



## Research paper

# Effects of environmental conditions on onset of xylem growth in *Pinus sylvestris* under drought

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We determined the influence of environmental factors (air and soil temperature, precipitation, photoperiod) on onset of xylem growth in Scots pine (*Pinus sylvestris* L.) within a dry inner Alpine valley (750 m a.s.l., Tyrol, Austria) by repeatedly sampling micro-cores throughout 2007–10 at two sites (xeric and dry-mesic) at the start of the growing season. Temperature sums were calculated in degree-days (DD)  $\geq 5$  °C from 1 January and 20 March, i.e., spring equinox, to account for photoperiodic control of release from winter dormancy. Threshold temperatures at which xylogenesis had a 0.5 probability of being active were calculated by logistic regression. Onset of xylem growth, which was not significantly different between the xeric and dry-mesic sites, ranged from mid-April in 2007 to early May in 2008. Among most study years, statistically significant differences ( $P < 0.05$ ) in onset of xylem growth were detected. Mean air temperature sums calculated from 1 January until onset of xylem growth were  $230 \pm 44$  DD (mean  $\pm$  standard deviation) at the xeric site and  $205 \pm 36$  DD at the dry-mesic site. Temperature sums calculated from spring equinox until onset of xylem growth showed somewhat less variability during the 4-year study period, amounting to  $144 \pm 10$  and  $137 \pm 12$  DD at the xeric and dry-mesic sites, respectively. At both sites, xylem growth was active when daily minimum, mean and maximum air temperatures were 5.3, 10.1 and 16.2 °C, respectively. Soil temperature thresholds and DD until onset of xylem growth differed significantly between sites, indicating minor importance of root-zone temperature for onset of xylem growth. Although spring precipitation is known to limit radial growth in *P. sylvestris* exposed to a dry inner Alpine climate, the results of this study revealed that (i) a daily minimum air temperature threshold for onset of xylem growth in the range 5–6 °C exists and (ii) air temperature sum rather than precipitation or soil temperature triggers start of xylem growth. Based on these findings, we suggest that drought stress forces *P. sylvestris* to draw upon water reserves in the stem for enlargement of first tracheids after cambial resumption in spring.

**Keywords:** dry inner Alpine valley, heat sum, phenology, Scots pine, wood formation, xylogenesis.

## Introduction

There is evidence that phenological responses to climate change occurred in recent decades, leading to an earlier start of the growing season in Europe (e.g., Badeck et al. 2004, Menzel et al. 2006). It is well known that annual growth cycles in trees of temperate and boreal climates are primarily influenced by temperature and photoperiod (Körner 2006, Lüttge and Hertel 2009), and several studies dealing with the physiology of cambial activity and xylem phenology underlined the importance of temperature in cambial reactivation and xylem growth after winter dormancy (e.g., Oribe et al. 2001, Gričar

et al. 2007, Lupi et al. 2010, Rossi et al. 2011). The date of onset of xylem growth in trees from cold environments where rainfall is abundant is assumed to be controlled by antecedent heat sums (e.g., Kramer 1994, Karlsson et al. 2003, Seo et al. 2008) or reaching a certain temperature threshold (Deslauriers et al. 2008, Rossi et al. 2008). To our knowledge, there are no reports on required heat sums or the level of critical temperature for xylem growth at sites where tree growth is strongly limited by drought at the start of the growing season.

Besides air temperature, the importance of soil temperature in controlling shoot growth and activity is well known (reviewed

in Tranquillini 1979, Körner 1998). Kirilyanov et al. (2003) reported that low root-zone temperature due to long-lasting snow cover retarded initiation of cambial activity at the northern treeline, and Gruber et al. (2009) found that soil temperatures were possibly involved in triggering cambial activity and xylem growth in *Pinus cembra* within the Alpine treeline ecotone. Within dry inner Alpine environments, differences in the temporal dynamics of xylem growth in *Pinus sylvestris* in spring were also suggested to be affected by earlier soil warming (Gruber et al. 2010).

Photoperiod is the second most important factor that triggers phenological phases in most long-lived plant species outside the tropics (Saxe et al. 2001, Badeck et al. 2004). Photoperiodism prevents premature growth onset during mild spells in late winter, which may cause heavy damage during subsequent periods of frost (Cannell and Smith 1986, Körner 2006). An influence of photoperiod on onset of growth was reported by Partanen et al. (1998), who found that bud burst in Norway spruce (*Picea abies*) was delayed when the natural photoperiod was shortened. Long days have also been shown to compensate partially for a lack of chilling during rest break in *P. sylvestris* (Jensen and Gatherum 1965, Hoffman and Lyr 1967) and other tree species (e.g., Garber 1983). Furthermore, a photoperiodic growth constraint was deduced from findings that maximum daily growth rates in conifers from cold environments peaked around summer solstice and not during the warmest period of the year (Rossi et al. 2006c, Gruber et al. 2009). Hence, it can be assumed that an increase in spring temperatures due to climate warming may induce earlier onset of phenophases (e.g., bud burst, onset of cambial activity) only if photoperiod does not override the temperature control (for a review see Jackson 2009, Körner and Basler 2010).

The dormant period in trees of cool-temperate climates, which is characterized by a lack of cell division, consists of two successive parts, a resting and a quiescent stage (e.g., Little and Bonga 1974, Hänninen 1995, Rensing and Samuels 2004). During the resting stage of cambial dormancy in late autumn and winter, environmental factors favourable for growth are ineffective for release from dormancy, which is maintained by internal agents or conditions (physiological dormancy). In mature trees of the boreal region, rest completion and transition to quiescence was found to take place around spring equinox (20 March), i.e., considerably after the time when the chilling requirement is usually met (e.g., Heide 1993b, Hänninen 1995, Partanen et al. 1998). During the quiescent stage of dormancy in early spring, development, i.e., growth onset, is controlled by environmental conditions (environmental dormancy), whereby actual temperature is regarded as most important (e.g., Oribe and Kubo 1997, Gričar et al. 2007, Begum et al. 2010). However, it is well known that drought stress in trees affects growth directly by inhibiting cell division

and, even more sensitively, cell enlargement (e.g., Hsiao and Acevedo 1974, Abe and Nakai 1999).

The present study focuses on the relationship between environmental factors and resumption of xylem growth after winter dormancy in *P. sylvestris* exposed to drought in the lower montane region of the Eastern Alps (Austria). Growth–climate relationships revealed that precipitation at the start of the growing season in spring limits radial stem growth of *P. sylvestris* in dry inner Alpine environments (e.g., Oberhuber et al. 1998, Rigling et al. 2002, Pichler and Oberhuber 2007). Recently published studies within the study area on cellular phenology of annual ring formation and climatic influences on intra-annual radial stem growth during two contrasting years revealed early culmination of wood formation in spring and influence of water availability on dynamics and duration of cell differentiation processes (Gruber et al. 2010, Oberhuber and Gruber 2010). Based on variability in timing of cambial activity and xylogenesis among contrasting years and sites, Gruber et al. (2010) also suggested that early spring temperature influences onset of xylem growth. Here we present a more in-depth evaluation of the effects of environmental factors on onset of xylem growth in *P. sylvestris* under drought stress, whereby we determined the variability in onset of xylem growth at two sites (xeric and dry-mesic) during a 4-year study period (2007–10) and analysed the impact of environmental factors, i.e., cumulative degree-days (DD) of air and soil, and precipitation sum on growth resumption. We expected that drought affects temperature control of onset of tracheid production due to inhibition of cell division and/or cell enlargement when water availability is limited in early spring. Because we also hypothesized that photoperiod is involved in controlling release from winter cambial dormancy, i.e., the transition from rest to quiescence, environmental triggers were accumulated from 1 January and 20 March (i.e., spring equinox). Furthermore, we aimed to define threshold air and soil temperatures above which xylem growth occurs in *P. sylvestris* at a xeric and a dry-mesic site.

## Material and methods

### Site description

The study site is part of a postglacial rock-slide area situated in the montane belt (~750 m a.s.l.) within the inner Alpine dry valley of the Inn River (Tyrol, Austria, 47°14'00" N, 10°50'20" E) and has a relatively continental climate with mean annual precipitation and temperature of 716 mm and 7.3 °C, respectively (long-term mean for 1911–2008 at Ötz, 812 m a.s.l., 5 km from the study area). *Pinus sylvestris* forms widespread forest ecosystems in the lower montane region within dry inner Alpine valleys in the central Austrian and Swiss Alps (Ellenberg and Leuschner 2010). Because trees within the study area responded quite differently to identical climatic conditions depending on the interaction of soil condition and topographic

features on water availability (Oberhuber and Kofler 2000), two sites differing in water availability were selected at the same elevation and within <200 m in linear distance: a more xeric open south-facing stand growing on shallow stony soil and a dry-mesic site with deeper soil and higher stand density in a hollow were selected (Table 1). Shallow soils, predominantly of protorendzina type, i.e., rendzic and lithic leptosols (FAO 1998), are developed and consist of unconsolidated, coarse-textured materials with low water-holding capacity. Distinct soil horizons are hardly ever developed and are restricted to small-scale areas within deep hollows. On the xeric site, pioneer vegetation prevails in the ground flora, whereas crowberry (*Vaccinium vitis-idaea* L.) and a thick moss layer dominate the understory in the hollow, which indicates slightly moist conditions at the latter site. All measurements were carried out on dominant trees to reduce the influence of competition on xylem growth. Whereas mean tree age at both study sites was statistically not significantly different (164 and 145 years at the dry-mesic and xeric sites, respectively;  $P > 0.05$ ), trees were twice as tall and had significantly wider rings at the dry-mesic compared with the xeric site ( $P < 0.05$ ), which indicates more favourable soil moisture conditions at the former site (Table 1). Student's *t*-test was applied to detect statistical significance of differences between mean values.

### Xylem sampling and determination of onset of xylem growth

The dynamics of wood formation were monitored by taking micro-cores (~20 mm in length and 2.5 mm in diameter) during 2007–10 from seven trees per site of the outermost tree rings (Deslauriers et al. 2003, Rossi et al. 2006a). Due to high variability in onset of xylem growth between trees, the data set gathered in a previous study for 2007–08 (see Gruber et al. 2010) was extended by analysing additional micro-cores. To determine the variability in onset of wood formation (xylem growth) between trees at each site, individual trees used for sampling micro-cores were randomly selected. However, trees with major stem or crown anomalies due to high mistletoe infection were excluded from the analysis. Micro-cores were taken at all study sites starting in early March at about weekly

intervals. At both study sites, micro-cores were sampled from different trees in successive years to avoid effects of wounding on wood formation. Samples were taken starting at ~1 m stem height on the slope-perpendicular side following a spiral trajectory up the stem. A distance of ~2 cm in tangential and longitudinal direction was kept to avoid lateral influence of wound reactions on adjacent sampling positions.

Immediately after extraction, cores were placed in a solution of 70% ethanol, propionic acid and 40% formaldehyde (mixing ratio: 90/5/5), subsequently embedded in glycol-methacrylate (Technovit 7100) and polymerized after adding an accelerator. Transverse sections of ~12 µm were cut with a rotary microtome, stained with a water solution of 0.05% cresyl fast violet and observed under a light microscope with polarized light to differentiate the development of xylem cells, i.e., the discrimination between tracheids in enlarging and cell-wall thickening phases (Antonova and Stasova 1993, Deslauriers et al. 2003, Rossi et al. 2006b). Xylem formation was considered to have begun when one horizontal row of tracheids was detected in the cell-enlargement phase. We refrained from defining onset of xylem growth on the basis of number of cells in the cambial zone, because (i) the exact date of increase in the number of cambial cells after dormancy (approximately four cells) cannot be determined unequivocally and (ii) the dynamics of cambial and enlarging cells are closely connected (see Gruber et al. 2010). Hence, determining onset of cell differentiation instead of cell division might have caused only a small systematic error in our analyses.

### Micro-climate records

During the study period, daily precipitation was collected automatically at 2 m height (ONSET, Pocasset, MA, USA) at the xeric site on an open ridge, i.e., in a non-vegetated area. Because the dry-mesic site was located at the same elevation and within <200 m in linear distance, records of precipitation from this site were regarded as representative of the whole study area. To determine differences in air temperature between study sites caused by varying topography and canopy coverage, air temperature sensors (HOBO; ONSET, Pocasset, MA, USA) shielded against solar radiation were installed at 2 m

Table 1. Site description and characteristics of *P. sylvestris* trees selected for micro-core sampling at the xeric and dry-mesic study sites during 2007–10 ( $n = 7$  trees per site, STD = standard deviation, RW = ring width). Statistically significant differences in mean values between sites are indicated by different letters ( $P < 0.05$ ; Student's *t*-test).

Site	Aspect	Slope (°)	Soil type	Humus type	Soil depth (cm)	Tree height <sup>1</sup> (m)	Canopy coverage (%)	Tree age <sup>2</sup> (year) mean ± STD	Stem diameter <sup>3</sup> (cm) mean ± STD	RW <sup>4</sup> (µm) mean ± STD
Xeric	SW	30–40	Syrosem	Xeromoder	0–10	4–5	33	145 ± 24 <sup>a</sup>	22.4 ± 3.9 <sup>a</sup>	272 ± 72 <sup>a</sup>
Dry-mesic	N	<10	Protorendzina	Raw humus	20–30	10	66	164 ± 26 <sup>a</sup>	29.2 ± 3.6 <sup>b</sup>	491 ± 87 <sup>b</sup>

<sup>1</sup>Tree height of dominant trees.

<sup>2</sup>Cambial age at sampling height.

<sup>3</sup>Mean tree diameter measured at 1 m stem height.

<sup>4</sup>Mean values for the period 2002–06.

height within both stands. Additionally, soil moisture dynamics (volumetric water content) and soil temperature in the top 510 cm– soil layer were continuously monitored at both sites. Moisture sensors are based on a capacitive method (Cyclobios, proprietary development at University of Innsbruck, Austria). Due to small-scale variability of soil structure with soil depth, records of three soil moisture and temperature sensors placed at each site were averaged. Measuring intervals for all sensors were 30 min. Mean daily air and soil temperature and soil water content (Vol.%) were calculated by averaging all measurements (48 values/day).

### Logistic regression

Binary logistic regressions (logit models) were calculated by applying SPSS 15.0.1 for Windows, to determine the probability of tracheid production being active at a given air and soil temperature. Binary responses were coded as non-active (value 0) or active (value 1), i.e., enlarging cells were missing or detectable, respectively, during January–July. For each tree, site and year, the model was fitted with the respective temperature series, i.e., mean, minimum and maximum air and soil temperature, and temperature thresholds were calculated when the probability of tracheid production being active was 0.5 (cf. Rossi et al. 2007). Fitting verification included  $\chi^2$  of the

likelihood ratio, Wald's  $\chi^2$  for regression parameter and goodness of fit, and Hosmer–Lemeshow  $\hat{C}$  for eventual lack of fit.

### Calculation of DD sum

Degree-day sum (DD) is an index representing a measure of accumulated heat and was calculated according to Baskerville and Emin (1969) as the integral of degrees  $\geq 5^\circ\text{C}$ , whereby the chosen threshold temperature was based on studies by Seo et al. (2008) and Vaganov et al. (2009). In particular, a sine curve was fitted to the recorded daily maximum and minimum temperatures and the area of the curve above the base temperature was calculated using calculus. Degree-days were calculated from records of air and soil temperature starting on 1 January and at spring equinox, i.e., from 20 March, until onset of xylem growth occurred. Selected dates are based on the finding that the dormant period of trees of cool-temperate climates consists of two successive parts and that rest completion takes place around spring equinox (e.g., Little and Bonga 1974, Heide 1993b, Hänninen 1995, Partanen et al. 1998). Hence, *P. sylvestris* is assumed to be in the rest and quiescence phases of dormancy on 1 January and 20 March, respectively. Degree-days were also calculated from both dates until 30 April, to determine differences in accumulated heat during late winter/early spring among study years.

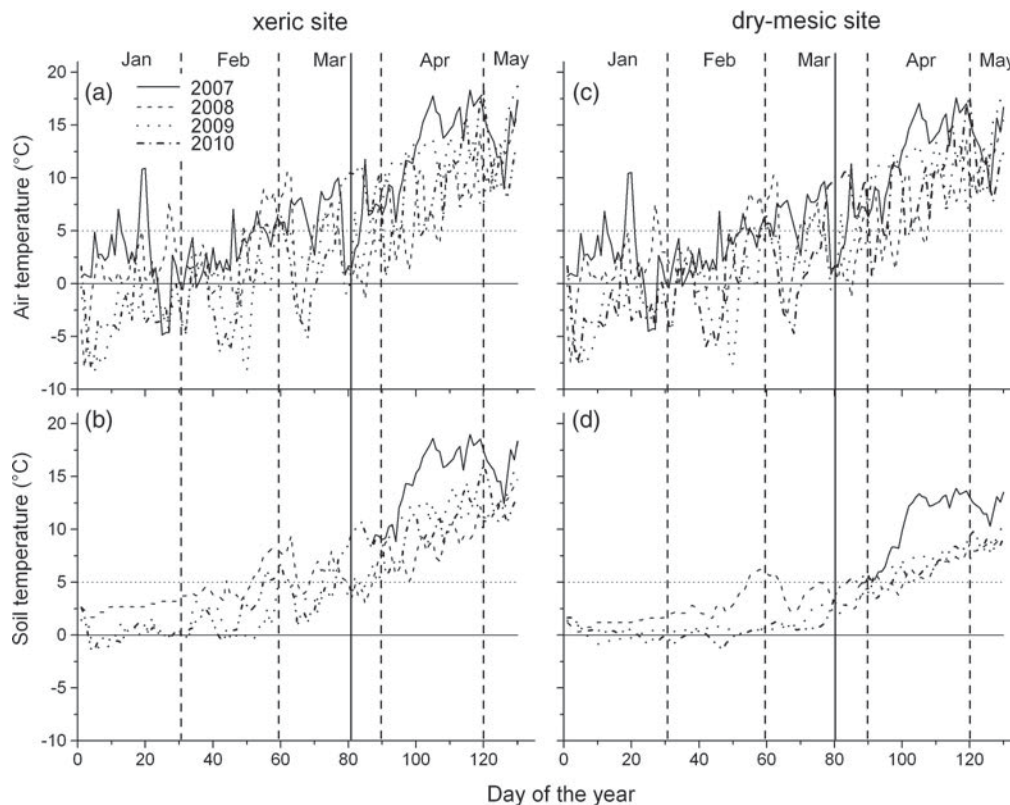


Figure 1. Mean daily air and soil temperature recorded during 2007–10 from January through May at the xeric (a, b) and dry-mesic sites (c, d). The study started in April 2007, which is why there are no soil temperature data before Day 87 in 2007. Solid vertical line indicates time of spring equinox.



## Results

### Micro-climatic conditions

During the 4-year study period, large variations between years in temperature and precipitation until start of the growing season were recorded (Figures 1 and 2, Table 2). Mildest air tem-

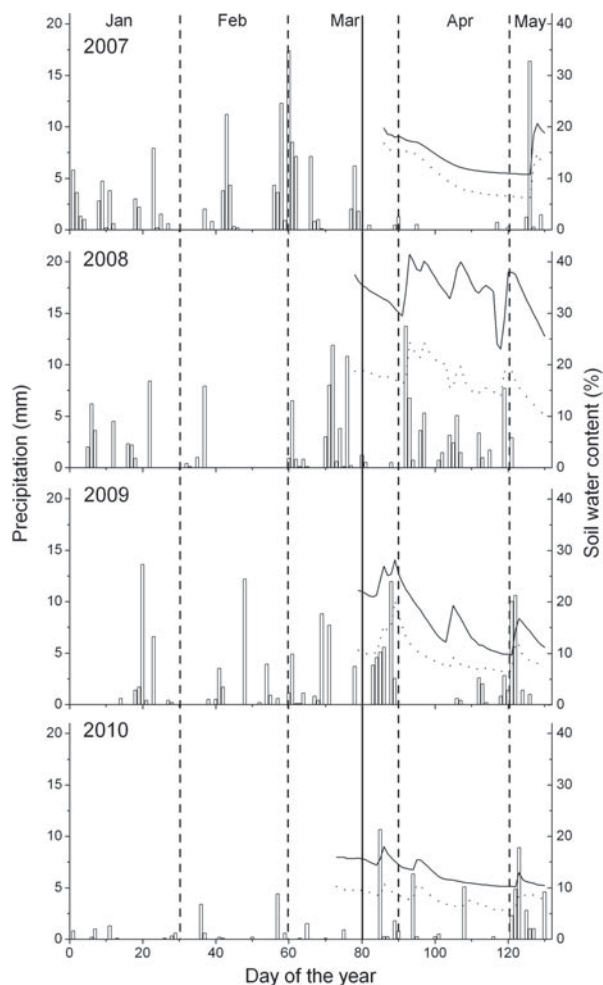


Figure 2. Daily precipitation sum (bars) and soil water content recorded during 2007–10 from January through May at study sites. Soil water content for the xeric and dry-mesic sites is denoted by dotted and solid lines, respectively. Solid vertical line indicates time of spring equinox.

Table 2. Accumulated heat (DD sum) in air and soil and precipitation sum (mm) from 1 January and 20 March (spring equinox) until 30 April during 2007–10. The study started in late March 2007, which is why there are no soil temperature data in 2007.

Year	DD sum (air)				DD sum (soil)				Precipitation sum (mm)	
	Xeric site		Dry-mesic site		Xeric site		Dry-mesic site		1 Jan	20 Mar
	1 Jan	20 Mar	1 Jan	20 Mar	1 Jan	20 Mar	1 Jan	20 Mar		
2007	459	320	422	307	–	–	–	–	139	4
2008	247	137	210	123	207	127	42	34	147	59
2009	276	234	251	221	258	229	60	60	122	44
2010	268	217	247	206	269	231	48	48	43	26

peratures during late winter and early spring occurred in 2007. Accumulated heat (DD) from 1 January until 30 April 2007 exceeded DD in the coldest year of our study period (2008) by ~85% at the xeric and 100% at the dry-mesic site. Exceptionally warm conditions in spring 2007 compared with 2008 are obvious when DD was calculated from 20 March (spring equinox) until 30 April (Table 2). Mean air temperature recorded from January through April 2007 amounted to 6.5 °C at the xeric site and exceeded records from 2008 through 2010 by 2.7, 3.5 and 3.6 °C, respectively (data not shown). While air temperatures and calculated heat sums differed only slightly between the study sites (Figure 1, Table 3), DD in soil was strikingly lower at the dry-mesic site compared with the xeric site (Table 2). The difference in soil temperature between the xeric and dry-mesic sites steadily increased from January through May, amounting to 0.5 °C in January and 4.1 °C in May. At the xeric site the difference between air and soil temperature ( $\Delta T$ ) gradually decreased from January through May. On the other hand,  $\Delta T$  at the dry-mesic site steadily increased from March through May, whereby  $\Delta T$  amounted to 3.6 °C in May (Table 3). January–April precipitation reached a maximum and minimum of 147 and 43 mm in 2008 and 2010, respectively. From 20 March until 30 April 2007 almost no rainfall was recorded (4 mm), whereas during the same period in 2008 precipitation sum reached a maximum of 59 mm (Table 2, Figure 2). Mean soil water content throughout growing seasons 2007 and 2009–10 was ~5–10% higher at the dry-mesic site compared with the xeric site. In 2008, soil water content differed by ~15% between study sites (Figure 2).

### Environmental factors and onset of xylem growth

Onset of xylem growth ranged from mid-April in 2007 to early May in 2008, but did not significantly differ between the xeric and dry-mesic sites. However, statistically significant differences ( $P < 0.05$ ) in onset of xylem growth were detected between all years, except between 2007 and 2009 (Table 4). The threshold air and soil temperatures at which there was a 0.5 probability of xylem growth at study sites are depicted in Figure 3. Air temperature thresholds differed only slightly between sites, whereby mean values of the minimum, mean and maximum amounted to 5.3, 10.1 and 16.2 °C (Figure 3a). On the other

Table 3. Mean monthly air and soil temperature from January through May 2008–10 at the xeric and dry-mesic sites and difference between air and soil temperature ( $\Delta T$ ). Because records of soil temperature were started in late March 2007, means from January through May 2007 were omitted in the comparison. Mean values  $\pm$  standard deviation are shown.

	Xeric site			Dry-mesic site		
	Air $T$ ( $^{\circ}\text{C}$ )	Soil $T$ ( $^{\circ}\text{C}$ )	$\Delta T$ ( $^{\circ}\text{C}$ )	Air $T$ ( $^{\circ}\text{C}$ )	Soil $T$ ( $^{\circ}\text{C}$ )	$\Delta T$ ( $^{\circ}\text{C}$ )
January	$-1.6 \pm 2.3$	$1.0 \pm 1.5$	$-2.6$	$-1.4 \pm 2.2$	$0.5 \pm 0.6$	$-1.9$
February	$0.8 \pm 1.6$	$2.8 \pm 1.9$	$-2.0$	$0.8 \pm 1.5$	$1.2 \pm 1.8$	$-0.4$
March	$4.1 \pm 0.3$	$5.5 \pm 0.7$	$-1.4$	$4.0 \pm 0.3$	$2.8 \pm 1.0$	$1.2$
April	$9.7 \pm 2.0$	$10.1 \pm 1.5$	$-0.4$	$9.3 \pm 1.9$	$6.4 \pm 0.5$	$2.9$
May	$14.0 \pm 2.0$	$14.0 \pm 1.3$	$0$	$13.5 \pm 1.9$	$9.9 \pm 0.6$	$3.6$

Table 4. Onset of xylem growth in *P. sylvestris* at study sites during 2007–10 ( $n = 7$  trees per site). Timing of xylem growth is given in days of the year (mean values  $\pm$  standard deviation). Statistically significant differences of mean values at  $P < 0.05$  (Student's *t*-test) between sites during 2007–10 and between years at the xeric and dry-mesic sites are indicated by different letters.

Year	Xeric site	Dry-mesic site
2007	$104 \pm 6^a$	$107 \pm 7^a$
2008	$119 \pm 9^b$	$122 \pm 6^b$
2009	$105 \pm 4^a$	$106 \pm 4^a$
2010	$114 \pm 3^c$	$115 \pm 4^c$

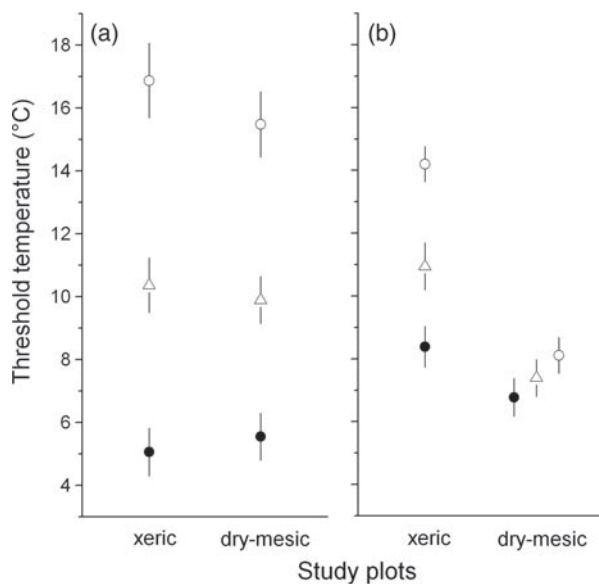


Figure 3. Threshold minimum (closed circles), mean (open triangles) and maximum (open circles) air (a) and soil temperature (b) corresponding with 0.5 probability of xylem growth at the xeric and dry-mesic study sites. Threshold air and soil temperatures were estimated for the periods 2007–10 and 2008–10, respectively. Standard deviations among trees are indicated.

hand, distinct differences in soil temperature thresholds were found between study sites. At the xeric site, the minimum, mean and maximum soil temperature thresholds were 1.6, 3.5 and 6.1  $^{\circ}\text{C}$  above temperature thresholds calculated for the dry-mesic site (Figure 3b).

Degree-day sums of air and soil temperature and precipitation sum calculated from 1 January and 20 March until onset of xylem growth are depicted in Figures 4 and 5 for both study sites. Among years, air temperature sum until the beginning of xylem growth at the xeric site ranged from 186 DD in 2009 to 287 DD in 2007 and 131 DD in 2008 to 154 DD in 2010, when DD was calculated from 1 January and 20 March, respectively. At the dry-mesic site, corresponding temperature sums ranged from 162 to 247 DD and 121 to 154 DD. Hence, accumulated heat in air from 1 January until onset of xylem growth varied strongly between study years at both sites (mean values  $\pm$  standard deviation (STD) were  $230 \pm 44$  and  $205 \pm 36$  DD at the xeric and dry-mesic sites, respectively), while mean amount of warmth necessary for initiation of xylem growth after spring equinox was  $144 \pm 10$  and  $137 \pm 12$  DD at the xeric and dry-mesic sites, respectively (Figure 6a).

Heat sum in 5–10 cm soil depth calculated from 1 January and 20 March until start of xylem growth was significantly different between study sites ( $P < 0.01$ ), whereby DD was strikingly lower at the dry-mesic site compared with the xeric site (Figure 6b). At the xeric site accumulated heat below ground calculated after spring equinox (mean  $\pm$  STD:  $141 \pm 29$  DD) was consistent with heat sum determined from records of air temperature. A strikingly high variability in precipitation sum until onset of xylem growth was detected between study years, ranging from 42.7 to 147.3 mm (mean  $\pm$  STD:  $110 \pm 48$  mm) and from 2.6 to 59 mm (mean  $\pm$  STD:  $30 \pm 24$  mm), when cumulated after 1 January and 20 March, respectively (Figures 5 and 6c).

## Discussion

In most long-lived plant species native to cool-temperate climates, photoperiod and temperature are considered to be the main environmental factors that synergistically control release from winter dormancy and onset of developmental processes in spring (e.g., induction of bud burst; Hay 1990, Körner 2006). Several authors reported that cambium activity and xylem growth are highly responsive to temperature (e.g., Deslauriers and Morin 2005, Rossi et al. 2007, Deslauriers et al. 2008, Gruber et al. 2009) and cambial reactivation can be induced

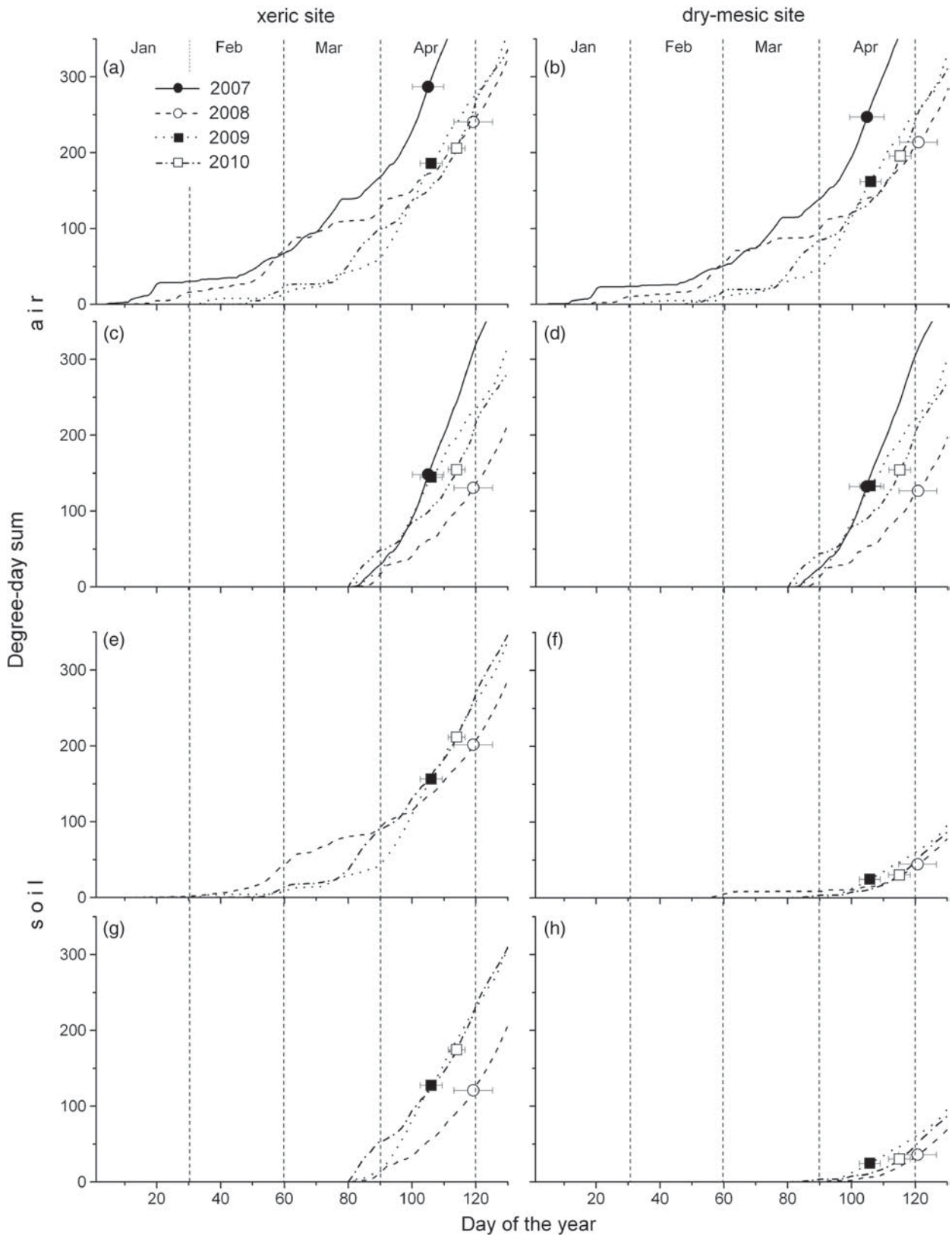


Figure 4. Degree-day sums of air (a–d) and soil (e–h) temperature during 2007–10 cumulated after 1 January (a, b and e, f) and 20 March (c, d and g, h) compared with onset of xylem growth at the xeric and dry-mesic sites. Symbols indicate onset of xylem growth (mean values  $\pm$  standard deviation) during study years. The study started in April 2007, which is why no DD of soil temperature could be calculated for 2007.

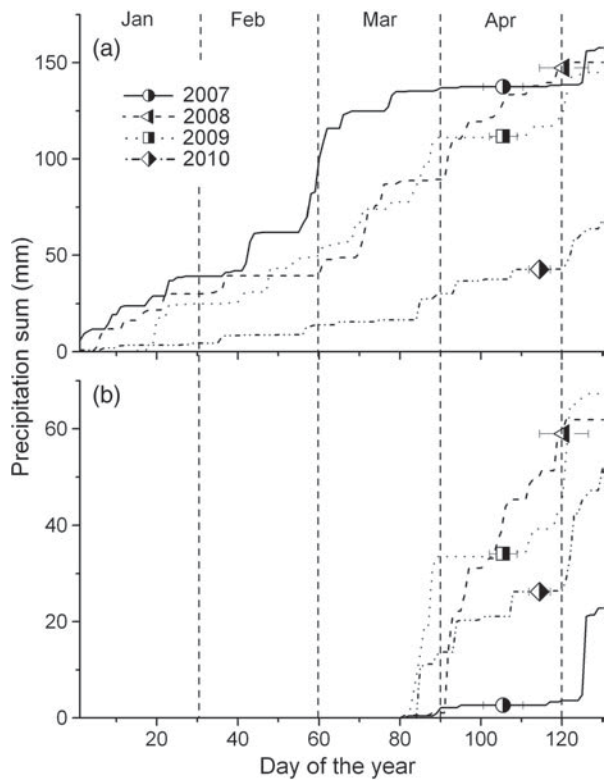


Figure 5. Precipitation sums within the study area during 2007–10 cumulated after 1 January (a) and 20 March (b) compared with mean onset of xylem growth. Because of close proximity of study sites records of precipitation were regarded as representative of both sites, which is why onset of xylem growth was averaged (split symbols).

during the quiescent stage by artificial heating (Oribe et al. 2001, Gričar et al. 2007). Plants from cold environments also require the experience of a period of cold weather, i.e., there is a chilling requirement before growth in spring is resumed (Cannell and Smith 1986, Heide 1993a, Myking and Heide 1995, Saxe et al. 2001). Within the study area a sufficient number of chilling days occurs every winter. This can also be deduced from the finding that although mean air temperature during winter 2007 was 2.9 °C higher than in 2009, date of onset of xylem growth was not significantly different between these years (mean daily air temperature from December through February was 1.2 and –1.7 °C in 2007 and 2009, respectively).

The existence of a temperature threshold in the range 5–7 °C above which significant tree growth occurs is well known for cold-adapted trees (for a review see Körner 2006). Rossi et al. (2008) estimated a daily minimum threshold air temperature of 4–5 °C for onset and ending of xylogenesis in conifers of cold climates. In our 4-year study period, mean daily minimum air temperature thresholds were 5.1 and 5.6 °C at the xeric and dry-mesic sites, respectively. Hence, results support the existence of a comparable temperature threshold for xylogenesis in *P. sylvestris* exposed to a montane dry inner

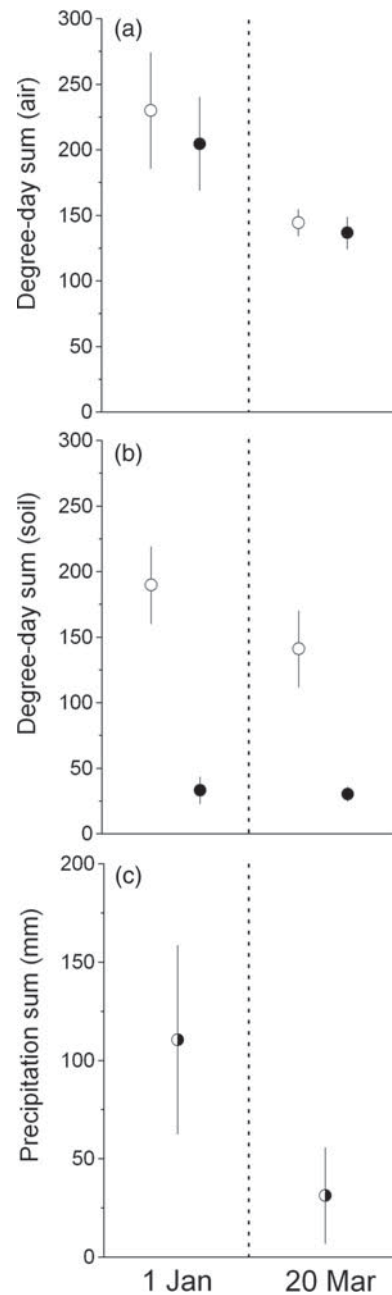


Figure 6. Degree-day sums of air (a) and soil temperature (b) and precipitation sums (c) (mean values  $\pm$  standard deviation) cumulated after 1 January and 20 March until onset of xylem growth for the xeric and dry-mesic sites (open and closed circles, respectively). For precipitation sums onset of xylem growth at the study sites was averaged (split symbols). Degree-day sum of air temperature was not significantly different between study sites ( $P > 0.05$ ; Student's *t*-test).

Alpine environment. Besides, evidence in mature trees of the boreal region was found that rest completion takes place around spring equinox after photoperiod requirements were met (e.g., Heide 1993b, Hänninen 1995, Partanen et al. 1998), while the speed of subsequent developmental processes is controlled by temperature (e.g., Saxe et al. 2001, Körner 2003). Onset of xylem growth in *P. sylvestris* in our study



varied from mid-April to early May during 2007–10. When the photoperiodic constraint was taken into account, the year-to-year variability in heat sum accumulated after spring equinox until onset of xylem growth was rather low. On the other hand, if timing of growth onset is assumed to be controlled solely by accumulated air temperature after 1 January, a much larger variability in DD until start of radial stem growth was found. The base temperature of  $\geq 5$  °C used for determining DD is in agreement with calculated air temperature threshold when there was a 0.5 probability of active xylem growth, which amounted to 5–6 °C. Our reasoning of photoperiodic control of growth onset is supported by Downs and Borthwick (1956), who reported that growth of *P. sylvestris* seedlings is strongly influenced by photoperiod. Furthermore, in boreal Norway spruce (*P. abies*), Slaney et al. (2007) also found smaller deviations from mean DD among years until bud burst, when temperature sums were calculated from early spring (1 April) instead of 1 January. Although our data might indicate that rest completion in *P. sylvestris* takes place close to spring equinox, while timing of onset of stem wood production is controlled by air temperature sum occurring afterwards, we are aware of shortcomings of our study, i.e., a short monitoring period and a limited sample size restricted to one location, which challenge the inference of photoperiodic control of increment onset in *P. sylvestris* within the study area. Because our results do not provide conclusive verification of the hypothesis of photoperiod requirement for rest completion, an experimental design with contrasting photoperiodic conditions is needed to properly separate temperature and photoperiodic effects on onset of xylem growth (cf. Partanen et al. 1998).

In a previous study on temporal dynamics of xylem growth within the study area (Gruber et al. 2010), we speculated that site-specific differences in onset of cambial activity and cell differentiation processes might be caused by earlier soil warming under open sparse canopy at the southwest-facing xeric site compared with shaded conditions prevailing at the slightly north-facing dry-mesic site. In this study we could show that soil temperature sums expressed as DD until onset of xylem growth and soil temperature thresholds for xylogenesis were significantly different between the xeric and dry-mesic sites. Because timing of onset of xylem growth was statistically not significantly different between sites during the 4-year study period, our results indicate that there is no leeway to assume that soil temperature determines start of xylem growth in *P. sylvestris* within a dry inner Alpine environment. This is in contrast to reports from high altitude treelines, where root-zone temperature was found to be critical to tree growth (Körner and Paulsen 2004) and possibly triggers aboveground cambial activity and xylem growth (Gruber et al. 2009). We suggest that in *P. sylvestris* under drought, stored water is used for enlargement of first tracheids after cambial resumption in spring rather than water transported from the soil. Our

suggestion is based on the finding that at the start of tracheid production, distinct differences were recorded in soil temperatures between study sites, which are expected to affect water uptake and transport and hence cell expansion differently, due to temperature dependence of both permeability of roots to water and viscosity of water (Pallardy 2008). Accordingly, the missing influence of precipitation on onset of xylem growth, which is in contrast to findings that a close relationship between spring precipitation and ring width of *P. sylvestris* was repeatedly detected within the study area and other dry inner Alpine environments (e.g., Oberhuber et al. 1998, Rigling et al. 2002, Schuster and Oberhuber 2005), can also be explained by adequate water storage capacity in the stem due to maintenance of a large proportion of sapwood to heartwood in pines (cf. Waring and Running 1978).

## Conclusion

Numerous studies have already reported earlier onset of phenological phases in spring and a lengthening of the growing season due to global warming (e.g., Menzel and Fabian 1999, Walther et al. 2002, Studer et al. 2005). Although in *P. sylvestris* earlier onset of increment growth with increasing spring temperatures can also be deduced from the results of our study, hot and dry conditions during the growing season were repeatedly found to cause early cessation of cambial activity and cell differentiation (e.g., Thabeet et al. 2009, Gruber et al. 2010, Eilmann et al. 2011). Hence, although a warmer climate may cause a gradual shift in onset of stem growth to periods of low evapotranspiration forcing in early spring, recently observed large-scale decline of *P. sylvestris* in dry inner Alpine environments (e.g., Oberhuber 2001, Rebetez and Dobbertin 2004, Bigler et al. 2006) indicates increasing sensitivity to drought stress rather than increasing productivity due to an extended growing season. Furthermore, episodic late frost events in spring are anticipated to become more frequent in a warmer climate (IPCC 2007), increasing the risk of lethal frost injuries to newly developed shoots (Cannell and Smith 1986). Rossi et al. (2009) reported a later resumption of needle and shoot growth with respect to xylem differentiation in the stem of three timberline conifers. Hence, a comparative assessment of cambial resumption at breast height and needle and shoot growth in the upper crown of *P. sylvestris* as well as *in situ* determination of frost resistance of emerging shoots and tissues (cf. Taschler et al. 2004) will be necessary to elucidate the risk of frost damage due to earlier onset of tree growth in a warmer climate.

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## References

- Abe, H. and T. Nakai. 1999. Effect of the water status within a tree on tracheid morphogenesis in *Cryptomeria japonica* D. Don. *Trees* 14:124–129.
- Antonova, G.F. and V.V. Stasova. 1993. Effects of environmental factors on wood formation in Scots pine stems. *Trees* 7:214–219.
- Badeck, F.-W., A. Bondeau, K. Böttcher, D. Doktor, W. Lucht, J. Schaber and S. Sitch. 2004. Responses of spring phenology to climate change. *New Phytol.* 162:295–309.
- Baskerville, G.L. and P. Emin. 1969. Rapid estimation of heat accumulation from maximum and minimum temperatures. *Ecology* 50:514–517.
- Begum, S., S. Nakaba, Y. Oribe, T. Kubo and R. Funada. 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.
- Bigler, C., O.U. Bräker, H. Bugmann, M. Dobbertin and A. Rigling. 2006. Drought as an inciting mortality factor in Scots pine stands of the Valais, Switzerland. *Ecosystems* 9:330–343.
- Cannell, M.G.R. and R.I. Smith. 1986. Climatic warming, spring budburst and frost damage in trees. *J. Appl. Ecol.* 23:177–191.
- Deslauriers, A. and H. Morin. 2005. Intra-annual tracheid production in balsam fir stems and the effect of meteorological variables. *Trees* 19:402–408.
- Deslauriers, A., H. Morin and Y. Begin. 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., S. Rossi, T. Anfodillo and A. Saracino. 2008. Cambial phenology, wood formation and temperature thresholds in two contrasting years at high altitude in southern Italy. *Tree Physiol.* 28:863–871.
- Downs, R.J. and H.A. Borthwick. 1956. Effects of photoperiod on growth of trees. *Bot. Gaz.* 117:310–326.
- Eilmann, B., R. Zweifel, N. Buchmann, E. Graf Pannatier and A. Rigling. 2011. Drought alters timing, quantity, and quality of wood formation in Scots pine. *J. Exp. Bot.* doi:10.1093/jxb/erq443.
- Ellenberg, H. and C. Leuschner. 2010. Vegetation Mitteleuropas mit den Alpen in ökologischer, dynamischer und historischer Sicht. Ulmer, Stuttgart, 1333 p.
- FAO. 1998. World references base for soil resources. FAO, Rome.
- Garber, M.P. 1983. Effects of chilling and photoperiod on dormancy release of container-grown loblolly pine seedlings. *Can. J. For. Res.* 13:1265–1270.
- Gričar, J., M. Zupančič, K. Čufar and O. Primož. 2007. Regular cambial activity and xylem and phloem formation in locally heated and cooled stem portions of Norway spruce. *Wood Sci. Technol.* 41:463–475.
- Gruber, A., D. Baumgartner, J. Zimmermann and W. Oberhuber. 2009. Temporal dynamic of wood formation in *Pinus cembra* along the alpine treeline ecotone and the effect of climate variables. *Trees* 23:623–635.
- Gruber, A., S. Stöbl, B. Veit and W. Oberhuber. 2010. Impact of drought on the temporal dynamics of wood formation in *Pinus sylvestris*. *Tree Physiol.* 30:490–501.
- Hänninen, H. 1995. Effects of climate change on trees from cool and temperate regions: an ecophysiological approach to modeling of bud burst phenology. *Can. J. Bot.* 73:183–199.
- Hay, R.K.M. 1990. The influence of photoperiod on the drymatter production of grasses and cereals. *New Phytol.* 116:233–254.
- Heide, O.M. 1993a. Dormancy release in beech buds (*Fagus sylvatica*) requires both chilling and long days. *Physiol. Plant.* 89:187–191.
- Heide, O.M. 1993b. Daylength and thermal time responses of budburst during dormancy release in some northern deciduous trees. *Physiol. Plant.* 88:531–540.
- Hoffman, G. and H. Lyr. 1967. Über die Wirkung der winterlichen Thermoperiode auf das Wurzel- und Sproßwachstum von *Pinus sylvestris* L. *Flora* 158:373–383.
- Hsiao, T.C. and E. Acevedo. 1974. Plant responses to water deficits, water-use efficiency, and drought resistance. *Agric. Meteorol.* 14:59–84.
- IPCC 2007. Technical report. *In Climate Change 2007: the Physical Science Basis*. Eds. S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor and H.L. Miller. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, 996 p.
- Jackson, S.D. 2009. Plant responses to photoperiod. *New Phytol.* 181:517–531.
- Jensen, K.F. and G.E. Gatherum. 1965. Effects of temperature, photoperiod and provenance on growth and development of Scots pine seedlings. *For. Sci.* 11:189–199.
- Karlsson, P.S., H. Bylund, S. Neuvonen, S. Heino and M. Tjus. 2003. Climatic response of budburst in the mountain birch at two areas in northern Fennoscandia and possible responses to global change. *Ecography* 26:617–625.
- Kirdyanov, A., M. Hughes, E. Vaganov, F. Schweingruber and P. Silkin. 2003. The importance of early summer temperature and date of snow melt for tree growth in the Siberian Subarctic. *Trees* 17:61–69.
- Körner, C. 1998. A re-assessment of high elevation treeline positions and their explanation. *Oecologia* 115:445–459.
- Körner, C. 2003. Alpine plant life: functional plant ecology of high mountain ecosystems. Springer, Berlin, 344 p.
- Körner, C. 2006. Significance of temperature in plant life. *In Plant Growth and Climate Change*. Eds. J.I.L. Morison and M.D. Morecroft. Blackwell Publishing Ltd, Oxford, UK, pp 48–69.
- Körner, C. and D. Basler. 2010. Phenology under global warming. *Science* 327:1461–1462.
- Körner, C. and J. Paulsen. 2004. A world-wide study of high altitude treeline temperatures. *J. Biogeogr.* 31:713–732.
- Kramer, K. 1994. Selecting a model to predict the onset of growth of *Fagus sylvatica*. *J. Appl. Ecol.* 31:72–181.
- Little, C.H.A. and J.M. Bonga. 1974. Rest in the cambium of *Abies balsamea*. *Can. J. Bot.* 52:1723–1730.
- Lupi, C., H. Morin, A. Deslauriers and S. Rossi. 2010. Xylem phenology and wood production: resolving the chicken-or-egg dilemma. *Plant, Cell Environ.* 33:1721–1730.
- Lüttge, U. and B. Hertel. 2009. Diurnal and annual rhythms in trees. *Trees* 23:683–700.
- Menzel, A. and P. Fabian. 1999. Growing season extended in Europe. *Nature* 397:659.

- Menzel, A., T.H. Sparks, N. Estrella, et al. 2006. European phenological response to climate change matches the warming pattern. *Glob. Change Biol.* 12:1969–1976.
- Myking, T. and O.M. Heide. 1995. Dormancy release and chilling requirement of buds of latitudinal ecotypes of *Betula pendula* and *B. pubescens*. *Tree Physiol.* 15:697–704.
- Oberhuber, W. 2001. The role of climate in the mortality of Scots pine (*Pinus sylvestris* L.) exposed to soil dryness. *Dendrochronologia* 19:45–55.
- Oberhuber, W. and A. Gruber. 2010. Climatic influences on intra-annual stem radial increment of *Pinus sylvestris* (L.) exposed to drought. *Trees* 24:887–898.
- Oberhuber, W. and W. Kofler. 2000. Topographic influences on radial growth of Scots pine (*Pinus sylvestris* L.) at small spatial scales. *Plant Ecol.* 146:229–238.
- Oberhuber, W., M. Stumböck and W. Kofler. 1998. Climate-tree-growth relationships of Scots pine stands (*Pinus sylvestris* L.) exposed to soil dryness. *Trees* 13:19–27.
- Oribe, Y. and T. Kubo. 1997. Effect of heat on cambial reactivation during winter dormancy in evergreen and deciduous conifers. *Tree Physiol.* 17:81–87.
- Oribe, Y., R. Funada, M. Shibagaki and T. Kubo. 2001. Cambial reactivation in locally heated stems of evergreen conifer *Abies sachalinensis* (Schmidt) Masters. *Planta* 212:684–691.
- Pallardy, S.G. 2008. *Physiology of woody plants*. 3rd edn. Elsevier, Oxford, UK, 454 p.
- Partanen, J., V. Koski and H. Hänninen. 1998. Effects of photoperiod and temperature on the timing of bud burst in Norway spruce (*Picea abies*). *Tree Physiol.* 18:811–816.
- Pichler, P. and W. Oberhuber. 2007. Radial growth response of coniferous forest trees in an inner Alpine environment to heat-wave in 2003. *For. Ecol. Manage.* 242:688–699.
- Rebetez, M. and M. Dobbertin. 2004. Climate change may already threaten Scots pine stands in the Swiss Alps. *Theor. Appl. Clim.* 79:1–9.
- Rensing, K.H. and A.L. Samuels. 2004. Cellular changes associated with rest and quiescence in winter-dormant vascular cambium of *Pinus contorta*. *Trees* 18:373–380.
- Rigling, A., O.U. Bräker, G. Schneiter and F.H. Schweingruber. 2002. Intra-annual tree-ring parameters indicating differences in drought stress of *Pinus sylvestris* forests within the Erico-Pinien in the Valais (Switzerland). *Plant Ecol.* 163:105–121.
- Rossi, S., T. Anfodillo and R. Menardi. 2006a. Trephor: a new tool for sampling microcores from tree stems. *IAWA J.* 27:89–97.
- Rossi, S., A. Deslauriers and T. Anfodillo. 2006b. Assessment of cambial activity and xylogenesis by microsampling tree species: an example at the Alpine timberline. *IAWA J.* 27:383–394.
- Rossi, S., A. Deslauriers, T. Anfodillo, H. Morin, A. Saracino, R. Motta and M. Borghetti. 2006c. Conifers in cold environments synchronize maximum growth rate of tree-ring formation with day length. *New Phytol.* 170:301–310.
- Rossi, S., A. Deslauriers, T. Anfodillo and V. Carraro. 2007. Evidence of threshold temperatures for xylogenesis in conifers at high altitudes. *Oecologia* 152:1–12.
- Rossi, S., A. Deslauriers, J. Gričar, et al. 2008. Critical temperatures for xylogenesis in conifers of cold climates. *Glob. Ecol. Biogeogr.* 17:696–707.
- Rossi, S., C.B.K. Rathgeber and A. Deslauriers. 2009. Comparing needle and shoot phenology with xylem development on three conifer species in Italy. *Ann. For. Sci.* 66:206.
- Rossi, S., H. Morin, A. Deslauriers and P.-Y. Plourde. 2011. Predicting xylem phenology in black spruce under climate warming. *Glob. Change Biol.* 17:614–625.
- Saxe, H., M.G.R. Cannell, B. Johnsen, M.G. Ryan and G. Vourlitis. 2001. Tree and forest functioning in response to global warming. *New Phytol.* 149:369–399.
- Schuster, S. and W. Oberhuber. 2005. Sensitivity of pine stands in the inneralpine dry valley Vinschgau (Italy) against drought and parasitic infection (in German). *Gredleriana* 5:171–190.
- Seo, J.-W., D. Eckstein, R. Jalkanen, S. Rickebusch and U. Schmitt. 2008. Estimating the onset of cambial activity in Scots pine in northern Finland by means of the heat-sum approach. *Tree Physiol.* 28:105–112.
- Slaney, M., G. Wallin, J. Medhurst and S. Linder. 2007. Impact of elevated carbon dioxide concentration and temperature on bud burst and shoot growth of boreal Norway spruce. *Tree Physiol.* 27:301–312.
- Studer, S., C. Appenzeller and C. Defila. 2005. Inter-annual variability and decadal trends in alpine spring phenology: a multivariate analysis approach. *Clim. Change* 73:395–414.
- Taschler, D., B. Beikircher and G. Neuner. 2004. Frost resistance and ice nucleation in leaves of five woody timberline species measured in situ during shoot expansion. *Tree Physiol.* 24:331–337.
- Thabeet, A., M. Vennetier, C. Gadbin-Henry, N. Denedle, M. Roux, Y. Caraglio and B. Vila. 2009. Response of *Pinus sylvestris* L. to recent climatic events in the French Mediterranean region. *Trees* 23:843–853.
- Tranquillini, W. 1979. *Physiological ecology of the alpine timberline. Tree existence at high altitudes with special references to the European Alps*. Ecological Studies 31, Springer, Berlin, 137 p.
- Vaganov, E.A., E.-D. Schulze, M.V. Skomarkova, A. Knohl, W.A. Brand and C. Roscher. 2009. Intra-annual variability of anatomical structure and  $\delta^{13}\text{C}$  values within tree rings of spruce and pine in alpine, temperate and boreal Europe. *Oecologia* 161:729–745.
- Walther, G.R., E. Post, P. Convey, A. Menzel, C. Parmesan, T.J.C. Beebee, J.-M. Fromentin, O. Hoegh-Guldberg and F. Bairlein. 2002. Ecological responses to recent climate change. *Nature* 416:389–395.
- Waring, R.H. and S.W. Running. 1978. Sapwood water storage: its contribution to transpiration and effect upon water conductance through the stems of old-growth Douglas-fir. *Plant, Cell Environ.* 1:131–140.