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Original article

Effects of climate variables on intra-annual stem radial increment in *Pinus cembra* (L.) along the alpine treeline ecotone

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Abstract

• Within the alpine treeline ecotone tree growth is increasingly restricted by extreme climate conditions. Although intra-annual stem growth recorded by dendrometers can be linked to climate, stem diameter increments in slow-growing subalpine trees are masked by changes in tree water status.

• We tested the hypothesis that intra-annual radial stem growth in *Pinus cembra* is influenced by different climate variables along the treeline ecotone in the Austrian Alps. Dendrometer traces were compared with dynamics of xylem cell development to date onset of cambial activity and radial stem growth in spring.

• Daily fluctuations in stem radius reflected changes in tree water status throughout the treeline ecotone. Extracted daily radial increments were significantly correlated with air temperature at the timberline and treeline only, where budburst, cambial activity and enlargement of first tracheids also occurred quite similarly. A close relationship was detected between radial increment and number of enlarging tracheids throughout the treeline ecotone.

• We conclude that (i) the relationship between climate and radial stem growth within the treeline ecotone is dependent on a close coupling to atmospheric climate conditions and (ii) initiation of cambial activity and radial growth in spring can be distinguished from stem re-hydration by histological analysis.

Résumé – Effets du climat sur les variation intra-annuelles de croissance radiale de *Pinus cembra* (L.) le long d'un écotone à la limite altitudinale de forêt alpine.

• La croissance des arbres est fortement limitée par les conditions climatiques extrêmes de la limite altitudinale de la forêt. Les variations interannuelles de croissance radiale enregistrées par des dendromètres de tige peuvent être reliées au climat. Mais la croissance radiale peut être masquée par des changements de l'état hydrique des arbres.

• Nous avons testé l'hypothèse selon laquelle les variations intra-annuelles de croissance radiale des troncs de *P. cembra* étaient contrôlées par différents facteurs climatiques le long de l'écotone de la limite altitudinale de la forêt dans les Alpes autrichiennes. Les enregistrements obtenus à partir de dendromètres ont été comparés à des dynamiques de développement des cellules cambiales, afin de détecter avec précision la date de départ de la croissance cambiale au printemps.

• Les variations diurnes de diamètre du tronc reflétaient des variations de l'état hydrique des arbres le long de l'écotone. Les incréments quotidiens en diamètre extraits de ces signaux étaient corrélés à la température de l'air à la limite forestière, mais ne l'étaient plus à des altitudes supérieures, alors que le débourrement des bourgeons, la reprise d'activité cambiale et l'expansion des premiers trachéides se produisaient pratiquement en même temps le long de l'écotone. Une forte corrélation a été détectée tout au long de l'écotone, entre la croissance radiale et le nombre de trachéides en expansion.

• Nous concluons que : (i) la croissance radiale le long de l'écotone de la limite altitudinale de la forêt est fortement couplée aux conditions climatiques dans l'atmosphère, et que : (ii) l'initiation de l'activité cambiale et de la croissance radiale au printemps peut être distinguée de la réhydratation des troncs en mobilisant des analyses histologiques.

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1. INTRODUCTION

Automatic point or band dendrometers have been used traditionally for continuous monitoring of stem radial variation throughout the year and determination of seasonal tree growth (e.g., Bouriaud et al., 2005; Carrer et al., 1998; Fritts, 1961; Herzog et al., 1995; Tardif et al., 2001). However, dendrometer measurements are strongly influenced by water-related swelling and shrinkage of the bark (Daudet et al., 2005; Zweifel and Häsler, 2000) and onset of wood formation in spring is masked by re-hydration of the stem (e.g., Downes et al., 1999; Kozlowski and Winget, 1964; Mäkinen et al., 2003; Zweifel and Häsler, 2001). A comparison with cellular analyses is therefore necessary to determine crucial phenological events such as cambial growth onset and ending and stem radius increment on the basis of dendrometer data (Deslauriers et al., 2003a; Mäkinen et al., 2008; Tardif et al., 2001). Mäkinen et al. (2008) and Zweifel and Häsler (2001) also reported that in slow-growing boreal tree species and subalpine Norway spruce (*Picea abies*), respectively, it was impossible to differentiate water-induced radius expansion from growth-induced radius expansion in spring, when replenishment of dehydrated bark and onset of radial stem growth occurred simultaneously.

Alternatively, temporal dynamics of cambial activity and xylem development can be monitored throughout the growing season by repeatedly extracting wood samples at short intervals (e.g., Antonova and Stasova, 1993; 1997; Bäucker et al., 1998; Deslauriers et al., 2003b; Loris, 1981; Rossi et al., 2006b) or by inserting a pin through the bark into the outer xylem to wound the cambium (pinning-technique; e.g., Kuroda, 1986; Schmitt et al., 2004; Wolter, 1968). Both techniques, which enable direct observation of the periodic process of cambial activity and tracheid differentiation (radial cell enlargement, secondary wall thickening, lignification and cell death; Plomion et al., 2001; Vaganov et al., 2006) are regarded to represent the most reliable techniques for monitoring wood formation (Mäkinen et al., 2008; cf. Rossi and Deslauriers, 2007). In a study on seasonal dynamics of wood formation in *Picea abies* Mäkinen et al. (2008) also found that pinning and micro-coring yielded rather similar results regarding cellular phenology of xylem growth ring formation.

A few papers already compared dynamic of wood formation monitored by automatic dendrometers with laborious histological analysis (Mäkinen et al., 2003; 2008; Rossi et al., 2006c; Zweifel et al., 2006), however, results from these studies were contradicting. Whereas Mäkinen et al. (2003; 2008) stated that dendrometers are of dubious value for measuring the timing of actual xylem formation, Rossi et al. (2006c) and Zweifel et al. (2006) reported that both methods are able to detect the course of intra-annual radial growth. On the other hand, both methods have been applied to link intra-annual dynamics of radial tree growth with climate (e.g., Antonova and Stasova, 1993; Bouriaud et al., 2005; Deslauriers et al., 2003a; Downes et al., 1999; Gruber et al., 2009; Rossi et al., 2008; Zweifel et al., 2006).

This paper tests the hypothesis that along the treeline ecotone, where steep environmental gradients cause stand density and tree vitality to decrease rapidly with increasing altitude (Körner, 2003; Tranquillini, 1979), intra-annual radial stem growth in cembran pine (*Pinus cembra*) is influenced by different climate variables. Because enlargement of tracheids is considered to represent the major driving force for radial stem growth (Deslauriers et al., 2003a), we compared seasonal changes in stem radius of *Pinus cembra* determined by point dendrometers with intra-annual dynamics of xylem cell development along an altitudinal gradient including the timberline, treeline and krummholz-site. After determination of onset of radial stem growth in dendrometer traces by histological analysis of xylogenesis, daily changes in stem radius and daily radial increments were correlated with environmental variables and xylem sap flow. Additionally, time of maximum increment growth was determined throughout the treeline ecotone by applying Gompertz modelled growth functions (e.g., Deslauriers and Morin, 2005; Zeide, 1993).

2. MATERIAL AND METHODS

2.1. Study area and climatic conditions

The study area is situated at Mt. Patscherkofel (2 246 m a.s.l.) near Innsbruck, in western Austria (47° 12' N, 11° 27' E). Mt. Patscherkofel is located in the Central Austrian Alps within an inneralpine dry zone, where the local climate is strongly influenced by warm and dry southerly winds (Föhn). This special situation is reflected in local climate. During the period 1967–2004 mean annual precipitation at the top of Mt. Patscherkofel was 890 mm with a maximum during summer (June–August: 358 mm) and minimum in winter (December-February: 147 mm). Mean annual temperature at timberline (Station Klimahaus and Alpengarten, 1 950 m a.s.l.) during the same period was 2.5 °C and the coldest and warmest months were February (–4.3 °C) and July (10.0 °C), respectively.

Since the dominant conifer in the study area and at the timberline in the central part of the Eastern Alps is *Pinus cembra* – European larch (*Larix decidua*) and *Picea abies* are scattered at some sites – we concentrated on the analysis of growth dynamics of this tree species.

Study plots were selected along a southwest facing altitudinal transect including the timberline at 1 950 m a.s.l. (upper limit of tall trees forming a closed canopy), the treeline at 2 110 m a.s.l. (isolated trees above the timberline ≥ 3 m height), and the krummholz-belt at 2 180 m a.s.l. (outposts of short-stature individuals). At the krummholz-belt only individuals, with almost no damage due to extreme environmental conditions, especially winter desiccation, were selected.

The geology of the Mt. Patscherkofel region (Tuxer Alpen as part of the Central Tyrolean Alps) is dominated by gneisses and schist. According to the World Base for Soil Resources (FAO, 1998), the soil at the study site is classified as a haplic podzol, a soil type typical for the Central Austrian Alps (Neuwinger, 1970).

At each study plot air temperature, relative humidity (HMP45C, Vaisala, Helsinki, Finland), solar radiation (SP-Lite, Campbell Scientific, Shepshed; UK), wind velocity (A100R, Campbell Scientific, Shepshed; UK), soil temperature (type-T thermocouples) and soil water potential (EQ3 Equitensiometer, Liu, Dachau, Germany) were monitored and recorded with a CR10X data logger (Campbell Scientific, Shepshed; UK) programmed to record 30-minute averages of measurements taken every minute throughout the growing season of

2007. Daily precipitation was recorded at a meteorological station on top of Mt. Patscherkofel (2 246 m a.s.l.).

2.2. Measurements of xylem sap flow density and changes in stem thickness

Xylem sap flow density (u) was monitored continuously throughout the growing season of 2007 with 2 cm long continuously heated sap flow gauges according to Granier (1985). The sensors were inserted into the xylem of three trees per plot 100 cm, 50 cm, and 15 cm above ground at the timberline, the treeline and the krummholz-site, respectively. The sensors were installed into the N-facing side of the trees under study and were shielded through a thermally isolating styrofoam cover. Data were recorded with a CR10X data logger (Campbell Scientific, Shepshed; UK) programmed to record 30-min averages of measurements taken every minute. Xylem sap flow density (u) was then calculated according to the equation:

$$u = 0.714 \times (\Delta T_{max}/\Delta T_{act} - 1)^{1.231} \times 1\,440$$

where ΔT_{max} is the maximum temperature difference recorded during the night when transpiration is close to zero and ΔT_{act} is the momentary temperature difference.

At the same trees we also installed point dendrometers (Loris, 1981) in summer 2006 and measurements were started in early March and mid April 2007 at timberline and at study plots above, respectively. Dendrometers consisted of an electronic displacement-sensor (linear motion potentiometer, MM10 Megatron, Putzbrunn Munich, Germany) mounted on a stainless steel rod anchored 5 cm to 10 cm above sap flow sensors. Dead outermost layers (periderm) of the bark were slightly removed at timberline and treeline to reduce the influence of hygroscopic swelling and shrinkage of the bark on dendrometer traces and to ensure close contact with the stem (cf. Zweifel and Häsler, 2001). Bark thickness determined after removing point dendrometers at the end of the growing season 2007 was about 5 mm, 4 mm and 1.5 mm at timberline, treeline and krummholz-site, respectively. Data were recorded with a CR10X data logger (Campbell Scientific, Shepshed; UK) programmed to record 30-minute averages of measurements taken every minute and daily increment of stem radius was calculated by averaging all daily measurements (48 values/day).

The daily stem radius variation was then determined by calculating the difference between mean values of two consecutive days ("daily mean approach", Deslauriers et al., 2007a), which represents a combination of water- and growth-induced radius expansion (e.g., Daudet et al., 2005; Herzog et al., 1995; Steppe et al., 2006). Additionally, we extracted daily radial increments from dendrometer records based on the methodology described by Deslauriers et al. (2003a) and Downes et al. (1999). Daily stem radial increment was defined as that part of the stem's circadian cycle, when the stem radius exceeded the morning maximum until the subsequent maximum, which in general was reached the following day (see Fig. 2). When several days were required until the previous cycle maximum was exceeded, the difference between maximum values was divided by the corresponding number of days. Considering the small amount of radial change represented by cambial cell division, cell enlargement was regarded the major driving force for radial stem increase (cf. Deslauriers et al., 2003a). Secondary wall thickening and lignification take place inside enlarged cells and therefore are not expressed as a radial increase. Therefore, dendrometer traces were set to zero

when first enlarging cells were detected. Pearson correlation coefficients were calculated between climate variables (relative air humidity, precipitation, air temperature) and xylem sap flow density, and daily stem radius variations and increments extracted from dendrometer records. Due to higher measurement variability than increment growth at the end of the growing season only the main period of linear radial stem increase, i.e. May and June (cf. Figs. 4, 7), was used for calculating relationships between daily radial increment and climate-parameters and xylem sap flow density (cf. Deslauriers and Morin, 2005; Deslauriers et al., 2007b).

2.3. Xylem sampling and determination of wood formation

Seasonal wood formation dynamics were monitored during the growing season 2007 by taking small punched cores from 5 trees/site of the outermost tree rings (micro-cores) with a diameter and length of 2.5 mm and *c.* 2 cm, respectively (Rossi et al., 2006a). To determine the variability in intra-annual wood formation between trees at each plot (i.e., timberline, treeline and krummholz-site), individual trees were randomly selected. Furthermore, to avoid influence of frequent wounding on dendrometer traces, different sample populations for determination of wood formation and dendrometer measurements were selected. Samples were taken on the slope-parallel side of the stem following a spiral trajectory up the stem and at timberline from *c.* 15 cm below breast height (1.3 m) to *c.* 15 cm above. At treeline and within the krummholz-belt, samples were taken above the ground at *c.* 50 (± 15 cm) and *c.* 15 cm (± 5 cm), respectively. A distance of *c.* 2 cm in tangential and longitudinal direction was kept to avoid lateral influence of wound reactions on adjacent sampling positions.

Micro-cores were taken at all study plots from late April through October in about 10-day intervals to include the whole dynamic of xylem formation. Because of small diameter of tree stems at the krummholz-site, sampling at this plot was reduced to about once every two weeks.

Collected core samples were prepared for light microscopy. Immediately after extraction cores were fixed in a solution of 70% ethanol, propionic acid and 40% formaldehyde (mixing ratio: 90/5/5), subsequently embedded in glycolmethacrylate (Technovit 7 100) and polymerized after adding an accelerator. Transverse sections of *c.* 12 μm were cut with a microtome, stained with a water solution of 0.05% cresyl fast violet and observed under a light microscope with polarised light to differentiate the development of xylem cells, i.e. the discrimination between tracheids in enlarging and cell-wall thickening phase (Antonova and Stasova, 1993; Deslauriers et al., 2003b; Rossi et al., 2006b). The number of cambial cells (i.e., fusiform cells lacking radial enlargement), radial enlarging cells, cells undergoing secondary wall thickening and mature xylem cells were counted on all sampled cores in three radial rows. Xylem formation was considered to have begun, when one horizontal row of cells was detected in the enlarging phase. Values, i.e. the number of cells in different zones of 5 cores (trees) per date and for each site were averaged.

2.4. Standardisation of cell number and fitting of radial growth

Circumferential variability in cambial activities including ring width, cell number, cell diameter and wall thickness exist at different

positions of the stem (Creber and Chaloner, 1984). Because micro-cores were taken from different positions within the tree circumference during the growing season, standardisation of cell number is therefore required (Rossi et al., 2003). For this purpose, the total cell number of the previous tree ring was recorded in every sample and used for a cell number correction for each tree. Number of enlarging cells in each sample (i.e., micro-cores taken throughout growing season 2007) was corrected as follows:

$$nc_i = n_i \times n_m / n_s$$

where:

nc_i = corrected number of enlarging cells
n_i = counted number of enlarging cells
n_m = mean cell number of previous ring of all samples
n_s = cell number of previous ring for each sample.

Short-term variation in dendrometer records were modelled with a Gompertz function using the nonlinear regression procedure included in the Origin software package (OriginLab Corporation, Northampton, MA, USA). The Gompertz equation proved its versatility to describe growth limiting processes (Camarero et al., 1998; Deslauriers and Morin, 2005; Rossi et al., 2006c; Zeide, 1993). Based on developed Gompertz models dynamics of daily radial growth throughout the growing season 2007 was determined at all study plots.

3. RESULTS

3.1. Microclimate during the growing season

In 2007 the growing period was extended due to occurrence of exceptionally mild temperatures (3.4 °C above long-term mean 1967–2004 recorded at 1 950 m a.s.l.; LTM) and low precipitation (23% below LTM recorded at the top of Mt. Patscherkofel) in spring (March–May, Tab. I). Thus, all the study plots were already snow free in early April. Mean temperature and total precipitation in summer (June–August) 2007 exceeded the LTM by 1.3 °C and *c*. 15%, respectively. Highest mean air temperature was recorded in July (Fig. 1, Tab. I). Irrespective of the study plot, the relative humidity of the air fluctuated around of 75% and with values down to 47% during a dry period lasting from 10 July to 1 August 2007 (Fig. 1). Soil water potential throughout the growing period 2007 did not decrease below –0.08 MPa throughout the treeline ecotone (data not shown).

Underneath the closed tree canopy at the timberline, mean soil temperature during May through August 2007 was at average 1.6 and 2.6 °C lower as compared to soil temperature recorded at treeline and within the krummholz-belt, respectively (Fig. 1, Tab. I). In May 2007, when cambial activity was recovered at all sites, mean daily soil temperature reached 4.9, 6.4 and 7.5 °C at the timberline, treeline and krummholz-site, respectively. Highest mean monthly soil temperatures were recorded in July (Fig. 1, Tab. I).

3.2. Dynamics of tree ring growth and relationship with xylogenesis

Age of trees selected for dendrometer measurements and determination of xylem cell dynamic differed by about 20 y at
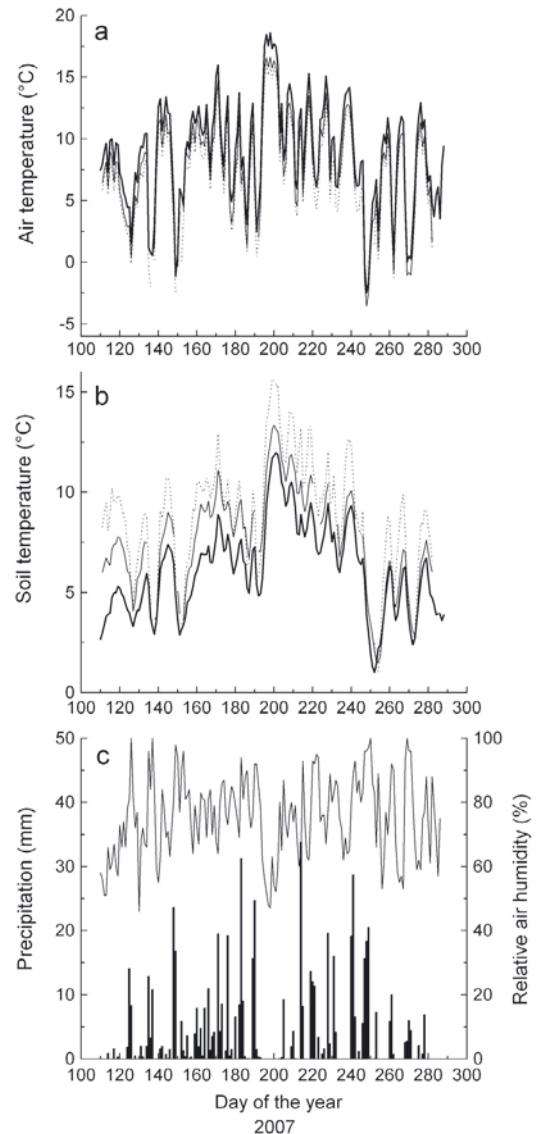

Figure 1. Climate variables recorded during the growing season 2007 within the treeline ecotone. (a) Mean daily air temperature. (b) Mean daily soil temperature. (c) Mean daily sum of precipitation (Mt. Patscherkofel summit) and mean relative air humidity averaged over the 3 sites within the treeline ecotone. In (a, b) study sites are denoted by thick and thin solid lines for timberline and treeline, respectively, and dotted lines for the krummholz-site.

the timberline and treeline and by about 5 y at the krummholz-site (Tab. II). The age-dependent decrease in ring width is typical for coniferous trees and is related to increasing tree size (see Bräker, 1981). Most krummholz-individuals, however, showed pronounced stem eccentricity, which impaired accurate ring width determination and comparison between sample populations. Typical diurnal cycles of stem shrinking and swelling throughout the treeline ecotone are depicted in Figure 2. Stem radius reached maximum and minimum values at all study plots in the morning (*c*. 6 a.m.) and afternoon

Table I. Monthly mean daily air and soil temperature (°C) and precipitation during the growing season 2007 (TB = timberline 1 950 m a.s.l., TR = treeline 2 110 m, K = krummholz-site 2 180 m). Long-term records (LTM ± standard deviation) of air temperature (recorded at 1 950 m a.s.l.; Station Klimahaus) and precipitation (Mt. Patscherkofel summit, 2 246 m) for the period 1967–2004, respectively.

	Air temperature (°C)			Soil temperature (°C)			Precipitation (mm)	
	LTM	TB	TR	K	TB	TR	K	LTM	2007
May	4.8 ± 1.90	6.9	6.2	5.2	4.9	6.4	7.5	76 ± 30	102
Jun	7.8 ± 1.87	10.1	9.1	8.0	6.4	8.4	9.2	109 ± 35	120
Jul	10.0 ± 1.90	11.3	10.0	9.1	8.5	10.3	11.2	127 ± 39	108
Aug	10.1 ± 1.89	10.4	9.9	8.5	8.0	9.3	10.5	121 ± 50	182

Table II. Characteristics of *Pinus cembra* trees selected for dendrometer measurements ($n = 3$ trees/site) and sampling micro-cores ($n = 5$ trees/site) throughout the treeline ecotone. Mean values ± standard deviation are given for age and ring width (RW).

					Dendrometer			Micro-coring		
Site	Altitude	Tree height	Crown	Stem	Age[3]	RW (mm)		Age[3]	RW (mm)	
	(m a.s.l.)	(m)	diameter[1] (m)	diameter[2] (cm)	(y)			(y)		
						2004–2006	2007		2004–2006	2007
Timberline	1 950	10–14	4–5	30–50	60 ± 7	2.34 ± 0.69	2.39 ± 0.42	80 ± 11	1.98 ± 0.65	1.84 ± 0.72
Treeline	2 110	3–5	2.5–3.0	15–20	49 ± 6	2.65 ± 0.83	3.14 ± 1.11	70 ± 15	2.20 ± 0.74	2.07 ± 0.92
Krummholz	2 180	0.5–1.0	0.35–0.60	5–7	16 ± 2	1.44 ± 0.44	1.49 ± 0.55	22 ± 5	2.00 ± 0.77	2.17 ± 0.63

[1] Range of maximum crown diameter.

[2] Range of tree diameter measured at sampling height (see Material and Methods).

[3] Approximate age of selected trees was calculated by adding 15, 10 and 5 y to mean number of measured tree rings of increment cores taken at *c*. 1.3 m (timberline), *c*. 50 cm (treeline) and *c*. 15 cm stem height (krummholz-site), respectively.
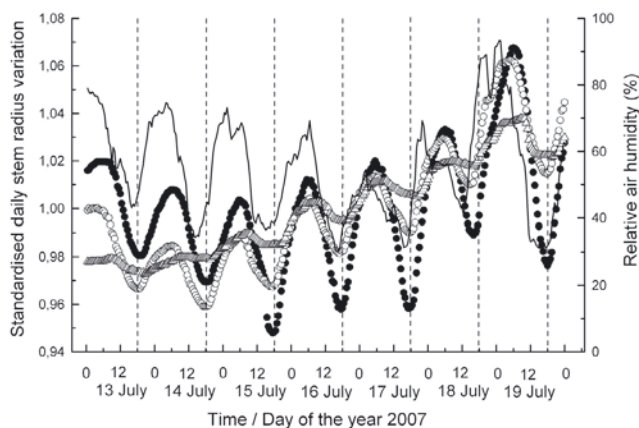

Figure 2. Diurnal cycles of standardised stem radius variation throughout the treeline ecotone ($n = 3$ trees/site) during a dry period in mid July compared to relative air humidity (mean value of all sites). Study sites are denoted by filled and open circles and open triangles for the timberline, treeline and krummholz-site, respectively.

(*c*. 6 p.m.), respectively, and were closely related to relative air humidity. Amplitude of diurnal fluctuations in stem radius during the growing season 2007 varied between *c*. 25 μm at the timberline and treeline, and *c*. 10 μm at the krummholz-site, respectively (Figs. 2, 3). Daily radius change showed closest direct correlations calculated over the whole measurement period with relative air humidity at the timberline ($r = 0.576$,

$p < 0.001$) and treeline ($r = 0.552$, $p < 0.001$) and with precipitation at the krummholz-site ($r = 0.350$; $p < 0.001$). At the timberline and treeline significant indirect relationships were also observed between daily radius change and air temperature and xylem sap flow. All variables showed lowest coefficients at the krummholz-site (Fig. 3).

Comparison between maximum stem radial increments recorded by dendrometers and cumulated daily radial increments calculated from differences between daily morning maximum values at study plots are depicted in Figure 4a. Calculated daily radial increment variations during the growing period 2007 were highly synchronous among timberline and treeline and reached lowest values at the krummholz-site (Fig. 4b). During the main growing period in 2007, i.e. May and June (cf. Fig. 7), daily radial increments calculated from dendrometer records at timberline and treeline were directly related to air temperature ($r = 0.465$ and 0.432, respectively; $p < 0.001$) and xylem sap flow ($r = 0.392$, $p < 0.01$ and $r = 0.352$, $p < 0.05$, respectively; Fig. 5). Additionally, significant indirect relationships were observed between daily radial increments at timberline and treeline and relative air humidity ($r = -0.260$ and -0.331, respectively; $p < 0.05$). No statistically significant relationships were found between daily radial increments and climate variables and xylem sap flow at the krummholz-site (Fig. 5).

The dormant cambium consisted of 7 to 8 cells, when there was no cambial activity from September 2006 through April 2007 (data not shown). In 2007 sampling started on 23 April
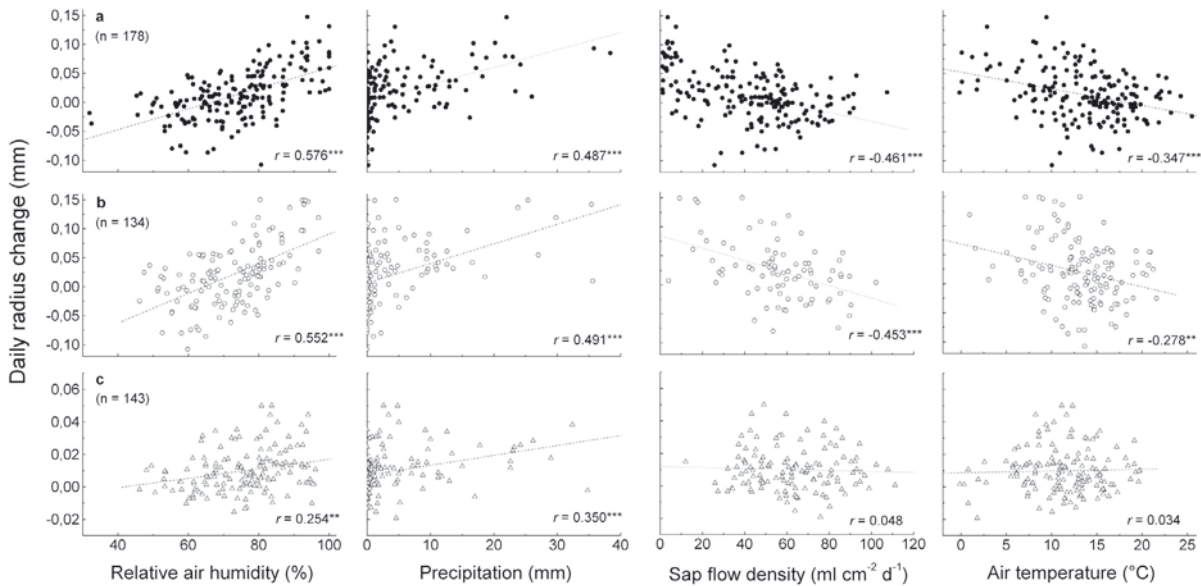
Figure 3. Pearson correlations between daily change in radial increment and climate parameters (relative air humidity, precipitation, mean air temperature) and xylem sap flow density at the timberline (a) (filled circles), treeline (b) (open circles) and krummholz-site (c) (open triangles). Due to incomplete sap flow records at the treeline and krummholz-site, number of samples used in calculations was 82 and 124, respectively. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

and in early May, the number of cells in the cambial zone rapidly increased to about 13 and then slowly decreased to reach initial values about mid July at timberline and treeline and mid August at the krummholz-site, respectively (Fig. 6).

The onset of radial enlargement in 2007 occurred simultaneously with budburst on 23 April at the timberline and treeline and on 2 May at the krummholz-site. In 2007 enlarging cells were detected throughout the treeline ecotone until end of August (243 d). Number of enlarging cells was significantly correlated with daily radial increments calculated on basis of modelled radial growth at all study plots (Fig. 6). As a base line for the comparison, dendrometer traces of individual trees were set to zero when first enlarging cells were detected. Correlation coefficients (r) were 0.973 ($p < 0.001$) and 0.972 ($p < 0.001$) at timberline and treeline and 0.698 ($p < 0.05$) at the krummholz-site, respectively. Because point dendrometers could not be installed at treeline and at the krummholz-site until mid April, spring re-hydration of stems was not recorded at these study plots. Maximum daily radial increment growth peaked before summer solstice throughout the treeline ecotone, although highest mean daily air temperatures were recorded in July (Tab. III). According to dendrometer measurements about one third of total annual increment in 2007 was already developed in May throughout the treeline ecotone (Fig. 7). During the warmest period in July only about 20% of total increment was developed.

4. DISCUSSION

In our study within the treeline ecotone we found a close relationship between daily variations in stem radius of *Pinus cembra* and climate variables, which reflects reversible
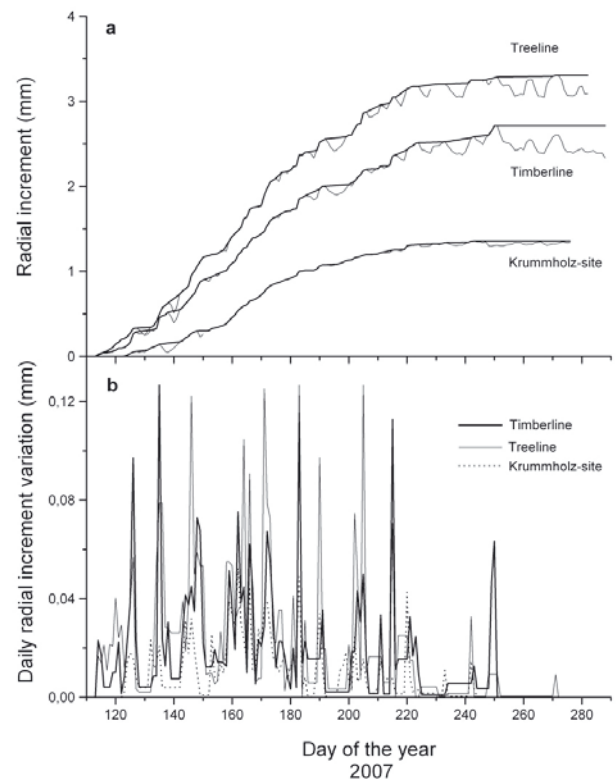

Figure 4. Time series of stem radial variations throughout the treeline ecotone from end of April to September 2007 ($n = 3$ trees/site). (a) Recorded daily stem radial variation (thin line) and extracted daily radial increment (thick line). (b) Daily radial increment variations (for details see Material and Methods).
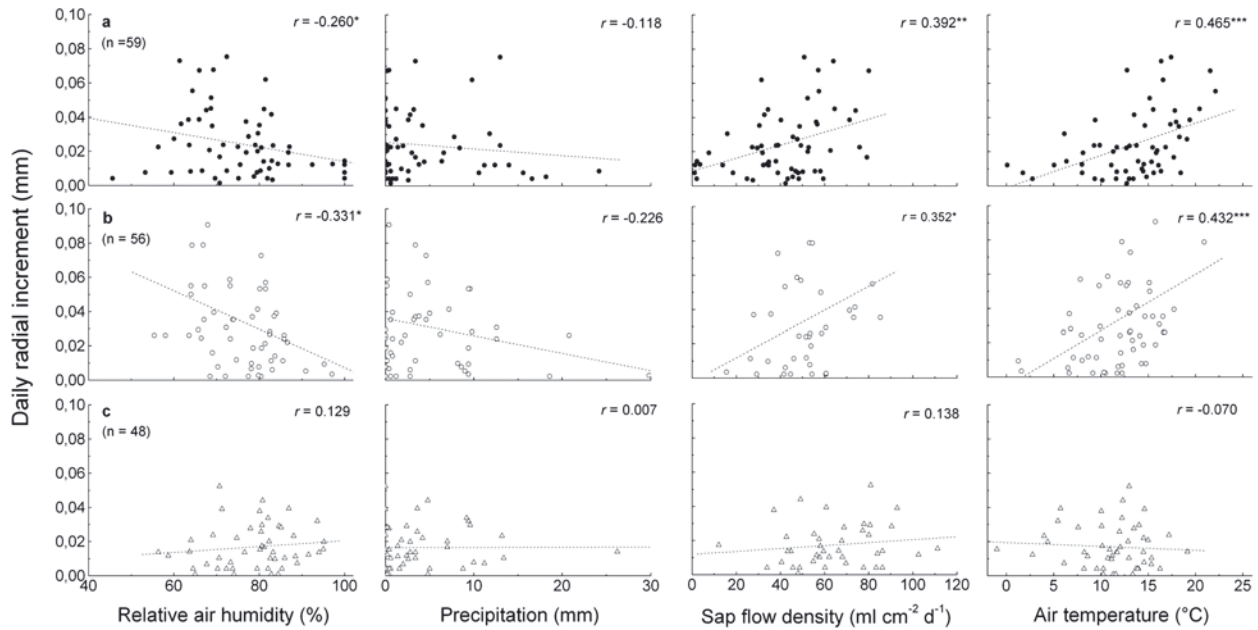
Figure 5. Pearson correlations between daily radial increment and climate parameters (relative air humidity, precipitation, mean air temperature) and xylem sap flow density during May and June at the timberline (a) (filled circles), treeline (b) (open circles) and krummholz-site (c) (open triangles). Due to incomplete sap flow records at the treeline and krummholz-site, number of samples used in calculations was 36 and 40, respectively. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.
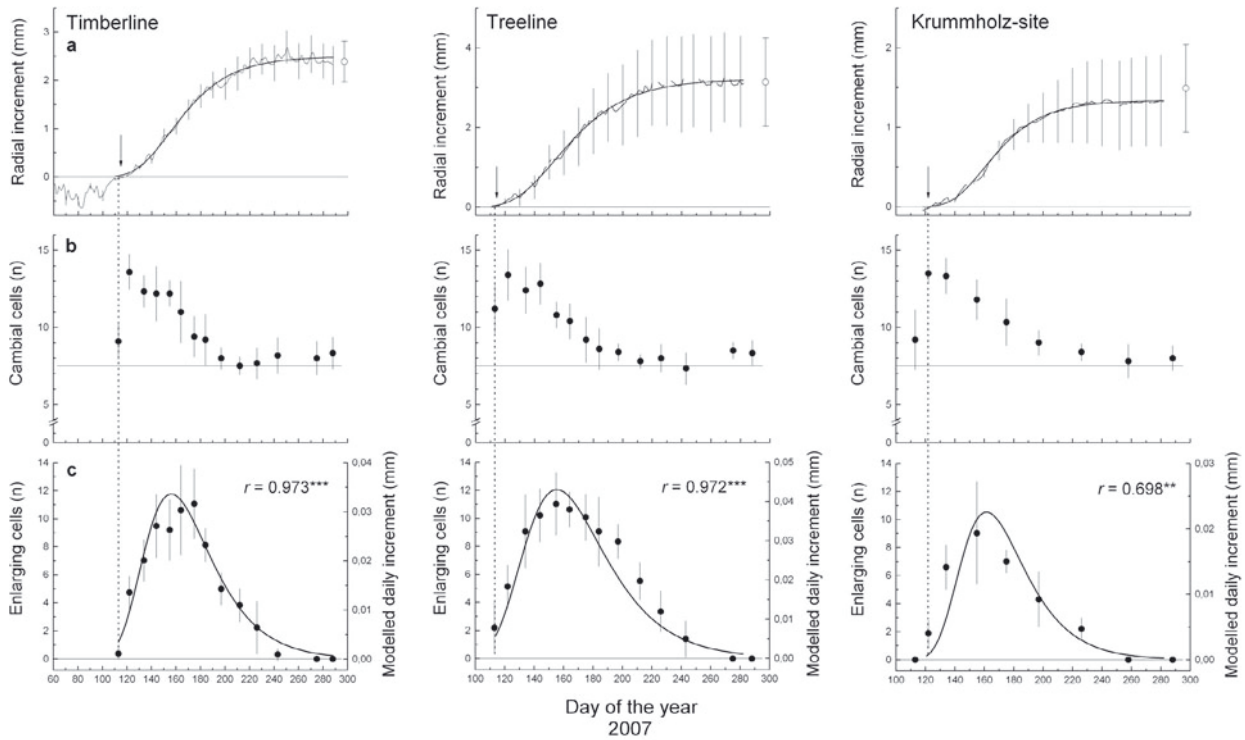

Figure 6. (a) Mean radial increment modelled by applying the Gompertz function ($n = 3$ trees/site). Dots indicate mean ring width determined end of October 2007 at position of point dendrometers. Bars represent standard deviations. Arrows indicate date of budburst. (b) Cambial cell dynamic in 2007 ($n = 5$ trees/site). (c) Number of enlarging cells determined during the growing season ($n = 5$ trees/site) and daily radial increment calculated on basis of modelled increment growth. Pearson correlation coefficients are indicated. *** $p < 0.001$; ** $p < 0.01$. Dotted vertical lines indicate first determination of enlarging cells in 2007. At that time, dendrometer records were set to zero.
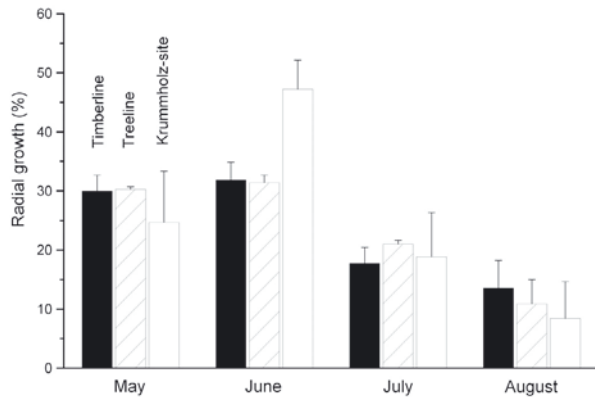
Figure 7. Mean monthly radial growth during the growing season 2007 based on dendrometer records. Bars indicate standard deviations.

Table III. Parameters of Gompertz functions for radial increment and R^2 of models (I_p = inflection point; κ = rate of change parameter; mean values ± standard deviation).

Site	I_p (day of the year)	κ	R^2
Timberline	155 ± 3.5	0.037 ± 0.005	0.991
Treeline	155 ± 1.5	0.037 ± 0.002	0.990
Krummholz	160 ± 7.2	0.051 ± 0.011	0.994

changes in stem hydration rather than changes in actual radial growth, as also observed in a study on seasonal changes in radial increment growth of *Picea abies* by Mäkinen et al. (2003). Furthermore, our results indicate that throughout the treeline ecotone stem re-hydration and radial cell enlargement occurred after stem radial minima were developed in the afternoon (6 p.m.), when water supply was adequate. Dünisch and Bauch (1994) also reported that radial cell enlargement of *Picea abies* seedlings takes place mostly during the night. Several authors reported that daily stem radius changes occur mainly in the elastic tissues outside the cambium (Dobbs and Scott, 1971; Molz and Klepper, 1973; Parlange et al., 1975; Zweifel et al., 2000), whereas only small diurnal fluctuations are assumed to be caused by swelling and shrinking of the hydroactive xylem (Irvine and Grace, 1997). Therefore, lower amplitude of diurnal stem fluctuations and lower correlation coefficients with environmental variables (precipitation, relative air humidity, temperature) found in krummholz trees can be explained by their thinner bark as compared to trees at timberline and treeline. The missing relationship between daily stem radius changes of krummholz trees and xylem sap flow might also be attributed to low transpiration rates of small trees at high altitude (Gruber et al., unpublished observations). That xylem sap flow is coupled with transpiration was reported by Steppe et al. (2006).

Calculated daily radial increments during the main growing period in 2007, i.e. May and June, have been found to be closely related to air temperature at the timberline and treeline. This is consistent with previous ecophysiological and dendroclimatological studies conducted within the treeline eco-

tone on Mt. Patscherkofel (Loris, 1981; Oberhuber, 2004). The missing influence of July temperature on radial growth in 2007 can be explained by early start of the growing season in late April due to occurrence of exceptionally mild temperatures in spring. Relative air humidity was inversely related to calculated daily radial increments at timberline and treeline, which indicates that extracted stem increments are to some extent masked by daily fluctuations in water status of trees. Hence, even stronger growth-temperature relationships can be expected at these sites. Significant direct relationships found between daily xylem sap flow and radial increment below the krummholz-belt are caused by highly significant correlation coefficients between sap flow and air temperature at timberline and treeline (r = 0.628 and 0.657, p < 0.001; respectively; data not shown; cf. Körner and Mayr, 1981; Tranquillini, 1979). Close correlations between these variables also indicate that drought stress during the growing period 2007 did not occur within the study area. Our results, however, are in contrast to findings by Deslauriers et al. (2007b), who reported no significant correlation between irreversible growth increment of *Pinus cembra* and sap flow.

On the other hand, daily radial increments of krummholz trees showed no relationship with air temperature, which is assumed to be caused by more extreme environmental conditions prevailing within the krummholz-belt (e.g., wind exposure) and/or by low tree height (≤ 1 m). Small stature trees are aerodynamically decoupled from atmospheric conditions, i.e. air temperature measured at 2 m height, but rather profit from more favourable microclimate conditions closer to the ground (cf. Grace et al., 2002; Körner, 2004; Smith et al., 2003). Non-significant correlation coefficients found at the tree-species limit might also indicate that daily stem radial increments of krummholz individuals are influenced by different microclimate conditions.

Mäkinen et al. (2008) suggested that water uptake (rehydration) in spring resulted in stem radius increase recorded by dendrometers, which is not related to the formation of new tracheids. Our study, however, revealed a close relationship between daily radial increment based on dendrometer records and number of enlarging cells in *Pinus cembra* throughout the growing season at all study plots. We therefore suggest that onset of radial growth can be distinguished from spring rehydration of internal tissues, which causes an abrupt increase in dendrometer traces, by taking micro-cores in weekly intervals before budburst and start of the growing season and detection of first enlarging tracheids. Additionally, in our study timing of maximum growth rates throughout the treeline ecotone peaked before summer solstice and not during the warmest period in July. Thus, the photoperiodic signal could regulate timing of maximum growth rate allowing tracheid differentiation to be completed before winter as already suggested by Rossi et al. (2006c). Premature cessation of cambial activity due to occurrence of drought stress in 2007 can be excluded, because soil water potential during a dry period in July was > −0.08 MPa (corresponds to a soil water content of 30% (vol.), Guggenberger 1980) even at the krummholz-site, where coarse textured shallow soils prevail (< 5 cm humus layer).

Furthermore, our results show that in 2007 mild spring temperatures and early snow melt allowed premature start of radial growth already in late April, whereas in the long-term, budburst of *Pinus cembra* within the study area occurs in mid May (Loris, 1981; Tranquillini, 1979). Deslauriers et al. (2007a) and Rossi et al. (2007) also reported that warmer spring temperatures induced an earlier resumption of xylem formation at the timberline. Several authors (Antonova and Stasova, 1993; 1997; Gričar et al., 2007; Kirdyanov et al., 2003) also found that primarily warm temperatures early in the growing season affect current cambial activity and tracheid production of conifers. The existence of thermal limits in xylogenesis of conifers, which corresponds with temperatures of 6–8 °C that are supposed to limit tree growth at high altitude (Grace et al., 1989; Körner, 1998; Körner and Paulsen, 2004), was reported by Rossi et al. (2007). Due to open canopy at the treeline and krummholz-site, root-zone temperatures in May exceeded those at timberline by 1.5 and 2.6 °C, respectively, and exceeded the lower threshold temperature reported for tissue growth at high altitudes. The observed lower soil temperatures at the timberline can mainly be attributed to the closed canopy conditions preventing soil heat flux and radiative warming of the rooting zone (Aulitzky, 1961; Körner and Paulsen, 2004) as compared to the open stands at the treeline and the krummholz-site. Because budburst, cambial activity and cell enlargement occurred quite similarly at the timberline and treeline and < 10 days later at the krummholz-site, we suggest that soil temperatures might play an important role for triggering onset of above ground stem growth within the treeline ecotone (see also Gruber et al., 2009). At north-facing slopes, however, delayed snow melt due to reduced insolation in spring could outweigh the advantage of open canopy within the treeline ecotone and cause retarded onset of stem growth at higher altitude. Direct influences of soil temperature on aboveground metabolism were reported by e.g., Day et al. (1989), DeLucia (1986), Havranek (1972), Hellmers et al. (1970), and Scott et al. (1987). Delayed bud opening and wood formation within the krummholz-belt might be explained by increased environmental constraints at higher elevations (e.g., wind exposure, frost desiccation) and can be regarded as an adaptation to prevent late frost injuries to current-year needles and to the developing xylem (cf. Cannell and Smith, 1986).

According to dendrometer measurements about two-thirds of total annual increment in 2007 was already developed end of June throughout the treeline ecotone. Hence, low correlation coefficients found between ring width series of *Pinus cembra* and July temperature (Carrer et al., 2007; Frenzel and Maisch, 1981; Oberhuber 2004) can partly be explained by early start of radial growth in years, when mild conditions prevail in spring. Additionally, several authors (e.g., Carrer et al., 2007; Oberhuber, 2004; Oberhuber et al., 2008; Pfeifer et al., 2005) found that besides July temperature other climate variables, like temperature in previous fall and during early spring, winter precipitation and climate extremes (e.g., late frost) influence radial growth of *Pinus cembra*. Therefore, complex climate-growth relationships might explain findings of this study that annual increments throughout the treeline ecotone were not significantly higher compared to previous years, although warm conditions in spring 2007 lengthened the growing season by about three weeks.

We conclude that throughout the treeline ecotone diurnal fluctuations in stem radius of *Pinus cembra* recorded by dendrometers are primarily coupled to the bark water content, rather than changes in xylem growth. On the other hand, we found a close relationship between extracted daily radial increment and air temperature below the krummholz-belt, which underscores the influence of tree stature, i.e., small krummholz-individuals are decoupled from atmospheric conditions (Grace, 1989; Körner, 1998), on sensitivity of tree growth to climate. Furthermore, our results suggest that in dendrometer traces of slow-growing subalpine *Pinus cembra*, growth-induced radial expansion of the stem can be distinguished from spring re-hydration of the bark by histological analysis of wood formation prior to budburst. By this way, a proper timing of phenological events in intra-annual stem radius increment, such as cambial growth onset and ending, can be accomplished.

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