeber CBK, Deslauriers A (2009) Comparing needle and shoot phenology with xylem development on three conifer species in Italy. Ann For Sci 66:206.

Rossi S, Morin H, Deslauriers A (2012) Causes and correlations in cambium phenology: towards an integrated framework of xylogenesis. J Exp Bot 63:2117–2163.

Rozas V, DeSoto L, Olano MJ (2009) Sex-specific, age-dependent sensitivity of tree-ring growth to climate in the dioecious tree *Juniperus thurifera*. New Phytol 182:687–697.

Sass U, Killmann W, Eckstein E (1995) Wood formation in two species of Dipterocarpaceae in Peninsular Malaysia. IAWA J 16:371–384.

Savidge RA (2000) Intrinsic regulation of cambial growth. J Plant Growth Regul 20:52–77.

Schweingruber FH (1996) Tree rings and environment: dendroecology. Paul Haupt, Berne.

Seo J-W, Eckstein D, Jalkanen R, Schmitt U (2011) Climatic control of intra- and inter-annual wood-formation dynamics of Scots pine in northern Finland. Environ Exp Bot 72:422–431.

Shi CM, Masson-Delmotte V, Risi C et al. (2011) Sampling strategy and climatic implications of tree-ring stable isotopes on the southeast Tibetan Plateau. Earth Planet Sci Lett 301:307–316.

Sundberg B, Uggla C, Tuominen H (2000) Cambial growth and auxin gradients. In: Savidge RA, Barnett JR, Napir R (eds) Cell and molecular biology of wood formation. BIOS Scientific Publishers Ltd., Oxford, pp 169–188.

Thibeault-Martel M, Krause C, Morin H, Rossi S (2008) Cambial activity and intra-annual xylem formation in roots and stems of *Abies balsamea* and *Picea mariana*. Ann Bot 102:667–674.

Uggla C, Mellerowicz E, Sundberg B (1998) Indole-3-acetic acid controls cambial growth in Scots pine by positional signaling. Plant Physiol 117:113–121.

Vanninen P, Mäkelä A (2000) Needle and stem wood production in Scots pine (*Pinus sylvestris*) trees of different age, size and competitive status. Tree Physiol 20:527–533.

Vieira J, Campelo F, Nabais C (2009) Age-dependent responses of tree-ring growth and intra-annual density fluctuations of *Pinus pinaster* to Mediterranean climate. Trees 23:257–265.

Wang XC, Zhang YD, McRae DJ (2009) Spatial and age-dependent tree-ring growth responses of *Larix gmelinii* to climate in northeastern China. Trees 23:875–885.

Woo HH, Hackett WP, Das A (1994) Differential expression of a chlorophyll a/b binding protein gene and a proline rich protein gene in juvenile and mature phase English ivy (*Hedera helix*). Physiol Plant 92:69–78.

Xiong W, Wang YH, Yu PT, Liu HL, Shi ZJ, Guan W (2007) The growth in stem diameter of *Larix principis-rupprechtii* and its response to meteorological factors in the south of Liupan Mountain. Acta Ecol Sin 27:432–441.

Yu GR, Liu YB, Wang XC, Ma KP (2008) Age-dependent tree-ring growth responses to climate in Qilian juniper (*Sabina przewalskii* Kom.). Trees 22:197–204.

Zar JH (1999) Biostatistical analysis, 4th edn. Prentice-Hall Press, New Jersey.

Zhu HF, Shao XM, Yin Z-Y, Xu P, Tian H (2011) August temperature variability in the southeastern Tibetan Plateau since AD 1358 inferred from tree rings. Palaeogeogr Palaeoclimatol Palaeoecol 305:84–92.