

Hydraulic constraints limit height growth in trees at high altitude

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Summary

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Received: 27 July 2010

Accepted: 2 August 2010

New Phytologist (2011) **189**: 241–252

doi: 10.1111/j.1469-8137.2010.03455.x

Key words: conduit tapering, hydraulic limitations, *Picea abies* (Norway spruce), sink limitations, treeline, xylem anatomy.

- Low temperatures limit the fixation of photosynthates and xylogenesis. Here, we hypothesized that reduced longitudinal growth in trees at high altitude is related to the lower hydraulic efficiency of the transport system.
- Apical buds of Norway spruce (*Picea abies*) trees at high and low elevation were heated during 2006 and 2007. At the end of the experiment, trees were felled. Longitudinal increments and tracheid lumen areas were measured along the stem. Apical hydraulic conductivity (k) was estimated from anatomical data.
- Before heating, high-altitude trees showed fewer ($P = 0.002$) and smaller ($P = 0.008$) apical conduits, and therefore reduced k ($P = 0.016$) and stem elongation ($P < 0.0001$), in comparison with trees at low elevation. After 2 yr of heating, k increased at both high ($P = 0.014$) and low ($P = 0.047$) elevation. Only high-altitude trees showed increased stem elongation, which reached the same magnitude as that of controls at low elevation ($P = 0.735$). Heating around the apical shoots did not appear to induce significant changes in conduit dimension along the rest of the stem.
- The total number and size of xylem elements at the stem apex are strongly constrained by low temperatures. Trees at high altitude are therefore prevented from building up an efficient transport system, and their reduced longitudinal growth reflects strong hydraulic limitations.

Introduction

Cold regions are characterized by a striking vegetation gradient between the limit of the closed forest and the uppermost patches of upright trees (i.e. the treeline) (Paulsen *et al.*, 2000; Körner & Paulsen, 2004). At a global scale, trees in cold regions present reduced tree ring width and growth rates (e.g. Körner, 2003a; Gamache & Payette, 2004), and therefore the mean tree height decreases with increasing altitude/latitude (Hoch & Körner, 2005; Miyajima & Takahashi, 2007).

Above the treeline, in the alpine belt, the 'tree' life-form (i.e. a plant taller than 3 m) appears not to be well adapted to cope with the severe climatic conditions: this means that critical constraints at the treeline probably do not occur in the first ontogenetic stages (e.g. seedlings), but later when trees are 'taller' (Hoch & Körner, 2005), and tree species have evolved morphological and functional modifications to resist environmental constraints. Treetops are particularly exposed to limiting environmental factors in all

seasons. During the summer, apical shoots, above the protected boundary layer of the dense crown below, come into direct contact with the much colder free atmosphere: tissues are therefore subject to temperature-mediated growth inhibition (Körner, 1998). In the winter, the crown protrudes from the deep snow and so is vulnerable to ice blasting, wind desiccation (Baig & Tranquillini, 1980; Smith *et al.*, 2003) and frost drought (Mayr *et al.*, 2006, 2007). Moreover, the hydraulic efficiency of the whole xylem transport system (i.e. the amount of water flow per unit of pressure difference between atmosphere and soil) is seriously constrained. Cold soils probably hamper the development of an efficient root system (Körner & Hoch, 2006; Alvarez-Uria & Körner, 2007). In addition, the frequent freeze–thaw cycles impose anatomical modifications to prevent the formation and spread of xylem embolisms (Mayr *et al.*, 2003) with potential sacrifices for hydraulic efficiency by virtue of the trade-off mechanism of safety vs efficiency (Pittermann & Sperry, 2003; Pittermann *et al.*, 2006).

Worldwide, both altitudinal and latitudinal treelines have a shorter vegetative season, with an average daily temperature of 6–8°C commonly considered to be a thermal boundary layer below which metabolic activities are inhibited (Vapaavuori *et al.*, 1992; Domisch *et al.*, 2002; Alvarez-Uria & Körner, 2007; Rossi *et al.*, 2007; Shi *et al.*, 2008). Vertical growth, spatial dynamics and altitudinal shifts of treeline ecotones can occur naturally in response to improved microsite environmental conditions when isolated patches of trees become denser (Smith *et al.*, 2009). Nevertheless, during recent decades, an altitudinal and latitudinal increase in treeline position has been observed on a global scale (Körner, 1998; Gamache & Payette, 2004; Harsch *et al.*, 2009). This has renewed interest in these sensitive environments within the context of possible effects of climate change. Primarily, the increase in CO₂ concentration, nitrogen availability and higher temperatures have been hypothesized and studied as potential causes of latitudinal and altitudinal treeline advance.

Experimental exposure of trees to increased CO₂ concentrations at the treeline has yielded ambiguous results, with a fertilization effect seen in some species, but not in others, and generally showing a progressive saturation in the photosynthetic response to the CO₂-enriched atmosphere (Hättenschwiler *et al.*, 2002; Körner, 2003a,b; Shi *et al.*, 2008). However, increased growth rate after exposure to higher concentrations of CO₂ does not directly imply a limitation in assimilation with photosynthesis (Millard *et al.*, 2007; Sala & Hoch, 2009). In fact, the atmospheric CO₂ concentration directly affects the chemical composition of cell walls, and therefore the extensibility of the developing organs (Gardner *et al.*, 1995).

Recent experiments on nitrogen availability at Himalayan treelines have revealed that nitrogen probably does not limit the growth of trees (Li *et al.*, 2008). On the contrary, warmer temperatures are likely to stimulate longitudinal growth rates in cold environments, thus leading to progressive morphological changes from shrubby to upright trees (Devi *et al.*, 2008). Moreover, the treeline advance appears to be strongly related to warmer temperatures (Harsch *et al.*, 2009). However, it is still not entirely clear what kind of structural and functional modifications are forced upon trees under the negative effect of low temperatures on plant metabolism.

The most robust hypothesis of the temperature-mediated process at the treeline is related to the effects of low temperatures on the formation of new tissues (Körner, 1998), thus suggesting that cambial activity (xylogenesis) might be the key process in determining growth inhibition. The 'sink limitation hypothesis' (Körner, 1998) appears to be consistent with the sharp thermal thresholds controlling cambial activity in high-altitude trees (Rossi *et al.*, 2007) and with the general increase in nonstructural carbohydrates (NSCs) with altitude (Körner, 1998; Hoch *et al.*, 2002; Hoch & Körner,

2005), even if the NSC trends seem to be less clear than previously thought (Li *et al.*, 2008; Bansal & Germino, 2009).

Among the morpho-anatomical modifications often observed in treeline trees, the reduction in conduit number and size (Körner, 1998; Gorsuch & Oberbauer, 2002; Körner, 2003a,b; Gričar *et al.*, 2006; Rossi *et al.*, 2008) causes an overall increase in sapwood density relative to that of low-elevation trees (Hoch & Körner, 2005). Consequently, this reduced xylogenesis induces a reduction in sapwood permeability (Gorsuch & Oberbauer, 2002), and therefore in the efficiency of water supply to the leaves. Moreover, the xylem anatomy of trees in cold environments ought to have evolved in order to prevent the spread of cavitation after freeze–thaw events (Davis *et al.*, 1999; Pittermann & Sperry, 2003), therefore potentially sacrificing the hydraulic efficiency of the whole system in favor of the trade-off mechanism of efficiency vs safety (Hacke & Sperry, 2001; Pittermann *et al.*, 2006). This reduced hydraulic efficiency of the xylem transport system of trees in cold environments is often coupled with a greater sensitivity to alterations in the water cycle throughout the year (especially at the time of snowmelt and summer precipitations) (Peterson & Peterson, 1994; Vaganov *et al.*, 1999) or (wind-related) wounds on leaf cuticles that expose trees to water losses by cuticular transpiration and to increased episodes of xylem failure by cavitation in winter when the soil is frozen (Wardle, 1971; Tranquillini, 1979; Baig & Tranquillini, 1980; Mayr *et al.*, 2006).

Indeed, phenomena potentially related to hydraulic limitations are often found in trees at the treeline. For instance, unless the vapor pressure deficit (VPD) is lower at high elevations, the Rubisco discrimination for ¹³CO₂ decreases, and thus δ¹³C increases, with altitude (Körner *et al.*, 1988, 1991; Hoch & Körner, 2005). Seemingly, δ¹³C increases with tree height because of the higher height-related hydraulic constraints (Koch *et al.*, 2004).

The amount of water passing through a surface unit of sapwood in a unit of time under a given pressure (hydraulic conductance, *K*) is strongly affected by the number and diameter of xylem conduits, as stated by the Hagen–Poiseuille formula (Tyree & Ewers, 1991):

$$K = \frac{\pi \times \rho}{128 \times \eta \times l} \times \sum_{i=1}^n d_i^4, \quad \text{Eqn 1}$$

(ρ , density; η , dynamic viscosity of water; l , length of the xylem conduits; d_i , diameter of the xylem conduits). As water flow is given by:

$$F = K \times \Delta P, \quad \text{Eqn 2}$$

(ΔP , pressure difference), it follows that a cylindrical pipe conducts the same amount of water as the other 256 pipes of the same length and four times smaller diameter, under the same ΔP ($F_{d=1} = 256 \times F_{d=1/4}$). For an increased pipe

length, as with the increase in root-to-leaf distance in real trees during ontogenesis, Eqn 1 states that K decreases accordingly, unless the conduit width varies longitudinally (see 'hydraulic limitation hypothesis': Ryan & Yoder, 1997): for high rates of conduit enlargement towards the base (conduit tapering), the negative effect of path length on K is markedly reduced (Becker *et al.*, 2000; Petit & Anfodillo, 2009), because K per unit length (hydraulic conductivity, k) increases from the stem apex downwards (Yang & Tyree, 1993; Petit *et al.*, 2008).

We tested the hypothesis that trees at the treeline undergo hydraulic limitations to height growth and that, specifically, cold temperatures, by direct hampering of the formation of many and large cells, lead to a reduced hydraulic efficiency of the whole transport system, which cannot sustain high rates of longitudinal growth.

We performed a 2-yr temperature manipulation experiment in different sites (treeline and low-altitude forest), altering the growth conditions of small *Picea abies* trees, and analyzed the effects on shoot elongation and anatomical features of the xylem transport system.

Materials and Methods

Site and plant material

The study area was located in the Dolomites (eastern Alps, Italy). One site (hereafter HA: high altitude) was set up at

Monte Rite (2100 m asl), inside a treeline formation composed primarily of *Picea abies* Karst. plus a few individuals of *Larix decidua* Miller. The other, a control site at low altitude (hereafter, LA), was delimited near the Research Center for Alpine Ecology of the University of Padova (1100 m asl), where individuals of *P. abies*, *L. decidua* and other secondary species formed a typical low-altitude mountain forest. The species selected for this study was *Picea abies* Karst. In each site, five specimens were selected for the application of a heating system to the stem's apical developing bud. Another five trees with growing features apparently similar to the others were selected as controls (Table 1). The apical developing buds were placed inside a transparent polycarbonate cylinder (length, 250 mm; diameter, 50 mm; thickness, 3 mm) with 20 spires of fine resistant wire coiled tightly around its inner side. Two sensors of fine wires of copper and constantane (type T) were connected to a Campbell CR10X data-logger (Campbell Scientific Inc., Logan, UT, USA) to switch on/off the low-voltage power supply (0–30 V, 0–10 A, with 35 W for each heated tube) to maintain the offset of temperature inside the cylinder at +5 (± 1)°C at HA and, to take into account the effect of elevation on the air temperature, at +10 (± 1)°C at LA. In addition, cylinders without resistance were applied to control trees to evaluate their possible glass-house effects.

Selected trees were provided with heated cylinders across the growing season of two successive years (2006 and 2007)

Table 1 Dendrometric and functional–anatomical features of sampled Norway spruce (*Picea abies*) trees: basal diameter post-treatment (D_{2007}); height pre- and post-treatment (H_{2005} and H_{2007}); longitudinal increment pre-treatment (ΔL_{2005}); mean diameter (d_{2005}) and number (NC_{2005}) of apical conduits pre-treatment; calculated apical hydraulic conductivity pre-treatment (k_{2005})

ID	Site	Treatment	D_{2007} (mm)	H_{2005} (cm)	H_{2007} (cm)	ΔL_{2005} (cm)	d_{2005} (μ m)	NC_{2005}	k_{2005} (cm ³ MPa ⁻¹ s ⁻¹)
1C	HA	Control	35.6	119.6	144.3	5.5	7.88	5356	9.28×10^{-12}
2C	HA	Control	25.4	65.7	77.4	5.3	6.24	2588	1.80×10^{-12}
3C	HA	Control	41.6	101	117.8	7.8	7.69	3693	5.86×10^{-12}
4C	HA	Control	52.0	82	95.8	4	8.48	4789	1.19×10^{-11}
5C	HA	Control	26.4	51.6	60.5	3.8	6.26	2150	1.34×10^{-12}
1H	HA	Heated	36.5	102.5	149.5	7.2	7.33	4335	5.38×10^{-12}
2H	HA	Heated	21.7	57.2	69.7	3.3	6	2429	1.39×10^{-12}
3H	HA	Heated	42.1	124.4	155.0	5.7	8.98	5429	1.57×10^{-11}
4H	HA	Heated	60.0	146.2	170.0	3.5	7.69	4601	6.40×10^{-12}
5H	HA	Heated	41.0	75.4	87.3	4.6	7.22	3714	5.07×10^{-12}
6H	HA	Heated	17	65.8	76.0	1	6.63	1689	1.41×10^{-12}
1C	LA	Control	32.0	147.5	161.7	15.8	8.94	8425	2.47×10^{-11}
2C	LA	Control	16.5	68.9	93.2	23.5	9.9	5848	2.50×10^{-11}
3C	LA	Control	38.3	176.1	213.3	30	7.65	5805	1.06×10^{-11}
4C	LA	Control	34.2	132.4	170.4	22.6	9.08	6206	2.08×10^{-11}
5C	LA	Control	50.8	221	284.3	30.3	11.52	14 703	1.03×10^{-10}
1H	LA	Heated	21.6	102.5	156.7	27.1	7.25	4712	5.99×10^{-12}
2H	LA	Heated	21.0	62.4	78.3	17.6	7.11	3721	4.62×10^{-12}
3H	LA	Heated	42.1	173	191.2	29.8	7.16	9348	3.25×10^{-11}
4H	LA	Heated	22.7	83.6	114.6	29.8	8.5	4461	1.00×10^{-11}
5H	LA	Heated	42.5	181.9	222.7	20.3	9.6	7952	3.07×10^{-11}
6H	LA	Heated	38.1	175.1	214.5	24.1	9.24	5129	1.61×10^{-11}

HA, high altitude; LA, low altitude.

to better evaluate the effect on tree growth, which is known to be very sensitive to the environmental conditions of the previous year, especially in coniferous species (e.g. Jalkanen & Tuovinen, 2001). At HA, heating was supplied from 19th May to 12th July in 2006 and from 10th May to 16th August in 2007, and, at LA, from 28th April to 13th September in 2006 and from 19th April to 21st August in 2007. Tree 2H at HA died at the end of the summer of 2006, and was replaced by tree 6H in 2007. Two other trees underwent accidental damage: tree 3H at LA (replaced with 6H at LA in 2007) and 1H at HA in 2007. All trees were felled at the beginning of September 2007 in order to analyse the structure of the xylem conduits.

Analyses

For each tree, the total height, basal diameter and stem annual longitudinal increments were measured. During the 2006 and 2007 growing seasons, the length of the developing apical shoot of each tree was measured once or twice per week.

Along the stem, wooden disks were carefully extracted at 2 cm from the distal node of the last three internodes. A total of 6–15 disks was extracted at different distances along the stem and their distance from the apex (L) was measured.

Wooden disks (or at least two or three opposite portions comprising zones with no reaction wood, macroscopic injuries or scars) were embedded in paraffin (Anderson & Bancroft, 2002); transverse sections of 10–12 μm were then cut with a rotatory microtome, stained with safranin (1% in water) and fixed permanently on microscope slides with Eukitt (BiOptica, Milan, Italy). Slides were observed at 100 \times magnification under a light microscope (Nikon Eclipse80i; Nikon, Tokyo, Japan) equipped with a digital photo camera. Digital images were carefully taken in order to analyze:

- the whole apical section (i.e. all the conduits) relative to the years 2005, 2006 and 2007;
- four to six radial strips (of *c.* 1.2 mm in width) of the 2005 and 2007 annual rings (from early- to latewood) for the remaining sections along the stem.

Lumen areas were measured by WINCELL software (Régent Instruments Inc., Sainte-Foy, QC, Canada). Although conduits were of different shapes (mostly rectangular), they have been considered to be circular. This helpful simplification should not strongly affect the estimation of the hydraulic properties of the system (Sperry *et al.*, 2005).

For each tree, we measured all conduits of the apical sections of 2005, 2006 and 2007 and estimated the total hydraulic conductivity (k , i.e. conductance per unit length, 1 cm) with the Hagen–Poiseuille formula (see Eqn 1), considering the conductance of each conduit connected in parallel.

In order to evaluate the effect of heating on k , we calculated the following ratios: $k_{2006} : k_{2005}$; $k_{2007} : k_{2005}$; $k_{2007} : k_{2006}$.

For the analysis of the vertical profile of the average conduit dimension (i.e. conduit tapering), for each section along the stem and for each annual ring (2005 and 2007) we estimated the hydraulically weighted diameter Dh (Kolb & Sperry, 1999):

$$Dh = \frac{\sum d_n^5}{\sum d_n^4}, \quad \text{Eqn 3}$$

(d_n , n th conduit diameter).

Statistical analyses

The software SAS 8.02 (SAS Institute, Cary, NC, USA) was used to analyze shoot development across the growing season and for the parametric and nonparametric analyses of variance.

The scaling parameters of the allometric (i.e. power) equations were determined from pairwise comparisons of \log_{10} -transformed data. Using reduced major axis (RMA) analysis, the scaling exponents and allometric constants were identified as the regression slopes (b -RMA) and y intercepts (a -RMA), respectively. Regression coefficients and their 95% confidence intervals were computed by standard methods (Sokal & Rohlf, 1981) using a bootstrap procedure with 100 000 replications (Davison & Hinkley, 1997).

Results

High altitude vs low altitude

Preliminary analyses on the morphology and anatomy of the stem apices revealed substantial differences between trees at the treeline and at low elevation (Table 1). Trees growing at HA showed a shorter longitudinal increment (ΔL_{2005} : $\chi^2 = 15.79$; $P < 0.0001$), smaller number (NC₂₀₀₅: $\chi^2 = 9.32$; $P = 0.002$) and mean diameter (d_{2005} : $\chi^2 = 7.08$; $P = 0.008$) of apical conduits, which therefore resulted in a lower hydraulic conductivity at the stem apex (k_{2005} : $\chi^2 = 5.77$; $P = 0.016$).

Phenology

The periodic measurements of the length of the apical shoot revealed that, at HA, the heating did not result in an early shift in bud burst, which was substantially simultaneous among trees, and stem elongation lasted for 48 d in both 2006 (from 31st May to 18th July) and 2007 (from 7th June to 25th July). At LA, bud burst was simultaneous among treatments in 2006, but the stem elongation of

heated trees was faster than that of controls. The time range for stem elongation varied from 29 to 50 d (from 11th May to 30th June) in heated trees and from 43 to 64 d (from 11th May to 14th July) in controls. However, bud burst in 2007 was anticipated by *c.* 2 wk in heated trees. The stem elongated for 28–61 d (from 17th May to 17th July) in plants provided with heating cylinders, and for 41–56 d (from 4th May to 29th June) in controls.

The development of the current apical shoot was well described by a logistic function (Fig. 1; Table 2):

$$L = x + \frac{m}{1 + e^{n-r(DY-x)}}, \quad \text{Eqn 4}$$

[x , day of the first measurement (unclosed buds); DY , Day of Year (i.e. the Julian day); m , upper asymptote (i.e. apical shoot length); n , translation coefficient along the x axis; r , shape coefficient describing the steepness of the curve between the two inflection points]. The parameter r was considered to be an index of the developmental speed of the terminal shoot. This was significantly higher in heated trees

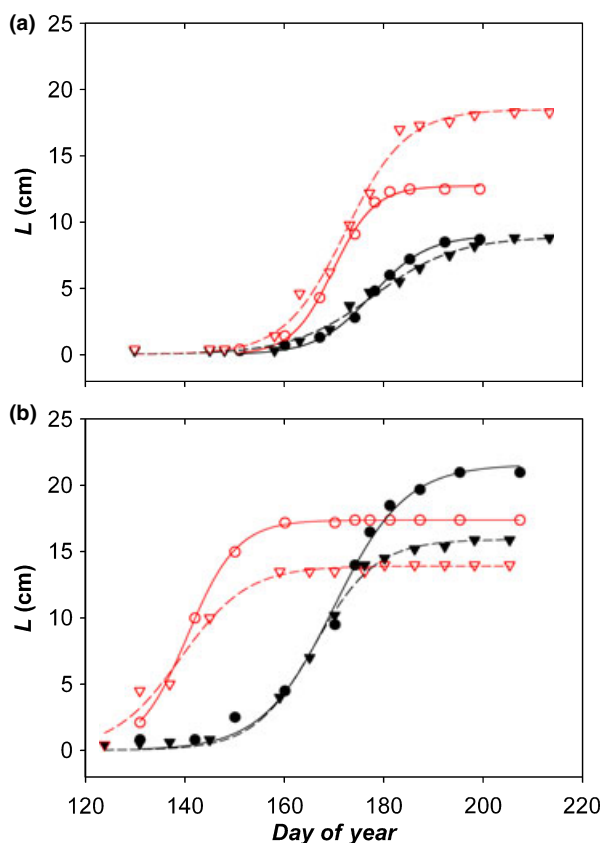


Fig. 1 Development of current apical shoot during the growing season in control (closed symbols) and heated (open symbols) Norway spruce (*Picea abies*) trees during 2006 (circles) and 2007 (triangles). Examples are given of the stem elongation of trees 3C and 3H at HA (high altitude; a) and of 4C and 4H at LA (low altitude; b). Details of fitting curves are given in Table 2.

at LA ($t = 3.58$, $P = 0.007$ in 2006; $t = 2.82$, $P = 0.042$ in 2007), whereas statistics did not reveal any difference between treatments at HA ($t = 1.86$, $P = 0.099$ in 2006; $t = 2.32$, $P = 0.075$ in 2007).

The current longitudinal increment (i.e. the length of the apical shoot after the second year of heat treatment, 2007), compared with the averaged increment of the 5 yr before the treatment (2001–2005), was significantly higher in heated trees at HA ($t = 3.56$, $P = 0.024$), whereas control trees did not present any significant changes in the current shoot length ($t = 1.80$, $P = 0.146$), nor did trees at LA, either heated trees ($t = 1.38$, $P = 0.239$) or those provided with unheated cylinders ($t = 1.07$, $P = 0.346$) (Fig. 2).

After the 2 yr of heating, stem elongation in heated trees at HA was not significantly different from that of control trees at LA ($t = 0.35$, $P = 0.735$).

Anatomy

At HA, trees provided with heated cylinders underwent some anatomical modifications. At the stem apex, either the conduit number or size, or both, changed after exposure to increased temperature (e.g. Fig. 3). Given the importance of both the dimension and number of conduits on hydraulics (Eqn 1), we evaluated the hydraulic response (i.e. the hydraulic conductivity, k) to anatomical modification of the xylem at the stem apex (Fig. 4). The assessment of k made it possible to incorporate the effect of both anatomical modifications (i.e. number and size of conduits) on the efficiency of the hydraulic system, and therefore on physiology. Table 3 summarizes the Kruskal–Wallis tests for the non-parametric analysis of variance among treatments (heated and controls) for the three ratios $k_{2006} : k_{2005}$, $k_{2007} : k_{2005}$ and $k_{2007} : k_{2006}$. The results revealed that the supplied heating stimulated a significant increase in k only after the second year at both HA and LA (Fig. 4).

In addition, we combined the conductivities of the last two terminal apices (i.e. of the current and previous year). Considering the two conductivities in series, the equivalent conductivity (k_{eq}) was calculated as:

$$k_{\text{eq}} = \frac{k_n \times k_{n-1}}{k_n + k_{n-1}}, \quad \text{Eqn 5}$$

(n , year of development of the apical shoot). We analyzed the stem longitudinal increment (ΔL) vs k_{eq} of the current and previous year. A positive correlation ($F = 34.544$; $R^2 = 0.657$; $P < 0.0001$) was found at HA, whereas, at LA, k_{eq} appeared not to affect ΔL ($P = 0.147$) (Fig. 5).

The results of the analyses on the vertical profiles of the hydraulically weighted conduit diameter (Dh) (i.e. conduit tapering) revealed that Dh increased monotonically from the stem apex to the base. The magnitude of variation of Dh with distance from the apex (L) was substantially similar

Table 2 Variation in current apical shoot length (L) of Norway spruce (*Picea abies*) trees with time (days from the first measurement) in 2006 and 2007 at both sites, Monte Rite and San Vito

ID	Site	Treatment	Year	N	m	n	r	R^2	F	P
1C	HA	Control	2006	9	14.99	4.51	0.17	0.99	1231	< 0.0001
2C	HA	Control	2006	9	6.58	4.01	0.15	0.97	236.61	< 0.0001
3C	HA	Control	2006	9	9.01	4.97	0.18	1.00	1610.54	< 0.0001
4C	HA	Control	2006	9	7.86	4.76	0.20	0.99	624.12	< 0.0001
5C	HA	Control	2006	9	4.91	4.66	0.18	0.97	278.78	< 0.0001
1C	HA	Control	2007	14	11.07	5.02	0.12	0.98	472.13	< 0.0001
3C	HA	Control	2007	14	5.78	6.17	0.13	0.99	885.00	< 0.0001
3C	HA	Control	2007	14	8.88	5.96	0.12	0.99	1209.69	< 0.0001
4C	HA	Control	2007	14	7.17	4.61	0.10	0.98	596.19	< 0.0001
5C	HA	Control	2007	14	4.47	5.95	0.13	0.99	1642.72	< 0.0001
1H	HA	Heated	2006	9	16.10	4.48	0.19	0.99	733.82	< 0.0001
2H	HA	Heated	2006	9	10.63	5.35	0.24	0.99	679.88	< 0.0001
3H	HA	Heated	2006	9	12.74	4.74	0.24	0.99	3232.54	< 0.0001
4H	HA	Heated	2006	9	5.61	4.34	0.20	0.97	368.69	< 0.0001
5H	HA	Heated	2006	9	4.24	2.60	0.17	0.98	609.65	< 0.0001
1H	HA	Heated	2007	14	24.89	7.10	0.17	0.99	1657.94	< 0.0001
3H	HA	Heated	2007	14	18.49	7.10	0.16	0.99	1925.04	< 0.0001
4H	HA	Heated	2007	14	17.27	5.05	0.12	0.99	1903.55	< 0.0001
5H	HA	Heated	2007	14	8.41	7.80	0.26	1.00	7557.23	< 0.0001
6H	HA	Heated	2007	14	6.90	5.65	0.17	1.00	11 132.10	< 0.0001
1C	LA	Control	2006	11	22.99	2.44	0.11	0.99	1892.48	< 0.0001
2C	LA	Control	2006	11	14.01	3.46	0.12	0.98	524.42	< 0.0001
3C	LA	Control	2006	11	27.30	3.30	0.11	0.99	1157.12	< 0.0001
4C	LA	Control	2006	11	21.59	5.57	0.14	0.99	1089.12	< 0.0001
5C	LA	Control	2006	11	40.14	4.53	0.12	1.00	4449.44	< 0.0001
1C	LA	Control	2007	13	6.18	4.28	0.15	0.99	1273.74	< 0.0001
2C	LA	Control	2007	13	11.32	3.38	0.14	0.99	1224.36	< 0.0001
3C	LA	Control	2007	13	10.73	5.14	0.12	0.99	1836.24	< 0.0001
4C	LA	Control	2007	13	15.91	7.06	0.16	1.00	3638.10	< 0.0001
5C	LA	Control	2007	13	24.11	5.79	0.14	0.99	2205.23	< 0.0001
1H	LA	Heated	2006	11	27.06	2.42	0.26	1.00	55 446.60	< 0.0001
2H	LA	Heated	2006	11	13.01	1.79	0.36	1.00	1 285 681.00	< 0.0001
3H	LA	Heated	2006	11	18.85	3.05	0.27	1.00	24 716.80	< 0.0001
4H	LA	Heated	2006	11	17.39	2.15	0.20	1.00	97 978.10	< 0.0001
5H	LA	Heated	2006	11	20.20	2.48	0.15	1.00	5414.52	< 0.0001
1H	LA	Heated	2007	13	26.96	3.28	0.26	1.00	29 424.80	< 0.0001
2H	LA	Heated	2007	13	3.51	3.47	0.17	0.99	5867.47	< 0.0001
4H	LA	Heated	2007	13	13.91	2.42	0.15	0.99	1679.90	< 0.0001
5H	LA	Heated	2007	13	20.36	5.87	0.30	1.00	9834.14	< 0.0001
6H	LA	Heated	2007	13	12.77	4.39	0.26	1.00	24 984.20	< 0.0001

Estimates for the parameters of the fitting logistic equation: m , n and r (see Eqn 4).
HA, high altitude; LA, low altitude.

among trees and well described by a power function (Fig. 6). Single-tree analyses of conduit tapering are summarized in Table 4. Power fitting explained > 85% of the total variance in 29 of 36 cases. However, the remaining seven trees showed a more complex pattern of conduit tapering, presenting wide areas of microscopic xylem damage, most probably caused by spring frosts, resulting in slides that were very hard to read and unreliable to measure.

Overall, the degree of conduit tapering (i.e. the scaling exponent b) was significantly lower ($\chi^2 = 11.11$; $P = 0.001$) at LA (mean $b = 0.13$; $s = 0.02$) than at HA (mean $b = 0.17$; $s = 0.03$). For both sites, all data fitting of each tree (Fig. 6), although used only for a graphical representation of

potential variability in the Dh profile, suggested that conduit tapering was slightly higher at HA than at LA (Table 4).

Discussion

Our experiment was designed to modify the thermal conditions of the most apical shoots, leaving all other parts unchanged. This has enabled us to test the effect of localized heating on longitudinal growth, and the importance of thermal environment in determining tree growth at high altitude.

The results appeared to be consistent with the hypothesis that the hydraulic efficiency of the xylem architecture

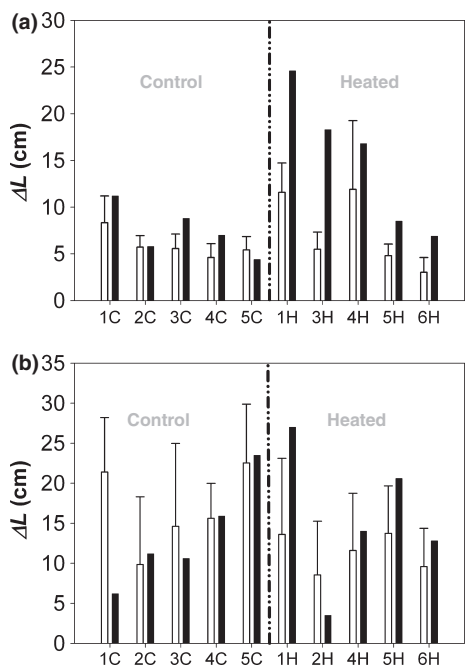


Fig. 2 Mean longitudinal annual increment (ΔL) over the period 2001–2005 (open columns; error bars are the standard deviations) and the annual increment in 2007 (closed columns) of Norway spruce (*Picea abies*) trees (excluding those accidentally damaged in 2006) at HA (high altitude; a) and LA (low altitude; b).

represents a relevant limiting factor for height growth at high altitude.

Overall, our observations confirmed that xylogenesis in trees living in cold environments is significantly stimulated by warmer temperatures (Gorsuch & Oberbauer, 2002; Danby & Hik, 2007). The increase in the number and size of conduits enhanced the xylem conductivity (i.e. conductance per unit length: see Eqn 1). A consequence of the observed tapered profile of conduit dimension along the stem is that the narrower conduits of the apical shoot represent a constraining bottleneck for the whole tree conductance (Becker *et al.*, 2000; Petit *et al.*, 2008). Artificial heating therefore substantially promoted the widening of this bottleneck by increasing both the number and size of apical conduits. This triggers a greater hydraulic efficiency of the whole transport system and higher rates of longitudinal growth.

At HA, heated trees grew significantly taller than controls, whereas those at LA showed no significant differences in the newly produced apical shoot length between the two treatments. This is consistent with the observation that the annual increment of leading shoots is particularly affected by low temperatures, and therefore leading shoots are generally much shorter in the uppermost trees compared with those growing at lower elevation (Hoch & Körner, 2005).

At LA, the most evident effect of the thermal manipulation on macromorphology and phenology was the anticipation of

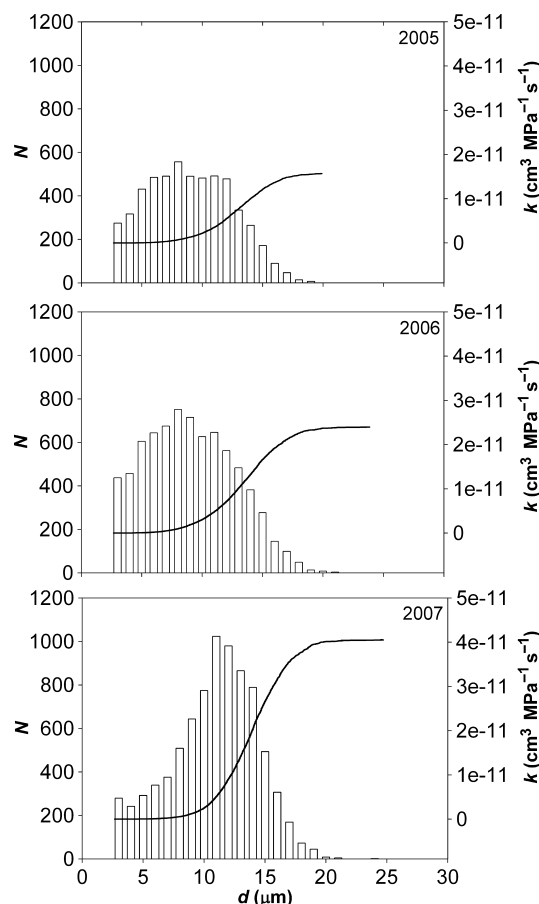


Fig. 3 Hydraulically functional anatomical modifications at the stem apex before (2005) and after (2006 and 2007) heating in Norway spruce (*Picea abies*) tree 3H at HA (high altitude). Histograms represent the distribution of conduit diameters (N vs d) and solid lines represent the cumulated apical hydraulic conductivity (k) calculated according to the Hagen–Poiseuille formula (see Eqn 1).

bud burst, which was consistent with previous observations (Roberntz, 1999).

The most relevant result of this study was that, after 2 yr, without manipulating any environmental factor other than temperature, heated trees from HA increased stem elongation at rates significantly similar to control trees from LA. This means that temperature alone so far represents the most limiting factor for height growth at high altitudes. Moreover, this effect was obtained by altering the thermal regimes only around the stem apex, which can therefore be considered as the thermal ‘sensor’ of the whole tree.

The results of anatomical analyses revealed that the artificial warming promoted hydraulically effective modification only in the newly produced shoots, whereas the remaining xylem tissue (stem) seemed not to show significant changes between pre- and post-treatment.

In the developing shoots, heating stimulated the production of either a larger number or size of conduits, or both, consistent with previous reports (Gorsuch & Oberbauer,

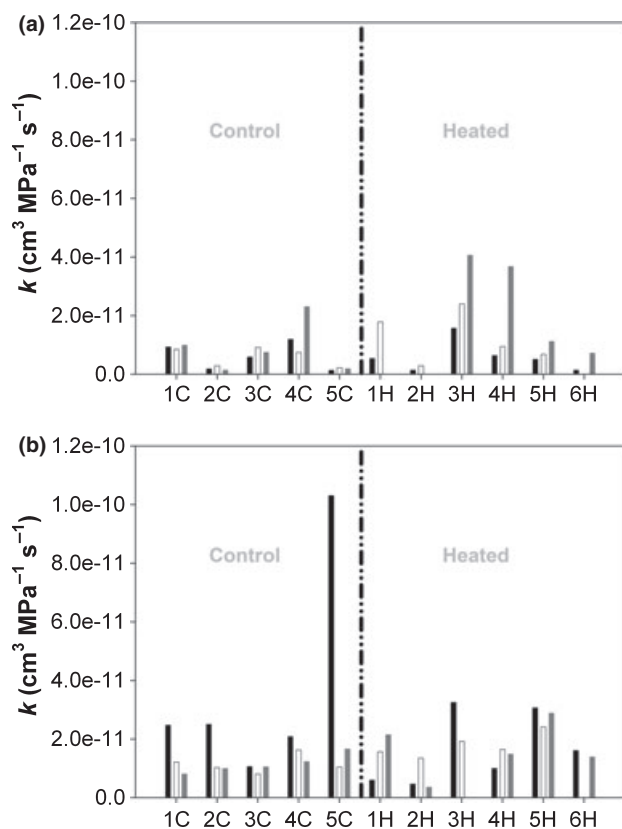


Fig. 4 Estimated hydraulic conductivity (k) of the apical section (2 cm from the apex) of the leader shoot of 2005 (black bars), 2006 (white bars) and 2007 (gray bars) in each control and heated Norway spruce (*Picea abies*) tree at HA (high altitude; a) and LA (low altitude; b).

Table 3 Results of Kruskal–Wallis test for the analysis of variance among treatments for the three ratios of apical conductivity (k):

$k_{2006} : k_{2005}$; $k_{2007} : k_{2005}$; $k_{2007} : k_{2006}$

Site	$k_{2006} : k_{2005}$	$k_{2007} : k_{2005}$	$k_{2007} : k_{2006}$
HA	$\chi^2 = 1.200$ $P = 0.273$	$\chi^2 = 6.000$ $P = 0.014^*$	$\chi^2 = 2.940$ $P = 0.086$
LA	$\chi^2 = 3.333$ $P = 0.068$	$\chi^2 = 3.938$ $P = 0.047^*$	$\chi^2 = 0.098$ $P = 0.754$

*, significant at 95%.

HA, high altitude; LA, low altitude.

2002; Danby & Hik, 2007). Such anatomical modifications probably improved the local xylem permeability, as reported in an experiment of artificial warming in trees at a latitudinal treeline (Gorsuch & Oberbauer, 2002). As the conducting properties of the stem apex significantly affect the hydraulic conductance of the whole tree (Yang & Tyree, 1993; Becker *et al.*, 2000; Petit *et al.*, 2008), xylem anatomical features, such as conduit number and size at the stem apex, were analyzed in terms of the apical hydraulic conductivity (k). k increased significantly in trees at both

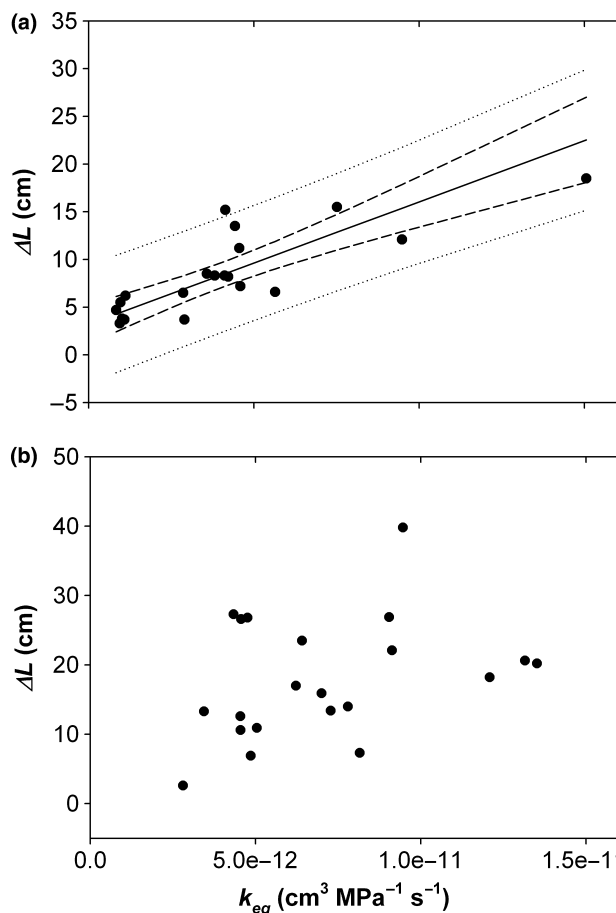


Fig. 5 Relationship between current stem longitudinal increment (ΔL) and the combined hydraulic conductivities of the current and previous year apices (k_{eq}) for Norway spruce (*Picea abies*) trees at HA (high altitude; a) and LA (low altitude; b). The regression line for trees at HA is: $y = 3.20 + x \times 1.28 \times 10^{12}$ ($F = 34.544$; $R^2 = 0.657$; $P < 0.0001$). Dashed lines indicate 95% confidence bands, dotted lines indicate 95% prediction bands.

HA and LA only after the second year of treatment (2007). At LA, we provided a temperature 10°C higher than the air temperature. We aimed to demonstrate that the effect of temperature on xylogenesis (i.e. conduit number and size) is less important at LA. Yet, the results suggested a significant effect of increased temperature on xylem anatomy in trees at LA. Therefore, as increased temperature stimulated xylogenesis, but not height growth, at LA, it can be speculated that photosynthetic rates had already achieved saturation at ambient temperatures or, rather, that warming took temperatures to supraoptimal values which had the effect of restricting height growth.

The analyses of the vertical profiles of conduit diameter revealed that the xylem transport system is organized according to a tapered architecture. Conduits increased continuously from the stem apex to the base according to a power function, as frequently observed in trees of different species and size (Anfodillo *et al.*, 2006; Weitz *et al.*, 2006;

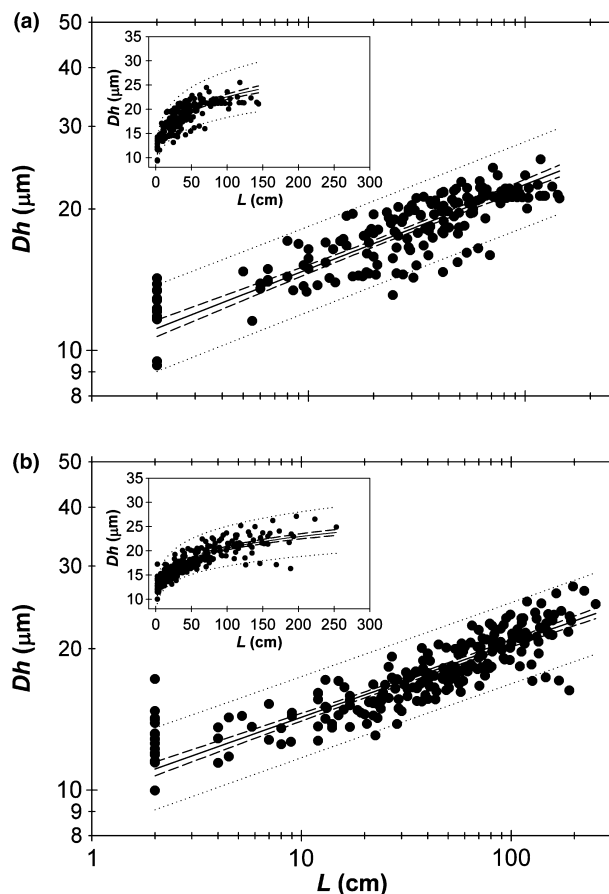


Fig. 6 Axial variation in the mean hydraulic diameter of xylem conduits (Dh) with distance from the apex (L) on a log–log scale (linear scale in inset) using data from all analyzed Norway spruce (*Picea abies*) trees at HA (high altitude; a) and LA (low altitude; b). Dashed lines indicate 95% confidence bands, dotted lines indicate 95% prediction bands. Details of regression parameters are given in Table 4.

Coomes *et al.*, 2007; Mencuccini *et al.*, 2007; Petit *et al.*, 2008, 2009). Overall, at HA, we observed narrower conduits coupled with higher degrees of tapering (scaling parameter b) compared with LA. Tapering results appeared to be in contrast with previous reports of higher rates of conduit tapering in trees at high altitudes (Coomes *et al.*, 2007; Petit *et al.*, 2009).

The lower degrees of tapering observed in trees at low elevation should reflect a minor compensation effect for the path length resistance with height growth (Becker *et al.*, 2000). Unless conduit tapering is the most effective mechanism of compensation for the increased resistance with the contextual growth in height (Petit *et al.*, 2008, 2010), other important mechanisms of compensation for the increase in path length resistance ought to have been adopted by trees from LA in order to prevent stronger constraints to the water supply to the leaves and, consequently, to the transpiration and photosynthetic assimilation of atmospheric CO_2 .

Comparisons of the vertical profiles of conduit diameters pre- and post-warming (2005 vs 2007) revealed that the effect of heat on xylem anatomy was localized in the last annual shoot. Therefore, heated trees showed a reduced degree of conduit tapering and a flattening in Dh profile towards the apex (increased scaling parameter a) (Table 4) as a result of the increased conduit dimension only around the apex.

To corroborate the results obtained, we combined the conductivities (k_{eq}) of the two terminal shoots and related these to the current longitudinal increment (Fig. 5). We found that k_{eq} explained 66% of the total variation in longitudinal growth (ΔL) at HA, whereas, at LA, the same relationship was not significant.

We therefore hypothesized that the xylem transport system in trees at HA is optimized for water transport according to the higher hydraulic efficiency compatible with the temperature regimes of that environment. Removing most of the hydraulic constraints (Yang & Tyree, 1993; Becker *et al.*, 2000; Petit *et al.*, 2008) by stimulating xylogenesis at the stem apex with artificial warming, we obtained similar height growth to control trees at LA. Instead, increased temperatures did not stimulate height growth at LA, probably because these trees are also very sensitive to other kinds of constraints, such as shading or competition with neighbors for resources, or because the applied warming led to supraoptimal temperatures for photosynthetic responses.

Modifications of apical xylem anatomy and their effect on hydraulics and shoot length were more evident after the second year of exposure to increased temperature, suggesting that anatomical adaptations to changing environment occur gradually over a period of years, rather than abruptly from 1 yr to the next.

The importance of this study is that we provided evidence that trees at the treeline undergo temperature-related hydraulic limitations to height growth because of the low efficiency of the transport system caused by narrow conduits. We demonstrated that localized warming to the apical shoots promoted a higher carbon investment in xylem conduits (i.e. increased number and/or size). Anatomical modifications occurred only around the heated zones and not along the rest of the stem. This suggests that the artificial warming, although applied around the leaves, did not produce an increase in photosynthetic assimilation of atmospheric CO_2 , as proposed by the ‘carbon limitation hypothesis’. Rather, this supports the hypothesis that low temperatures hamper the fixation of NSCs into biomass (i.e. wood, i.e. n number of xylem cells of x dimensions: ‘sink limitation’) (Körner, 1998, 2003a; Hoch *et al.*, 2002; Hoch & Körner, 2005). Artificial warming probably stimulated an increase in respiration rates (Tjoelker *et al.*, 1999) and, possibly, an enhancement of cell wall extensibility (i.e. changes in cell wall biochemistry) (Nakamura *et al.*, 2003), resulting in the production of wider and more hydraulically

Table 4 Parameters of the reduced major axis (RMA) linear regressions $\log_{10}Dh$ vs $\log_{10}L$ estimated for 2005 and 2007 for each Norway spruce (*Picea abies*) tree and for all the data for each site: a is the y intercept (i.e. $\log_{10}Dh$ at $L = 1$ cm) and b is the regression slope (i.e. degree of conduit tapering)

ID	Site	Treatment	Year	N	a	b	R^2	a -95% CI	b -95% CI
1C	HA	Control	2005	11	1.07	0.15	0.87	1.04–1.22	0.05–0.17
3C	HA	Control	2005	9	1.00	0.18	0.91	0.89–1.05	0.15–0.27
4C	HA	Control	2005	9	1.02	0.18	0.95	0.93–1.06	0.16–0.24
5C	HA	Control	2005	8	0.91	0.27	0.97	0.89–1.02	0.19–0.29
1C	HA	Control	2007	14	1.06	0.14	0.86	1.03–1.11	0.12–0.17
3C	HA	Control	2007	11	0.96	0.18	0.87	0.76–1.01	0.15–0.31
4C	HA	Control	2007	11	1.05	0.16	0.91	0.95–1.09	0.13–0.22
5C	HA	Control	2007	11	0.92	0.20	0.96	0.90–0.97	0.17–0.21
1H	HA	Heated	2005	10	1.07	0.16	0.91	1.04–1.19	0.08–0.19
3H	HA	Heated	2005	10	1.04	0.16	0.95	1.00–1.12	0.11–0.19
4H	HA	Heated	2005	8	0.99	0.20	0.98	0.95–1.03	0.11–0.20
5H	HA	Heated	2005	10	0.99	0.15	0.63	0.76–0.12	0.12–0.30
1H	HA	Heated	2007	13	1.01	0.16	0.80	0.93–1.13	0.10–0.21
3H	HA	Heated	2007	14	1.06	0.14	0.87	0.93–1.10	0.11–0.22
4H	HA	Heated	2007	11	1.04	0.13	0.89	0.92–1.08	0.11–0.20
5H	HA	Heated	2007	13	1.09	0.06	0.63	1.02–1.11	0.04–0.09
1C	LA	Control	2005	10	1.04	0.13	0.87	0.89–1.08	0.10–0.22
2C	LA	Control	2005	8	1.13	0.05	0.84	1.09–1.15	0.04–0.08
3C	LA	Control	2005	8	1.06	0.12	0.99	1.01–1.07	0.11–0.15
4C	LA	Control	2005	10	1.06	0.13	0.87	0.91–1.10	0.10–0.21
5C	LA	Control	2005	7	1.15	0.09	0.73	1.00–1.21	0.06–0.16
1C	LA	Control	2007	15	0.97	0.17	0.96	0.90–1.01	0.15–0.21
2C	LA	Control	2007	12	1.03	0.13	0.90	0.95–1.06	0.11–0.18
3C	LA	Control	2007	13	0.10	0.15	0.95	0.93–1.04	0.13–0.19
4C	LA	Control	2007	14	1.03	0.15	0.94	0.97–1.07	0.13–0.19
5C	LA	Control	2007	15	1.06	0.15	0.93	0.95–1.10	0.13–0.21
1H	LA	Heated	2005	8	0.99	0.13	0.89	0.78–1.03	0.10–0.25
2H	LA	Heated	2005	8	0.99	0.14	0.81	0.77–1.02	0.10–0.28
4H	LA	Heated	2005	6	1.09	0.06	0.85	1.03–1.16	0.01–0.1
5H	LA	Heated	2005	10	1.11	0.12	0.94	1.01–1.13	0.10–0.17
6H	LA	Heated	2005	10	1.05	0.13	0.96	0.95–1.07	0.12–0.18
1H	LA	Heated	2007	14	1.02	0.12	0.93	0.93–1.04	0.11–0.17
2H	LA	Heated	2007	12	0.95	0.16	0.97	0.89–0.96	0.14–0.19
4H	LA	Heated	2007	11	1.04	0.12	0.99	1.02–1.05	0.11–0.13
5H	LA	Heated	2007	15	1.05	0.14	0.91	1.01–1.13	0.10–0.17
6H	LA	Heated	2007	14	1.05	0.10	0.64	1.01–1.10	0.07–0.12
All data	HA	Heated + Control	2005 + 2007	173	0.99	0.18	0.74	0.96–1.02	0.16–0.20
All data	LA	Heated + Control	2005 + 2007	220	1.00	0.16	0.75	0.98–1.02	0.14–0.17

95% confidence intervals are also given for both a and b .

HA, high altitude; LA, low altitude.

efficient xylem cells. Moreover, it seemed that the use of an increased amount of NSCs to build more and wider conduits was possible only around the heated zones, whereas the remaining portion of the stem at ambient temperature seemed to have received no stimuli to enhance xylogenesis.

This study may also be relevant within the context of predictions of global warming scenarios. Our findings support the hypothesis that increased growth rates of trees near the treeline (Salzer *et al.*, 2009) and the treeline advance in altitude and latitude (Körner, 1998; Gamache & Payette, 2004; Harsch *et al.*, 2009) are phenomena driven by the increase in atmospheric temperatures. The production of wider cells would certainly increase the hydraulic efficiency of the transport system and stimulate tree growth. On the

other hand, it would expose treeline trees to higher risks of xylem failure by cavitation after freeze–thaw cycles (Mayr *et al.*, 2003, 2006), thus compromising the survival success. The forecasting of future scenarios of the potential speed of further altitudinal and latitudinal treeline advance therefore remains a challenge.

In conclusion, this study essentially represents an extension of the ‘sink limitation hypothesis’ (Körner, 1998, 2003a; Hoch *et al.*, 2002; Hoch & Körner, 2005): we analyzed the phenomenon of reduced height growth in trees at high altitude under a different perspective, that of hydraulic limitations. High-altitude trees presented a xylem architecture optimally tapered to effectively compensate for the negative effect of height growth on the total hydraulic

conductance, thus confining most of the resistance close to the stem apex (Yang & Tyree, 1993; Becker *et al.*, 2000; Petit *et al.*, 2008). Nevertheless, this hydraulic efficiency would allow for only small longitudinal increments because of the negative effect of cold temperatures on xylogenesis (Gričar *et al.*, 2006; Rossi *et al.*, 2007, 2008). In addition, any constraint to xylogenesis around the stem apex would determine a negative effect on the whole tree hydraulics and, ultimately, on height growth.

Acknowledgements

This study received financial support from the University of Padova ('EXTRA'-CPDA071953 and CPDR081920) and MIUR Prin 2007. Francesco Grani was supported by Fondazione Cassa di Risparmio di Padova e Rovigo (PhD grant). We sincerely thank Fausto Fontanella and Roberto Menardi for technical support.

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