FROST DROUGHT IN CONIFERS AT THE ALPINE TIMBERLINE: XYLEM DYSFUNCTION AND ADAPTATIONS

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Abstract. Drought stress can cause xylem embolism in trees when the water potential (Ψ) in the xylem falls below specific vulnerability thresholds. At the alpine timberline, frost drought is known to cause excessive winter embolism unless xylem vulnerability or transpiration is sufficiently reduced to avoid critical Ψ.

We compared annual courses of Ψ and embolism in *Picea abies*, *Pinus cembra*, *Pinus mugo*, *Larix decidua*, and *Juniperus communis* growing at the timberline vs. low altitude. In addition, vulnerability properties and related anatomical parameters as well as wood density (D_t) and wall reinforcement (wall thickness related to conduit diameter) were studied. This allowed an estimate of stress intensities as well as a detection of adaptations that reduce embolism formation.

At the alpine timberline, Ψ was lowest during winter with corresponding embolism rates of up to 100% in three of the conifers studied. Only *Pinus cembra* and *Larix decidua* avoided winter embolism due to moderate Ψ. Minor embolism was observed at low altitude where the water potentials of all species remained within a narrow range throughout the year. Within species, differences in Ψ_{50} (Ψ at 50% loss of conductivity) at high vs. low altitude were less than 1 MPa. In *Picea abies* and *Pinus cembra*, Ψ_{50} was more negative at the timberline while, in the other conifer species, Ψ_{50} was more negative at low altitude. *Juniperus communis* exhibited the lowest (-6.4 ± 0.04 MPa; mean $\pm$ SE) and *Pinus mugo* the highest Ψ_{50} (-3.34 ± 0.03 MPa). In some cases, D_t and tracheid wall reinforcement were higher than in previously established relationships of these parameters with Ψ_{50}, possibly because of mechanical demands associated with the specific growing conditions.

Conifers growing at the alpine timberline were exposed to higher drought stress intensities than individuals at low altitude. Frost drought during winter caused high embolism rates which were probably amplified by freeze–thaw stress. Although frost drought had a large effect on plant water transport, adaptations in hydraulic safety and related anatomical parameters were observed in only a few of the conifer species studied.

Key words: alpine timberline; drought-induced embolism; frost drought; hydraulic safety; tracheids; vulnerability; wood density.

INTRODUCTION

The alpine timberline marks the altitudinal limit of the tree life form. Plants growing at this ecotone are exposed to various stress factors like a short growing season, high wind speeds, intense radiation, and high snow loads (e.g., Tranquillini 1979, Körner 2003). This extreme environment also influences plant water relations: In the summer, soil water contents are normally sufficiently high and evaporative forces are moderate due to low temperatures. During winter, however, water uptake is blocked by the frozen soil and frozen stems while warm atmospheric temperatures and solar radiation increase evaporative demand leading to a condition known as "frost drought." (e.g., Michaelis 1934, Pisek and Larcher 1954, Larcher 1972, Tranquillini 1976, 1980). This clearly contrasts with low altitude sites within alpine landscapes, where drought is more likely to occur in the summer than during winter.

Drought can affect the water transport system of plants by the formation of embolism (e.g., Sperry and Tyree 1990, Tyree et al. 1994). In embolized xylem conduits, the transport of water is blocked by gas bubbles, which decrease hydraulic conductivity (Sperry at al. 1988) and interrupt the transmission of tension to the soil ("cohesion theory"; e.g., Boehm 1893, Dixon and Joly 1894). Drought leads to embolism when the water potential (Ψ) in conduits falls below xylem-specific thresholds at which air enters from adjacent, already air-filled spaces ("air seeding"; Tyree and Zimmermann 2002). At Ψ less negative than the air-seeding threshold, the surface tension of water stabilizes air–water interfaces and prevents the spread of embolism. Conifers have a special pit anatomy with a central thickening, the torus, which seals the pit apertures of an air-filled tracheid, preventing the spread of embolism. Therefore, vulnerability to drought-induced embolism depends on

the properties of the pits, where air-seeding normally occurs (Sperry and Hacke 2004, Hacke et al. 2004). Mesic species show 50% loss of conductivity at moderately negative Ψ (Ψ_{50}), while xeric species often show very low (negative) Ψ_{50}.

The embolism resistance (the hydraulic safety), of a species is reflected in its xylem anatomy. Hacke et al. (2001) reported a trade-off between the resistance to drought-induced embolism and construction costs to avoid the implosion of xylem conduits at low Ψ. Wood density and wall reinforcement $(t/b)_h^2$, which relates the wall thickness (t) to the span (b) of conduits for the hydraulic (h) mean diameter (D_h), tended to be higher in embolism-resistant xylem, in both conifers and angiosperms. Recently, Cochard et al. (2004) showed that decreasing xylem pressure provoked a progressive collapse of tracheids in needles of four pine species. The pressure at which collapse began was more negative for species with smaller tracheid diameter (a proxy of b) and thicker walls. Similar findings were observed in needles of *Podocarpus grayi* (Brodribb and Holbrook 2005). While this situation may be specific to xylem in needles, the observations give experimental support to the proposed link between the demands of cohesion-driven water transport and xylem anatomy, specifically wall reinforcement.

Analyses of water stress intensities, vulnerability properties, and related anatomical parameters, and the extent of embolism formation across environmental gradients provide important insights into adaptations of the xylem that maintain long-distance water transport. During the cold season, such analyses are complicated by the fact that, in addition to tension, freeze–thaw events may also induce embolism (e.g., Sperry et al. 1994, Sperry and Sullivan 1992). Freezing forces air out of the xylem sap and produces bubbles in frozen conduits. During thawing, these bubbles may expand and trigger embolism (Ewers 1985, Davis et al. 1999, Pittermann and Sperry 2003). At the alpine timberline, frost drought as well as frequent freeze–thaw events (e.g., Groß et al. 1991, Mayr et al. 2003*d*) were shown to induce winter embolism (Mayr et al. 2002, 2003*a, b, c, d*).

In previous studies, we found that several adaptations reduce the risk of embolism at the alpine timberline: *Picea abies* showed a consistent increase in resistance to drought-induced embolism with increasing altitude (Mayr et al. 2002). This pattern was in agreement with more negative Ψ during winter at greater elevations. Increasing resistance to embolism was associated with smaller tracheid diameters. However, despite these adjustments in xylem structure and function, embolism rates in twigs of *Picea abies* were up to 100% at the highest elevations where water potentials were most negative in the winter (Mayr et al. 2002). Leader shoot xylem was more resistant than the wood of lateral branches (Mayr et al. 2003*c*) and refilling processes were

observed in *Picea abies* during late winter and spring (Mayr et al. 2002, 2003*d*).

The broad spectrum of adaptations and the high values of observed winter embolism in *Picea abies* indicate that drought-induced embolism is an important stress factor for this species at the timberline. However, previous studies focused on the winter season while information on annual courses of Ψ and embolism is lacking. Furthermore, a general ecological assessment would require an analysis of other alpine tree species. We therefore studied key hydraulic parameters and related anatomical traits in five conifer species growing in the European Central Alps, three trees (*Picea abies, Pinus cembra, Larix decidua*) and two shrubs (*Pinus mugo, Juniperus communis*). Intensities of drought stress and embolism formation at the timberline were analyzed throughout an entire year. Hydraulic safety was characterized by measuring the vulnerability to drought-induced embolism and related anatomical parameters as well as wood density. Parallel measurements were also made in low altitude stands to allow comparison of strains on water transport at low vs. high altitudes, and for identifying adaptations in hydraulic safety that were related to differences in altitude.

METHODS

Plant material and study sites

Twigs of *Picea abies* L. Karst., *Pinus cembra* L., *Pinus mugo* Turra, *Larix decidua* Mill., and *Juniperus communis* L. were harvested at sites located at the alpine timberline as well as at low altitude (Table 1). Harvested plant material was situated above the ground snow cover.

For the analysis of annual courses, terminal segments of sun-exposed twigs (length up to 10 cm, $n \geq 5$ per species) were collected and transported in a plastic bag to the laboratory for water potential (Ψ) determination. Samples were always taken on clear days (after a period of at least three sunny days) between 11:00 and 13:00 hours to enable measurement of minimum Ψ. For conductivity measurements, pieces of the main axes about 40 cm long were harvested, wrapped in a plastic bag, and transported to the laboratory ($n \geq 3$ per species).

For the vulnerability analysis, twigs 0.5–2.5 m long were harvested between September and May, transported to the laboratory of the Institut für Botanik, Innsbruck, Austria in a dark plastic bag and re-cut under water (about 2 cm) at least three times to release xylem tension gradually. Twigs were hydrated for 24 h.

Twig sections previously used for vulnerability measurements (see *Methods: Loss of hydraulic conductivity*), were express mailed to the University of Utah in an ethanol-glycerol-water solution (1:1:1, v/v/v) for wood density and anatomical analysis.

Water potential (Ψ)

Water potential (Ψ) was measured with a pressure chamber (Model 1000 Pressure Chamber, PMS Instru-

TABLE 1.   Conifer species and study sites.

Species and growth form	Site of harvest	Altitude (m)	Tree height (m)
Picea abies, tree, evergreen	Götzens, 47°14′ N,11°18′ E	750	25
	Birgitz Köpfl, 47°11′ N, 11°19′ E	2035	2
Pinus cembra, tree, evergreen	Innsbruck, 47°16′ N, 11°22′ E	600	20
	Praxmar, 47°09′ N, 11°07′ E	2100	3
	Birgitz Köpfl, 47°11′ N, 11°19′ E	2035	3
Pinus mugo, shrub, evergreen	Innsbruck, 47°16′ N, 11°22′ E	600	3
	Seegrube, 47°18′ N, 11°22′ E	1970	3
	Birgitz Köpfl, 47°11′ N, 11°19′ E	2035	3
Larix decidua, tree, deciduous	Götzens, 47°14′ N, 11°18′ E	750	25
	Birgitz Köpfl, 47°11′ N, 11°19′ E	2035	4
Juniperus communis, shrub, evergreen	Innsbruck, 47°16′ N, 11°22′ E	600	2
	Praxmar, 47°09′ N, 11°07′ E	1700	0.3
	Birgitz Köpfl, 47°11′ N, 11°19′ E	2035	0.4

Notes: Twigs were harvested at low altitude (first site) and at the timberline (second and third site) in Tyrol, Austria, Central European Alps. Information on the ecology and tree size at the harvesting sites is given.

ment Company, Corvallis, Oregon, USA) on up to 10 cm long terminal segments of twigs. This was also done with leafless samples of *Larix decidua* during winter. The pressure chamber technique also allows a correct determination of Ψ in these samples, because the xylem is in contact with living tissues of the buds, the xylem, and the bark of the twig segment.

Loss of hydraulic conductivity

Loss of hydraulic conductivity was quantified by measuring the increase in hydraulic conductivity after removal of xylem embolism by repeated high pressure flushes (Sperry et al. 1988, Chiu and Ewers 1993, Vogt 2001). Samples 3–8 mm in diameter and up to 4 cm long, were prepared as described in Mayr et al. (2002). Measurement pressure was set to 4 kPa. The flow rate was determined with a PC-connected balance (Sartorius BP61S, 0.1 mg precision, Sartorius AG, Göttingen, Germany) by recording weight every 10 s and fitting linear regressions over 200-s intervals. Flushing (at 130 kPa) and conductivity measurements were done with distilled, filtered (0.22 μm), and degassed water containing 0.005% (volume/volume) "Micropur" (Katadyn Products, Wallisellen, Switzerland) to prevent microbial growth. Flushing was repeated until measurements showed no further increase in conductivity. Loss of conductivity as a percentage (PLC) was calculated from the ratio of initial to maximal conductivity (Sperry et al. 1988).

For vulnerability analysis, embolism in *Juniperus* was quantified using the "paired segment method" (Sperry and Sullivan 1992) because, in samples with low Ψ, it was not possible to restore full conductivity by flushing. For each twig, hydraulic conductivities of samples taken before and after dehydration were determined for the calculation of the PLC.

Vulnerability to drought-induced embolism

Vulnerability curves were obtained for twigs dehydrated to various extents by plotting the percentage loss of hydraulic conductivity vs. Ψ. Curves were fitted with an exponential sigmoidal equation (Pammenter and Vander Willigen 1998):

$$PLC = 100/\{1 + \exp[a(\Psi - \Psi_{50})]\} \qquad (1)$$

where PLC is the percentage loss of conductivity, Ψ is the corresponding water potential, and a is a constant related to the curve slope. Ψ_{50} corresponds to the Ψ at 50% loss of conductivity. Curves were calculated using Fig.P 2.98 (Biosoft, Cambridge, UK) and based on the statistic options Ψ_{10}, Ψ_{90}, and the corresponding SE were calculated. We also plotted PLC vs. Ψ values obtained from field measurements of *Picea abies*, *Pinus mugo*, and *Juniperus communis* growing at the alpine timberline. These "field vulnerability curves" were compared with vulnerability curves analyzed in the laboratory.

Wood density

Total wood density (D_t, dry mass per fresh volume) was measured in ~2 cm long segments after removal of the bark. In most cases, the pith was also removed after segments had been cut longitudinally. The D_t was measured on segments previously used for measuring Ψ_{50}, using the method of Hacke et al. (2000). Density was averaged for $n = 3$–7 samples per species. Fresh volume was determined by Archimedes' principle. Using a needle, segments were immersed in a water-filled tray, which was placed on a balance. Displacement weight was related to fresh volume. Samples were then stored in a dry oven at 75°C for 48 h, and their dry mass was measured.

Anatomical measurements

Anatomical measurements were done on samples previously used for vulnerability analysis. Cross sections were stained with phloroglucinol-HCl. Individual conduit diameters (d_c) were measured in radial sectors of recent growth rings, incorporating both late- and earlywood. A total of 220–450 conduits from three to seven stems per species and site were analyzed with a light microscope interfaced with a digital camera and image analysis software (Image-Pro Plus, Media Cyber-
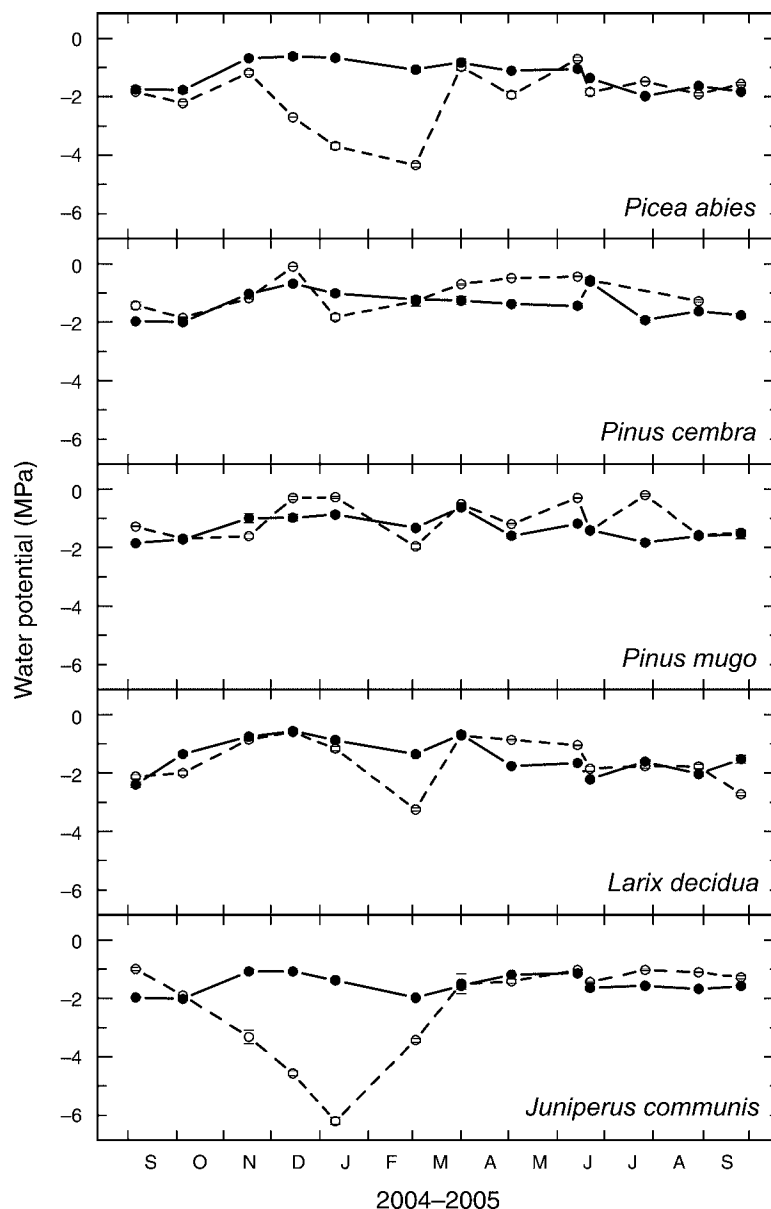
FIG. 1. Midday water potential (mean ± SE) of sun-exposed twigs of conifers growing at low altitude (solid symbols) and at timberline (open symbols) from September 2004 to September 2005.

netics, Silver Spring, Maryland, USA). The hydraulic mean conduit diameter (D_c) was calculated as $\Sigma d_c^5/\Sigma d_c^4$ (Sperry and Hacke 2004). We also measured the "thickness to span ratio" $(t/b)_h^2$ (Hacke et al. 2001, 2004) to characterize conduit wall reinforcement. The thickness of interconduit walls (t) was measured for tracheid pairs with average diameters within $D_h \pm 1$ μm. The thickness to span ratio was determined in $n = 20$–45 conduits per species and site; t was only measured in tangential walls, where pits were absent. The width of the common wall (b) was assumed equal to the side of a square of equal area to the conduit lumen (Hacke et al. 2001).

Statistics

All differences were tested at 5% probability level with Student's t test after testing for normal distribution and variance of the data.

RESULTS

At the alpine timberline, the most negative water potentials were found during winter (Fig. 1). The winter minimum Ψ was −6.2 ± 0.12 MPa (11 January; mean ± SE) in *Juniperus communis*, −4.34 ± 0.07 MPa in *Picea abies* (3 March), and −3.25 ± 0.05 MPa in *Larix decidua* (3 March). Regardless of season or altitude, Ψ of *Pinus cembra* and *Pinus mugo* did not fall below −2 MPa.
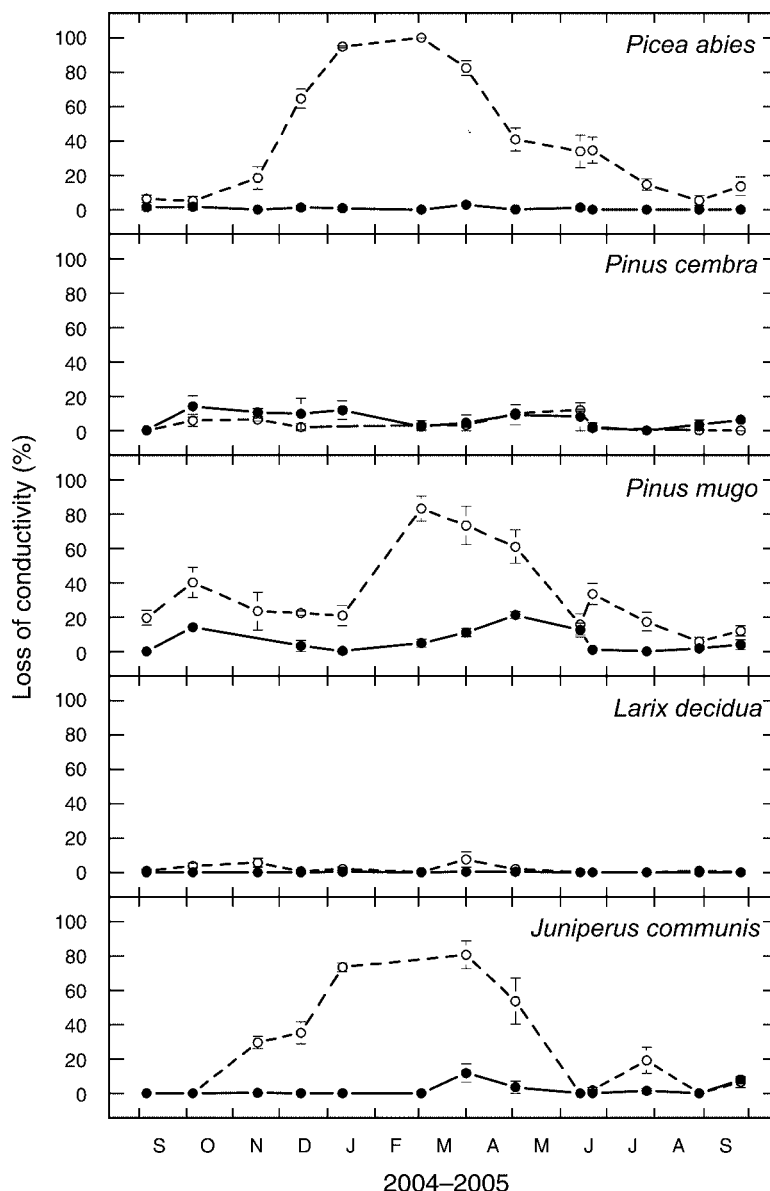
FIG. 2. Loss of conductivity (mean ± SE) in sun-exposed twigs of conifers growing at low altitude (solid symbols) and at timberline (open symbols) from September 2004 to September 2005.

Water potentials at low altitude remained above −2 MPa during winter in all species. During summer, Ψ at low altitudes was similar or more negative (e.g., Fig. 1; *Pinus cembra*, *Juniperus communis*) than at the timberline. The lowest Ψ at low altitude in the summer was observed in *Larix decidua* (−2.4 ± 0.1 MPa, 6 September).

The highest losses in conductivity in *Picea abies*, *Pinus mugo*, and *Juniperus communis* were observed during winter at the timberline sites (Fig. 2). PLC reached 100% (3 March) in *Picea abies*, 80.6 ± 8.23% in *Juniperus communis* (1 April), and 83.2 ± 7.2% in *Pinus mugo* (3 March). In *Pinus cembra* and *Larix decidua*, PLC

remained below 12% throughout the year. In the summer, embolism reached 33.4 ± 6.3% in *Pinus mugo*, but was below 20% in *Juniperus communis* and *Picea abies*. At low altitude, embolism rates remained low throughout the year. The highest PLC at low altitude was observed in *Pinus mugo* (21.1 ± 1.9%, 3 May).

The five conifer species exhibited values of Ψ_{50} between −6.4 and −3.3 MPa (Fig. 3 and Table 2). The highest resistance to drought-induced embolism was found in *Juniperus communis* growing at low altitude (Ψ_{10} at −5.0 MPa). A comparison between timberline stands and low-altitude study sites (including data of previous studies, see Mayr at al. 2002, 2003b, d) revealed
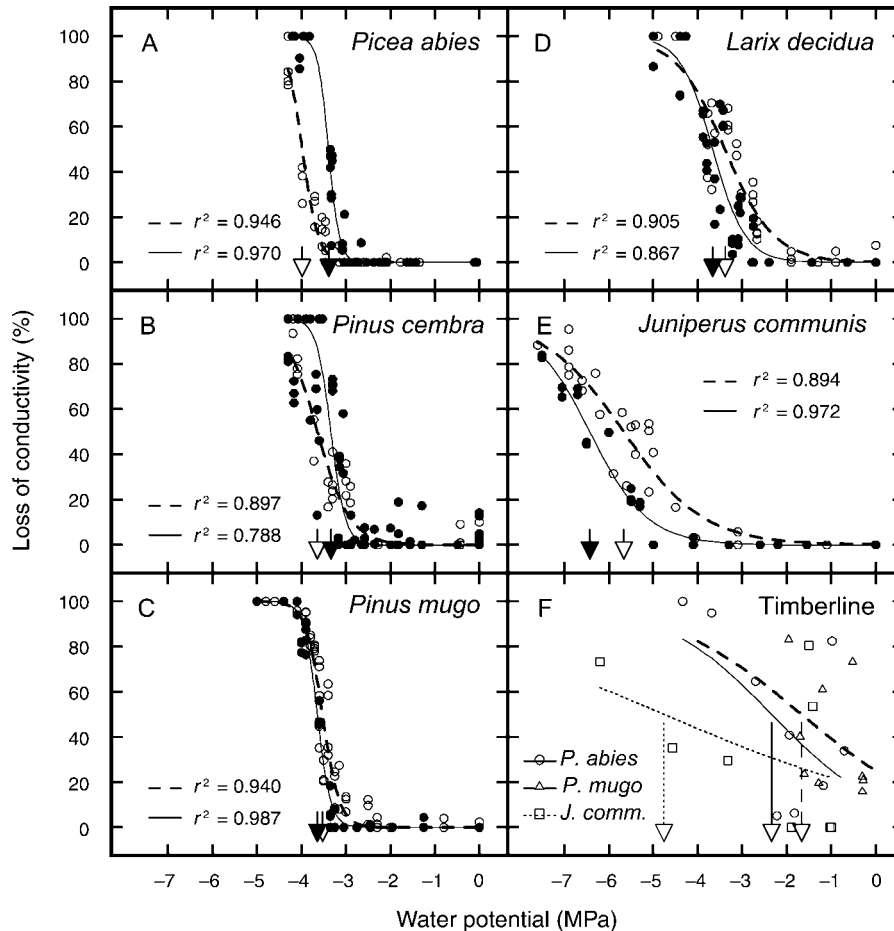
FIG. 3.   Vulnerability to drought-induced embolism (loss of conductivity vs. water potential) of (A) *Picea abies*, (B) *Pinus cembra*, (C) *Pinus mugo*, (D) *Larix decidua*, and (E) *Juniperus communis* twigs. Solid symbols and solid curves indicate stands at low altitude; open symbols and dashed curves show stands at the timberline. Arrows show Ψ_{50} (the water potential at 50% loss of conductivity) for the two elevations. Data for *Picea abies* are taken from Mayr et al. (2002) and Mayr et al. (2003d), for *Pinus mugo* from Mayr et al. (2003b), and for *Pinus cembra* (timberline) from Mayr et al. (2003d). Panel (F) shows loss of conductivity vs. water potentials observed at the timberline between September 2004 and May 2005 in *Picea abies* ($r^2 = 0.354$), *Pinus mugo* ($r^2 = 0.203$), and *Juniperus communis* ($r^2 = 0.166$). Data were fit according to the vulnerability curves. Arrows indicate Ψ_{50}.

a more negative Ψ_{50} at the timberline in only two out of five species (Table 2). In the other conifers, the trend was reversed and more negative Ψ_{50} were found at lower altitude. In all species, differences in Ψ_{50} between high and low altitude sites were <1 MPa. Although differences in Ψ_{50} across sites were significant within all species tested, the same was not true for Ψ_{10} and Ψ_{90}. Overall, no consistent trend of vulnerability properties was observed across species when low and high altitudes were compared.

A plot of PLC vs. Ψ values measured at the timberline during winter months revealed greater embolism levels than expected from vulnerability curves established in the laboratory. The latter curves (Fig. 3A–E) did not account for the effect of freezing on water transport. Curves were fitted through the field data (Fig. 3F) according to Eq. 1, and had less negative Ψ_{50} than curves measured in the laboratory. The difference in Ψ_{50}

between field and laboratory curves was 1.64 MPa in *Picea abies*, 2.27 MPa in *Pinus mugo,* and 0.90 MPa in *Juniperus communis*.

Consistent with the lack of a clear trend of Ψ_{50} with altitude, no clear pattern was found regarding variation of wood anatomy with altitude (Table 3). Within species, higher wood densities tended to occur at low altitude. This trend was significant in three species. In four out of five species, D_h was slightly smaller at the timberline than at low altitude, but differences were not significant. Tracheid wall reinforcement showed no consistent variation with altitude. The densities and thickness to span ratios showed considerable variation. The $(t/b)_h^2$ varied from 0.06 to 0.29 (Table 3, Fig. 4B) while Ψ_{50} remained within a relatively narrow range. When data of our species were pooled with a previous data set, which spanned a much broader range in embolism resistance (cross hairs, Fig. 4), significant

TABLE 2. Xylem vulnerability to embolism for the five conifer species studied.

Species and altitude	Parameter a	Ψ_{10} (MPa)	Ψ_{50} (MPa)	Ψ_{90} (MPa)	n
Picea abies					
Low	8.30* ± 1.23	−3.13* ± 0.05	−3.39* ± 0.01	−3.65* ± 0.03	67
High	5.55 ± 0.61	−3.59 ± 0.07	−3.98 ± 0.03	−4.38 ± 0.02	33
Pinus cembra					
Low	6.21* ± 1.06	−2.99 ± 0.1	−3.34* ± 0.03	−3.69* ± 0.03	78
High	2.74 ± 0.35	−2.84 ± 0.15	−3.64 ± 0.05	−4.44 ± 0.05	30
Pinus mugo					
Low	6.75* ± 0.40	−3.31* ± 0.03	−3.64* ± 0.01	−3.97 ± 0.01	49
High	5.29 ± 0.53	−3.11 ± 0.06	−3.53 ± 0.02	−3.94 ± 0.02	60
Larix decidua					
Low	2.74* ± 0.33	−2.86* ± 0.14	−3.66* ± 0.05	−4.46 ± 0.05	53
High	1.81 ± 0.23	−2.18 ± 0.22	−3.39 ± 0.06	−4.61 ± 0.09	36
Juniperus communis					
Low	1.54* ± 0.10	−5.00* ± 0.13	−6.43* ± 0.04	−7.85 ± 0.05	36
High	1.11 ± 0.15	−3.69 ± 0.37	−5.66 ± 0.10	−7.64 ± 0.17	27

Notes: Vulnerability parameters of sun-exposed twigs from conifers growing at low altitude and at the alpine timberline (high altitude). Vulnerability curve parameter a, Ψ_{10}, Ψ_{50}, and Ψ_{90} (water potential at 10%, 50%, and 90% loss of conductivity) were calculated according to Pammenter and Van der Willigen (1998). Data for *Picea abies* are taken from Mayr et al. (2002) for low elevation and from Mayr et al. (2003*d*) for high elevation. Data for *Pinus mugo* are from Mayr et al. 2003*b*; data for *Pinus cembra* (high elevation) are from Mayr et al. 2003*d*. Values are mean ± SE; *n*, number of samples.

* Indicates significant difference between low and high altitude at $P \leq 0.05$ (Student's *t* test).

correlations were found between Ψ_{50} and wood density (Fig. 4A) as well as Ψ_{50} and $(t/b)_h^2$ (Fig. 4B). However, the densities and $(t/b)_h^2$ values of several of our study species were higher at a given Ψ_{50} than those observed previously. Tracheid hydraulic mean diameters were correlated with Ψ_{50} (Fig. 4C, squares), and were within the range of a previously established relationship between D_h and Ψ_{50} (Fig. 4C, cross hairs).

TABLE 3. Xylem anatomy of studied species.

Species and altitude	D_h (μm)	$(t/b)_h^2$	Density (g/cm³)
Picea abies			
Low	13.74	0.117 ± 0.016	0.669* ± 0.029
High	13.09	0.101 ± 0.006	0.552 ± 0.018
Pinus cembra			
Low	13.91	0.097* ± 0.008	0.547* ± 0.017
High	12.13	0.169 ± 0.007	0.479 ± 0.015
Pinus mugo			
Low	12.93	0.133* ± 0.010	0.590 ± 0.025
High	11.26	0.237 ± 0.023	0.644 ± 0.013
Larix decidua			
Low	16.06	0.081 ± 0.006	0.586 ± 0.014
High	15.65	0.063 ± 0.007	0.575 ± 0.016
Juniperus communis			
Low	9.29	0.289* ± 0.015	0.680* ± 0.018
High	11.53	0.147 ± 0.010	0.581 ± 0.024

Notes: Anatomical parameters of sun-exposed twigs from conifers growing at low altitude and at the alpine timberline (high altitude). Mean hydraulic diameter (D_h) was calculated according to Kolb and Sperry (1999); the parameter $(t/b)_h^2$ was calculated from the wall thickness t and the conduit wall span b according to Hacke et al. (2001). Values are mean ± SE.

* Indicates significant differences of $(t/b)_h^2$ and wood density between low and high altitude at $P \leq 0.05$ (Student's *t* test).

DISCUSSION

At the alpine timberline, plant water relations are strongly influenced by pronounced seasonal changes in environmental conditions. This is also demonstrated by the annual courses of Ψ and PLC shown in Figs. 1 and 2, with characteristically low Ψ during winter months. Similar annual courses of Ψ were shown for *Picea engelmannii* and *Abies lasiocarpa* by Lindsay (1971) and winter courses for *Picea abies* and *Pinus cembra* by Mayr et al. (2003*d*). In the present study, *Juniperus communis* also exhibited extremely low Ψ in winter months and even *Larix decidua*, despite its deciduous strategy, showed less than −3 MPa (Fig. 1). Richards and Bliss (1986) also found very low Ψ, down to −5.3 MPa during winter for another deciduous *Larix* species, *Larix lyallii*. Only the *Pinus* species studied here showed relatively moderate Ψ during winter. *Pinus cembra* is known to form an efficient cuticular shield which kept Ψ above −2 MPa by limiting cuticular transpiration when stomata were closed (also see Wieser 2000, Mayr et al. 2003*d*). *Pinus mugo* may have similar cuticular protection. Furthermore, *Pinus mugo* is a very flexible shrub so that twigs, although situated above the snow cover at the time of harvest, may have been below the snow during previous periods. This would decrease water losses, and may even allow a rehydration of twigs.

In contrast to winter months, only moderate water potentials were observed in all species during summer at the timberline. Anfodillo et al. (1998) published similar summer courses of minimum Ψ in *Picea abies*, *Pinus cembra*, and *Larix decidua*. The summer of 2005 was relatively humid and lacked extended drought periods. This might explain why we observed only small differences in Ψ between low and high altitude. We

suggest that in dry summer seasons, Ψ may be significantly lower at low altitude than at the timberline.

In agreement with the annual pattern of Ψ, embolism rates were highest at the timberline during winter months. All conifer species showed conductivity losses when Ψ fell below their vulnerability threshold (cf. Table 2, Fig. 3). However, as demonstrated in previous studies (Mayr et al. 2002, 2003d), native embolism rates were higher than predicted from the drought induced vulnerability curves. When native embolism was plotted vs. Ψ values of winter field measurements, the Ψ_{50} of these "field vulnerability curves" was less negative in all species exhibiting embolism (Fig. 3F). This was probably due to freeze–thaw-induced embolism which contributed to the observed conductivity losses. In *Pinus mugo,* the observed effect was probably caused in part by rehydration of twigs below the snow which can lead to an increase in Ψ within few days. In contrast, embolism repair requires several weeks (S. Mayr, *unpublished data*). A combinatorial induction of embolism by drought and freeze–thaw events in conifers was described by Sparks and Black (2000) and Mayr et al. (2003a, d). Embolism formation resulting from drought combined with freeze–thaw events was also shown in chaparral shrubs (e.g., Davis et al. 2005, Pratt et al. 2005). Pittermann and Sperry (2003, 2006) demonstrated that low Ψ in the xylem is the prerequisite for freeze–thaw-induced embolism and Mayr et al. (2003a) showed that embolism formation upon repeated freeze–thaw events is most pronounced when drought-induced embolism formation already takes place (also see Sparks and Black 2000). We conclude that frost drought is the key stress factor affecting plant hydraulics in the alpine winter, and that it is amplified by freeze–thaw stress.

In the summer season, the trees' water transport system was fully functional, implying that the increase in conductivity during late winter and spring must have been related to refilling processes rather than formation of new xylem, because cambial growth had not yet started. In twig cross sections, the first rows of new earlywood were visible in June at the timberline. Furthermore, dye experiments revealed the whole cross section to be conductive during summer (data not shown). At low altitude, significant conductivity losses occurred neither in winter nor in summer. However, prolonged drought periods in the summer might lead to water potentials low enough to trigger embolism.

Xylem vulnerabilities of our study species (Fig. 3, Table 2) were similar to values published for stems and twigs by other authors: *Pinus* species showed a broad range of Ψ_{50} from −2.3 to −7.0 MPa (Hacke et al. 2004, Martinez-Vilalta et al. 2004, Pittermann et al. 2006). Pinol and Sala (2000) measured a Ψ_{50} of −4.5 MPa in *Larix occidentalis,* Sparks and Black (2000) found Ψ_{50} to be −3.6 MPa in *Larix occidentalis* and −2.8 MPa in *Larix lyallii.* The genus *Juniperus* is characterized by particularly drought-resistant xylem. The most resistant
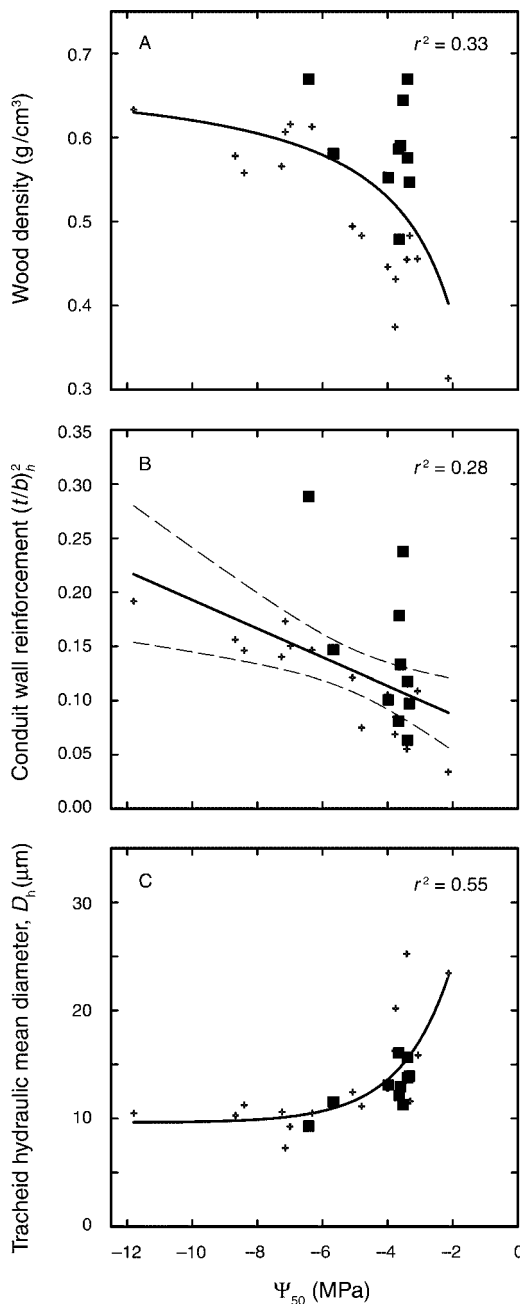

FIG. 4. Anatomical and biomechanical parameters vs. embolism resistance (Ψ_{50}) of conifer twigs (solid squares). Plus (+) symbols show stems from recent studies (Hacke et al. 2004, 2005) for comparison. While most data points fall within the range of previous analyses, some samples showed relatively high ratios of wood density and thickness to span $[(t/b)_h^2]$, which may be related to mechanical demands associated with the particular growing conditions. The r^2 values are for pooled data.

xylem of any conifer was found in *Juniperus scopulorum* (Ψ_{50} at −11.8 MPa; Pockman and Sperry 2000).

Three of the five species in the present study showed a less negative Ψ_{50} at timberline than at low altitude (Table 2). A study dealing with vulnerability properties

of conifers from upper (2500 m) and lower treeline (1005 m) in Montana, USA also revealed higher resistances to embolism in species from the lower treeline (Sparks and Black 2000). However, a direct comparison with the present study is difficult: The authors compared different species (of the genus *Pinus* and *Larix*, respectively) at upper vs. lower treelines. Furthermore, they compared two extreme tree habitats and not a treeline ecotone with a low-altitude stand of lower stress intensity as in our study. In *Pinus ponderosa*, Maherali and DeLucia (2000) found no difference in vulnerability of a desert and montane population.

As previously observed in *Picea abies* (Mayr et al. 2002), tracheid diameters tended to decrease with altitude (Table 3). Whether this is an adaptation to increase the hydraulic safety or just a result of the slower growth and smaller stature associated with life at the timberline is unknown. Tracheid size was correlated with Ψ_{50} and agreed well with results from a previous analysis, which covered a much broader range of Ψ_{50} (Fig. 4C).

Some of our conifer samples showed higher D_t and $(t/b)^2_h$ values than those found by Hacke et al. (2004, 2005; see Fig. 4A, B). Wood has hydraulic and mechanical functions. Depending on their size and specific location, the plants may have been exposed to varying degrees of mechanical stresses resulting from snow and ice loads and wind. Adaptations to such mechanical stresses may lead to changes in xylem anatomy (e.g., formation of compression wood), which could influence its vulnerability (e.g., Gartner 1995, Mayr and Cochard 2003) and mask relationships between parameters related to water transport and xylem structure. We suggest that some of the high densities found here result from the specific mechanical demands associated with the alpine environment. The data show that density was not influenced by plant size: the highest density in conifers was found in a shrub (*Juniperus communis*).

The relationships shown in Fig. 4A and B are best interpreted as setting a lower boundary on density and wall reinforcement for a given Ψ_{50}. While embolism-resistant conduits cannot have low wall reinforcement without risking collapse (Hacke et al. 2001, Cochard et al. 2004, Brodribb and Holbrook 2005), the opposite is possible: regardless of its Ψ_{50}, wood can show higher densities and greater wall reinforcement than required from the standpoint of cohesion-tension driven transport (Pittermann et al. 2006). In the context of this present study, this could be the case if snow loads were to induce the formation of extensive compression wood.

Features like compression wood and the different tracheid structure of late- and earlywood tracheids also explain why D_t (Fig. 4A) and $(t/b)^2_h$ (Fig. 4B) did not always vary in concert. Although there is a general correlation between these two parameters in conifers (U. Hacke, *unpublished data*), a few of our samples showed

disproportionately high densities for a given $(t/b)^2_h$. This was the case in *Larix decidua* at both sites. This species showed a pronounced heterogeneity in tracheid anatomy within growth rings. Earlywood tracheids were wide and had thin walls, which resulted in relatively high conduit hydraulic mean diameters, and thus low $(t/b)^2_h$ values. Yet, a large latewood fraction with particularly thick walls and small lumina made for high densities, despite a negligible effect on D_h.

Embolism resistance is usually correlated with the physiological Ψ range of a species (Brodribb and Hill 1999) where Ψ is the independent variable, influencing various parameters related to xylem structure and function. In contrast, the timberline populations in our study showed similar or even greater xylem vulnerability than low altitude populations despite higher intensity of drought stress at high altitude. How can this apparent discrepancy be explained?

First of all, the species studied here differ in their ecological strategies. Two of them avoid critical Ψ (Fig. 1) by a reduction of transpiration. *Pinus cembra* has an efficient cuticular shield (Wieser 2000, Mayr et al. 2003d) and *Larix decidua* reduces its transpiring surface by needle shedding. Adaptations in vulnerability of the latter species are therefore probably optimized for summer conditions. During the summer, sites at high altitudes usually receive more precipitation (Anfodillo et al. 1998, Körner 2003). For deciduous species, greater resistance to drought-induced embolism may thus be even more advantageous at lower elevation, where summer drought is likely to be more pronounced (Sparks and Black 2000). Shrubs are often protected from frost drought (and freeze–thaw events) by the snow pack (Mayr et al. 2003b), which can persist during the whole winter season at the timberline but not at low altitude. This may be the reason why both conifer shrubs, *Juniperus communis* and *Pinus mugo*, at the timberline showed even higher vulnerability to drought-induced embolism. However, twigs at timberline sites not covered by snow (e.g., windy sites) risk high embolism rates as shown in this study (Fig. 2). It is remarkable that Ψ in *Juniperus communis* can reach the extremely low vulnerability threshold of this species.

Another complicating factor is that the studied species show differences in their altitudinal distribution: Natural populations of *Pinus cembra* and *Pinus mugo* are restricted to high altitudes, so that "low-altitude specimen" had to be planted in the Botanical Garden of Innsbruck. Transplanted trees and shrubs may undergo changes in growth and anatomy of wood which probably led to altered vulnerability properties. *Juniperus communis* even forms a subspecies (ssp. *alpina*; Fischer et al. 2005) at the alpine timberline, which was compared with common *Juniperus communis* specimen in the Botanical Garden in Innsbruck.

Refilling also seems to be important to overcome xylem dysfunction (cf. Mayr et al. 2003d). All three timberline species that exhibited winter embolism were

able to repair their water transport system (Fig. 2) during late winter and spring. Refilling started when water potentials had recovered to values around −1 MPa. The mechanism of this process is not yet understood. Vogt (2001) demonstrated in a study on *Sambucus nigra* and *Sorbus aucuparia* that refilling can be important for restoring hydraulic conductivity when embolism avoidance mechanisms fail.

The observed refilling processes may be essential for some timberline tree species to restore xylem function before the beginning of the vegetation period. In addition, the present and previous studies demonstrate that trees may lower the risk of embolism formation with various avoidance strategies. Some species reduced transpirational water losses by optimized cuticular layers or leaf shedding and some showed an adapted hydraulic safety due to their xylem anatomy. All these adaptations are probably cost-intensive for trees. Increasing stress intensities at higher altitudes may require increasing energetic or structural investments of the trees to improve their adaptational potential. Winter drought may therefore be a limiting factor for tree life and survival at the alpine timberline.

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