

Winter at the Alpine Timberline. Why Does Embolism Occur in Norway Spruce But Not in Stone Pine?¹

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Conifers growing at the alpine timberline are exposed to frost drought and freeze-thaw cycles during winter—stress factors known to induce embolism in tree xylem. The two dominant species of the European Central Alps timberline were studied: Norway spruce (*Picea abies* [L.] Karst) and stone pine (*Pinus cembra*), which usually reaches higher altitudes. We hypothesized to find embolism only at the timberline and to observe less embolism in stone pine than in Norway spruce due to avoidance mechanisms. Seasonal courses of embolism and water potential were studied at 1,700 and 2,100 m during two winter seasons and correlated to vulnerability (to drought-induced embolism), leaf conductance, and micrometeorological data. Embolism was observed only at the timberline and only in Norway spruce (up to 49.2% loss of conductivity). Conductivity losses corresponded to low water potentials (down to -3.5 MPa) but also to the number of freeze-thaw events indicating both stress factors to contribute to embolism induction. Decreasing embolism rates—probably due to refilling—were observed already in winter. Stone pine did not exhibit an adapted vulnerability (50% loss of conductivity at -3.5 MPa) but avoided critical potentials (minimum -2.3 MPa): Cuticular conductance was 3.5-fold lower than in Norway spruce, and angles between needles and axes were found to decrease in dehydrating branches. The extent of conductivity losses in Norway spruce and the spectrum of avoidance and recovery mechanisms in both species indicates winter embolism to be relevant for tree line formation.

During the winter season, trees growing at the alpine timberline have to withstand conditions extremely unfavorable for plant water status. Water supply is permanently blocked because soil and stem are frozen, on the other hand, the shoot is exposed to water losses (“Frosttroknis”; e.g. Michaelis, 1934; Pisek and Larcher, 1954; Larcher, 1972; Tranquillini, 1980) and to frequent freeze-thaw events (Gross et al., 1991).

Drought and freeze-thaw cycles are known to induce the formation of gas bubbles in the water transport system of trees. This “embolism” interrupts the transmission of negative pressure to the soil and subsequently the flow of water through xylem conduits (“cohesion theory”; e.g. Boehm, 1893; Dixon and Joly, 1894; Richter, 1972; Jackson and Grace, 1994). Drought stress leads to high tensions in the water columns causing entry of air bubbles (air seeding) from adjacent air-filled conduits through the pits (e.g. Zimmermann, 1983; Tyree et al., 1994). Vulnerability analysis revealed species-specific water potential (ψ) thresholds for the onset of cavitation, whereby conifers were found to be very resistant due to their special pit anatomy (see e.g. Sperry and Tyree, 1990; Cochard, 1992; Jackson et al., 1995; Brodribb and Hill, 1999). Freeze-thaw events induce em-

bolism because air is not soluble in ice—remaining gas bubbles can expand during thawing and lead to cavitation. However, this effect was reported to be of minor importance in conifers (e.g. Sucoff, 1969; Robson and Petty, 1987; Robson et al., 1988; Sperry and Sullivan, 1992; Sperry et al., 1994).

In a previous study (Mayr et al., 2002), the occurrence of in part extreme conductivity losses during winter in Norway spruce growing at the alpine timberline was demonstrated: We found increasing embolism rates along an altitudinal transect up to the tree line despite an anatomically based adaptation in vulnerability to drought-induced embolism. Our data showed drought to be relevant for winter embolism formation, although we could not exclude a role of freeze-thaw events. Furthermore, we found some indications for refilling processes during winter.

Because these findings are based on few field measurement dates in late winter only, we now studied embolism and relevant parameters (e.g. ψ , vulnerability, cuticular leaf conductance, and micrometeorological data) during the whole winter season. Thereby, measurements were carried out directly at the alpine timberline (2,100 m) and at a stand 400 m below on Norway spruce (*Picea abies* [L.] Karst) and stone pine (*Pinus cembra*), two dominant species of the European Central Alps timberline. Due to the depression of the potential timberline by agricultural use during the past, even at the upper study site, stone pine and (rare) Norway spruce specimens occur, although stone pine usually reaches higher altitudes (highest stand at 2,390 m in Engadin, Switzerland; Mattes, 1982) within the timberline ecotone.

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Under the assumption that embolism is a limiting factor for trees at the timberline, we hypothesized (a) to find embolism only at the timberline and to (b) observe less embolism in stone pine than in Norway spruce due (c) to avoidance mechanisms of stone pine. Avoidance of embolism in stone pine was expected to be based on an adapted vulnerability or reduced water losses due to an effective transpiration protection. A comparison of sun-exposed and shaded twigs thereby should allow us to prove that drought is the main reason for winter embolism. Furthermore, investigations should give further indications for possible refilling processes expected for late winter.

RESULTS

Micrometeorological Measurements

Winter 2000/2001 was relatively mild with a lowest air temperature (1,700 m) of -15.5°C at February 27 (Table I). The number of days with freeze-thaw cycles was always slightly higher in sun-exposed twigs. In the xylem of these twigs, 95 d, and in shaded twigs, 78 d with freeze-thaw cycles between -1°C and $+1^{\circ}\text{C}$ were registered over the whole winter season 2000/2001 (Table I). The stem showed only 2 d with temperatures exceeding these thresholds (end of April), the soil (5 cm depth) was totally frozen from December until March and remained unfrozen from the end of April on (Fig. 1). No October values are available for season 2000/2001.

In season 2001/2002, extraordinarily low temperatures in early winter (December) and a relatively mild period in February were observed. Lowest air temperature (-20.9°C) was reached at December 14, and mean air temperature in February 2002 was only

-0.3°C (Table II). The stem thawed three times during winter, the soil remained frozen from December to the middle of April (Fig. 2). In the xylem of sun-exposed and shaded twigs, great differences in the number of days with freeze-thaw cycles were observed: At 1,700 m during January, only 14 d with freeze-thaw cycles were counted in the xylem of shaded twigs, but in sun-exposed twigs, 25 d were registered. For the whole winter season, 82 d with freeze-thaw cycles in shaded and 119 d in sun-exposed twigs were observed (Table II). Even greater differences were found at 2,100 m, where 20 d with freeze-thaw cycles in sun-exposed but only 3 d in shaded twigs (Norway spruce) were observed in January. During the winter season, 73 d with freeze-thaw cycles in shaded and 133 d in sun-exposed twigs were registered at the timberline (Table II).

Vulnerability Curves

Stone pine exhibited a significantly less negative vulnerability threshold than Norway spruce (Fig. 3). The 50% vulnerability value (constant *b*; Pammenter and Vander Willigen, 1998) was -3.52 ± 0.03 MPa for stone pine and -4.12 ± 0.02 MPa for Norway spruce trees growing at the alpine timberline. Curve slope of the vulnerability curve (constant *a*; Pammenter and Vander Willigen, 1998) was significantly lower in stone pine (3.05 ± 0.25) than in Norway spruce (6.10 ± 0.80). In both tree species, no significant difference between sun-exposed and shaded twigs was detected.

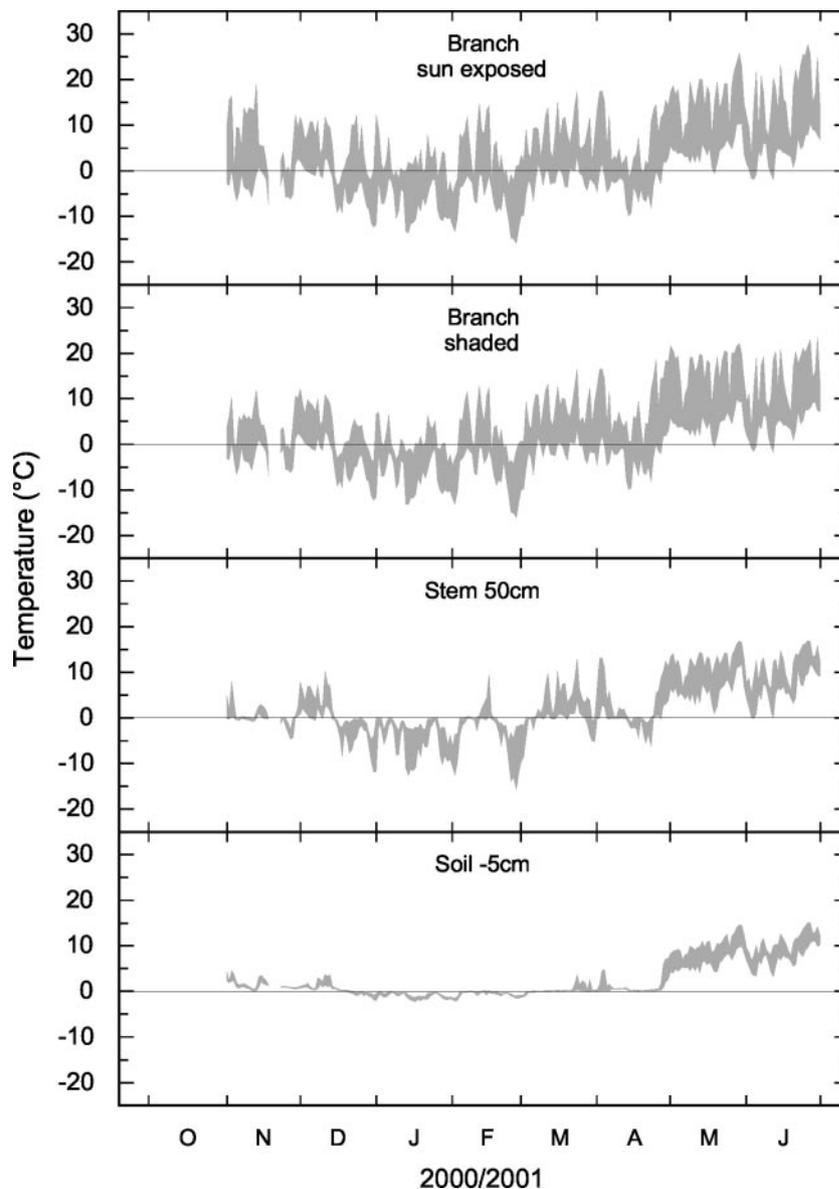
Plotting conductivity losses versus ψ_s from field data (sun-exposed Norway spruce twigs, 2,100 m, season 2001/2002) revealed higher embolism rates at

Table I. Temperatures and days with freeze-thaw cycles 2000/2001

Air and xylem temperatures (sun-exposed and shaded twig) of a stone pine growing at Praxmar, Tyrol (1,700 m) from November 2000 to June 2001. Days with xylem minimum temperature below -1°C and maximum above $+1^{\circ}\text{C}$ are counted as days with freeze-thaw cycles.

Stone Pine at Praxmar, 1,700 m	2000/2001								
	O	N	D	J	F	M	A	M	J
Temperatures $^{\circ}\text{C}$									
Air									
Mean	-0.2	-0.8	-4.2	-3.4	1.0	0.6	9.5	8.6	
Minimum	-7.2	-12.0	-13.0	-15.5	-9.8	-10.0	-0.4	-1.9	
Maximum	11.7	9.6	5.1	10.6	12.9	14.0	22.4	23.3	
Xylem, twig sun									
Mean	0.2	-0.6	-4.1	-3.0	1.5	1.0	9.7	9.1	
Minimum	-7.5	-12.3	-13.6	-15.9	-9.9	-9.9	-0.6	-1.9	
Maximum	19.1	12.1	12.3	14.7	17.4	17.6	25.8	27.8	
Xylem, twig shade									
Mean	-0.1	-0.8	-4.1	-2.9	1.8	1.9	10.2	9.1	
Minimum	-7.2	-12.2	-13.3	-16.0	-10.0	-10.0	-0.6	-1.7	
Maximum	11.9	12.1	7.0	12.9	16.5	18.6	22.1	23.4	
Freeze-thaw d									
Twig sun		12	12	16	13	19	20	0	3
Twig shade		11	10	10	11	16	18	0	2

Figure 1. Seasonal courses of temperatures 2000/2001. Daily maximum and minimum temperatures of air and soil (5 cm depth) and from the xylem of a sun-exposed and shaded twig as well as of the stem (50 cm height) of a stone pine at Praxmar, Tyrol (1,700 m) during winter 2000/2001 (November–June).



comparable water potentials (ψ) (Fig. 3). Differences to the experimentally determined vulnerability curve increased with the number of freeze-thaw events.

Seasonal Course of Embolism and ψ

At 1,700 m in winter 2000/2001 and 2001/2002, conductivity losses did not exceed 10% in both species (Fig. 4). ψ s from November to March were lower in Norway spruce (lowest value: -1.75 MPa at February 19, 2001, and -2.12 MPa at January 23, 2002) than in stone pine (lowest values: -1.21 MPa at December 13, 2000, and -1.15 MPa at January 23, 2002), whereby sun-exposed and shaded twigs always exhibited similar values (Fig. 4).

At 2,100 m, embolism rates in stone pine also were negligible (highest value: 13.1% at January 8, 2002), whereas conductivity losses of up to 49.2% (March 4,

2002) were found in Norway spruce (Fig. 5). Thereby, sun-exposed twigs showed higher conductivity losses than shaded twigs in winter 2000/2001. In winter 2001/2002 only sun-exposed but not shaded twigs of Norway spruce were embolized. In both years, embolism rates of Norway spruce had already decreased during winter and reached about 10% or less in June. ψ s at 2,100 m were lower in Norway spruce (lowest value: -3.53 MPa at January 29, 2001) than in stone pine (lowest value: -2.34 MPa at January 29, 2001). At most sampling dates, ψ s of shaded twigs were similar or slightly less negative than of sun-exposed twigs except at January 8, 2002, when shaded branches of Norway spruce exhibited an about 0.8 MPa lower ψ . ψ s—always taken at about 10 AM—measured in autumn and spring are not taken into consideration because they were influenced by stomatal transpiration.

Table II. Temperatures and days with freeze-thaw cycles 2001/2002

Air and xylem temperatures (sun-exposed and shaded twig) of a stone pine growing at Praxmar, Tyrol (1,700 m) from October 2001 to June 2002. Days with xylem minimum temperature below -1°C and maximum above $+1^{\circ}\text{C}$ are counted as days with freeze-thaw cycles. Days with freeze-thaw cycles were also registered at a Norway spruce tree growing at the timberline (2,100 m).

	2001/2002								
	O	N	D	J	F	M	A	M	J
Temperatures of stone pine at Praxmar, 1,700 m °C									
Air									
Mean	8.3	-1.5	-6.5	-2.8	-0.3	0.9	2.3	8.1	12.7
Minimum	-0.9	-11.6	-20.9	-16.4	-14.2	-10.9	-6.4	-0.6	0.9
Maximum	20.4	11.9	3.5	11.7	-12.6	11.8	13.6	20.4	26.7
Xylem, twig sun									
Mean	9.4	-0.7	-6	-1.9	0.6	2.5	3.7	9.2	13.8
Minimum	-1.6	-12.4	-20.9	-17.1	-14.5	-12.4	-8.3	-4.1	0.7
Maximum	30.6	22.8	13.5	19.8	20.5	25.7	25.8	28.5	33.6
Xylem, twig shade									
Mean	8.5	-1.3	-6.3	-2.7	0.1	1.4	2.8	8.9	13.5
Minimum	-0.9	-12.0	-21.1	-16.6	-14.6	-11.3	-7.1	-0.6	0.7
Maximum	20.9	12.9	5.1	13.1	14.4	13.2	15.6	22.2	28.2
Freeze-thaw d									
Praxmar, 1700 m, stone pine									
Twig sun	2	18	12	25	21	17	19	5	0
Twig shade	1	12	4	14	19	16	16	0	0
Timberline, 2100 m, Norway spruce									
Twig sun	2	20	13	20	23	25	25	4	1
Twig shade	1	10	2	3	18	16	19	4	0

Cuticular Leaf Conductance

Cuticular leaf conductance of both tree species decreased asymptotically with decreasing ψ (Fig. 6). At moderate ψ s (down to -1 MPa), Norway spruce exhibited a significantly higher leaf conductance by about 3.5-fold (37.9 ± 4.1 $\text{mmol m}^{-2} \text{s}^{-1}$) than stone pine (10.2 ± 0.7 $\text{mmol m}^{-2} \text{s}^{-1}$). In the critical range between -2 and -3 MPa (near the upper vulnerability threshold for drought-induced embolism), leaf conductance of Norway spruce (12.6 ± 1.0 $\text{mmol m}^{-2} \text{s}^{-1}$) was even 4.4 times higher than that of stone pine (2.7 ± 0.3 $\text{mmol m}^{-2} \text{s}^{-1}$). Due to the differences in leaf conductance, dehydration of stone pine twigs lasted about three times longer (-5 MPa after up to 3 weeks) than of Norway spruce. Significant differences between sun-exposed and shaded twigs were only observed in Norway spruce (between -2 and -3 MPa: sun-exposed 37.9 ± 4.1 $\text{mmol m}^{-2} \text{s}^{-1}$, shaded 33.7 ± 2.6 $\text{mmol m}^{-2} \text{s}^{-1}$).

Needle Angle

Needle angle (angle between needle and axes) in dehydrating twigs of stone pine was found to decrease significantly with decreasing ψ s (Fig. 7). This leads to a visible closure of flushes (see photos in Fig. 7).

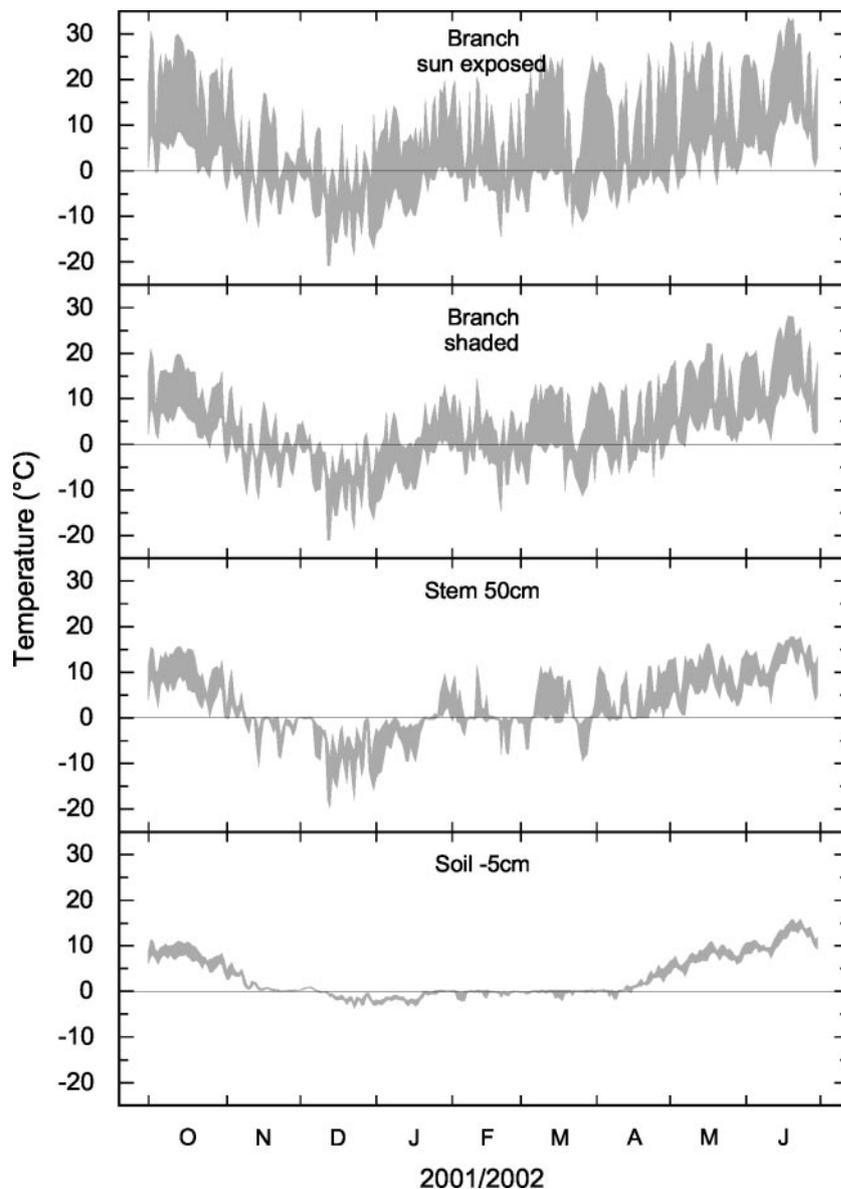
Sap Flow

Although the measurement system was switched on for several times during both winters when stem temperatures exceeded 4°C (see "Materials and methods"), no sap flow was observed during winter months. After thawing of soil and stem in spring, sap flow started within few days at the end of April (Fig. 8).

DISCUSSION

Winter at the alpine timberline causes a special and complex situation for trees of this ecotone. The permanently frozen soil and frozen parts of the stem create a static hydraulic system with blocked influx, whereas intensive radiation (leading to overheating of needles and in combination with low air temperatures to steep vapor pressure gradients) and high wind speeds (Baig and Tranquillini, 1980) lead to water losses continuously impairing plant water status. During this period, no mass transport of water through the system occurs, which has to be considered when correlations of drought and embolism are interpreted. Under these circumstances, conductivity losses always are the consequence and not the cause of low ψ s, because embolism cannot further impair an already blocked water transport system (Mayr et al., 2002). Furthermore (in contrast to stomatally tran-

Figure 2. Seasonal courses of temperatures 2001/2002. Daily maximum and minimum temperatures of air and soil (5 cm depth) and from the xylem of a sun-exposed and shaded twig as well as of the stem (50 cm height) of a stone pine at Praxmar, Tyrol (1,700 m) during winter 2001/2002 (October–June).



spirating plants), ψ measurements directly reflect water status of the tree (or part of the tree) also when not taken before dawn. Within the tree, freezing and thawing of xylem sections as well as embolism (and possibly refilling) create a complex pattern of separated subsystems that may differ in water status during winter. This period is extremely long at the timberline and lasts until soil and stem thaw—then (under the precondition that no embolism remained) reactivation of water transport within few days as demonstrated in Figure 8 is possible.

Winter embolism was reported for several dicotyls in various habitats (e.g. Lo Gullo and Salleo, 1993; Magnani and Borghetti, 1995; Hacke and Sauter, 1996; Lipp and Nilsen, 1997), but there are only few studies about conifers: Sperry et al. (1994) found winter embolism of up to 30% in *Abies lasiocarpa* at an elevation of 2,825 m in Utah (see also Sperry and

Sullivan, 1992) as well as in *Picea glauca* (up to 30%) and *Larix laricina* (up to 20%) in Alaska. Sparks et al. (2001) observed about 25% loss of conductivity in *Pinus contorta* (Idaho). In the present study, we could demonstrate winter embolism in the extreme habitat of Central Alps timberline—according to the hypothesis formulated in the introduction section we thereby found a contrasting situation for the two conifer species studied:

(a) Seasonal courses of both winters prove that embolism is a typical phenomenon of the alpine timberline. No relevant conductivity losses (and less negative ψ s) were found in trees growing at an only 400 m lower elevation (Figs. 4 and 5). This corresponds to data presented by Mayr et al. (2002) where in Norway spruce, a gradient from high embolism rates to zero values within only 200 m down from the timberline was demonstrated.

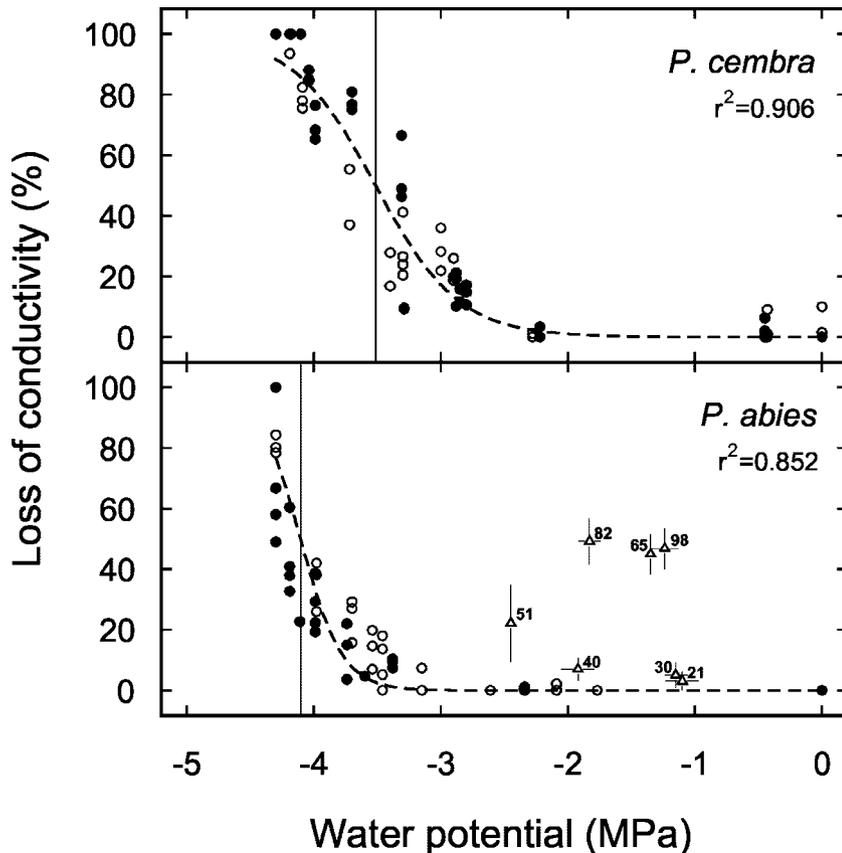


Figure 3. Vulnerability analysis. Drought-induced vulnerability (loss of conductivity versus ψ) of sun-exposed (white symbols) and shaded (black symbols) twigs of stone pine (*P. cembra*) and Norway spruce (*P. abies*) growing at the alpine timberline (2,100 m) near Praxmar, Tyrol. Curves were fitted by the equation described in Pammenter and Vander Willigen (1998). Fifty percent loss of conductivity is indicated by vertical lines. Data are from 63 Norway spruce (34 sun-exposed and 29 shaded twigs) and 72 stone pine twig segments (30 sun-exposed and 40 shaded twigs). Conductivity losses versus ψ s from field measurements (sun-exposed Norway spruce twigs, 2,100 m, November 2001–March 2002) are indicated by triangles (mean \pm SE). The number of days with freeze-thaw events is indicated for each sampling date.

(b) At the timberline (2,100 m), clear differences between Norway spruce and stone pine were observed (Fig. 5): The latter did not exhibit relevant conductivity losses even in the harsh season 2001/2002, whereas in Norway spruce embolism was observed in both winters with highest values in 2001/2002. Because both tree species are exposed to identical climatic and environmental conditions, we have to discuss by which causes Norway spruce is embolized but stone pine is not.

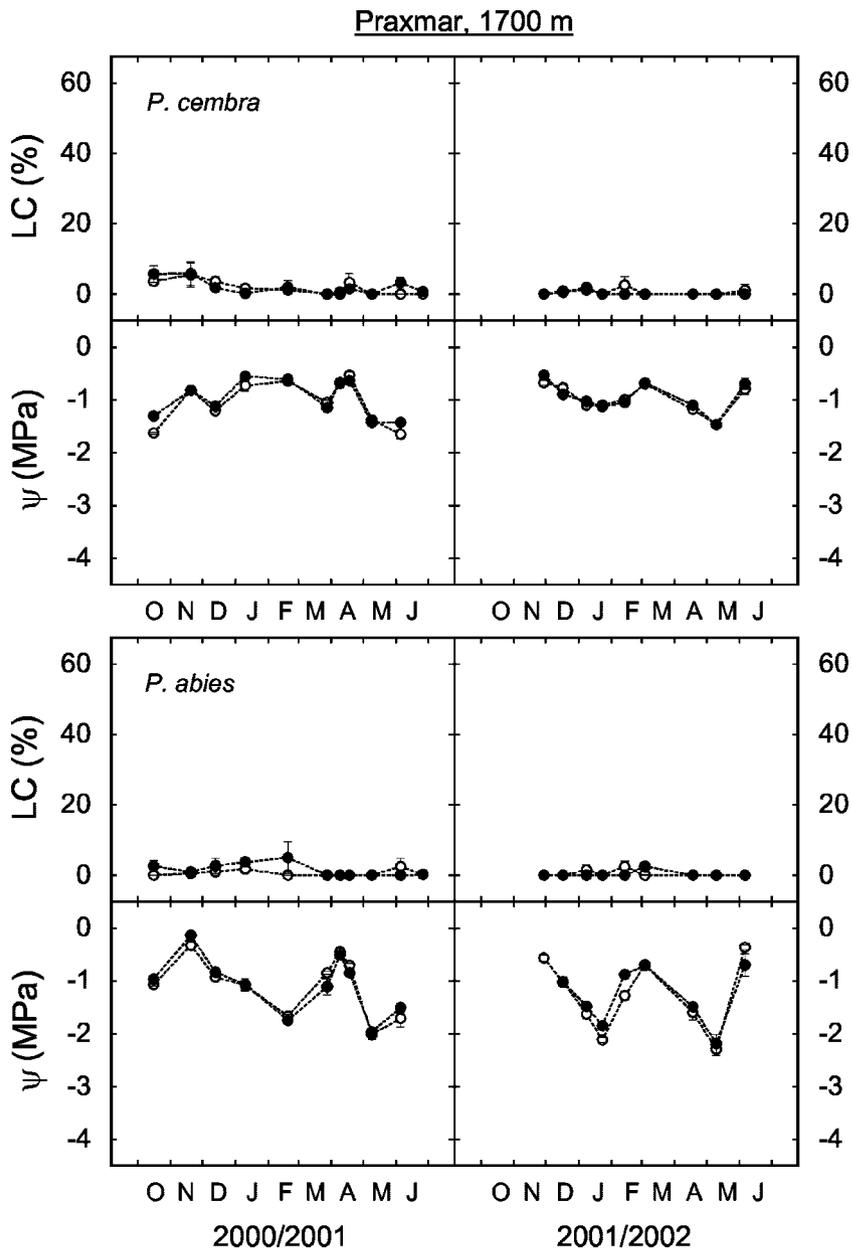
(c) Vulnerability to drought-induced embolism was hypothesized to be one possible reason for different embolism rates of the investigated species. Nevertheless, Norway spruce was found to be even more resistant against drought-induced embolism (Fig. 3)—the upper vulnerability threshold is reached at more negative ψ s than in stone pine. The high resistance of Norway spruce against drought-induced embolism thereby fits to the observed trend of altitudinally decreasing vulnerability thresholds and to anatomical data presented by Mayr et al. (2002). But the adaptation in vulnerability found in Norway spruce obviously failed to prevent embolism.

When vulnerability properties can be excluded as explanation for the observed differences in embolism rates, ψ s reached during winter were expected to differ in the two species. Frost-drought was hypothesized to be the main cause for observed conductivity losses in Norway spruce, and therefore

low ψ s should be associated with embolism during seasonal courses. In fact, when data of the first winter season are analyzed, embolism occurred from the end of January on when ψ s decreased below -3.3 MPa, which is the upper vulnerability threshold of Norway spruce (Fig. 5). But in the second winter, something striking was observed at January 8: Sun-exposed twigs in Norway spruce exhibited ψ s less negative than shaded twigs. We suggest, that this was due to the extreme cold period from December on (Fig. 2), when water could shift from stem to twigs only in sun-exposed and thawed parts of the tree. Shaded twigs remained frozen and insulated from stem water resources even during sunny days. Surprisingly, although in sun-exposed twigs ψ s were about 1.3 MPa above the upper vulnerability threshold, only in these twigs was embolism observed. Shaded twigs exhibited no conductivity losses, although ψ s (-2.7 MPa) were closer to (but still above) the upper vulnerability threshold (vulnerabilities of sun-exposed and shaded twigs were identical, see Fig. 3). This can only be explained taking into consideration another factor inducing embolism: freeze-thaw events.

Table II shows an extraordinary high number of days with freeze-thaw events in sun-exposed twigs especially during December and January 2002 in Praxmar (1,700 m) and also at the timberline (2,100 m). In contrast, shaded twigs often remained frozen

Figure 4. Seasonal courses of embolism and ψ at 1,700 m. Embolism rates (loss of conductivity [LC]) and Ψ in sun-exposed (white symbols) and shaded twigs (black symbols) of stone pine (*P. cembra*) and Norway spruce (*P. abies*) at Praxmar, Tyrol (1,700 m) in winter 2000/2001 and 2001/2002 (October–June). ψ s of five and conductivity measurements of at least three samples of each species and exposition. Mean \pm SE.



during this period leading to less frequent freeze-thaw cycles. Another indication for a role of freeze-thaw events are the observed differences between embolism- ψ properties observed at the timberline and experimentally determined vulnerability: Figure 3 shows accumulating freeze-thaw events in the xylem to cause increasing embolism rates far above rates induced by drought only. Conductivity losses significantly correlate with the number of freeze-thaw events ($r^2 = 0.876$). The increase of embolism compared with drought-induced vulnerability curves is strikingly similar to curve shifts found along an altitudinal transect (Mayr et al., 2002) and in an experimental approach, where dehydrated conifers were exposed to numerous freeze-thaw cycles (S. Mayr, A. Gruber, and H. Bauer, unpublished

data). Therefore, we hypothesize that embolism in studied conifers cannot only be induced by drought but also by a combination of low ψ s and repeated freeze-thaw events. Freeze-thaw events can obviously impair xylem conductivity when ψ is near but still above the drought-induced vulnerability threshold.

Sparks et al. (2001) also reported ψ as well as the number of freeze-thaw events to be correlated to rates of winter embolism in *P. contorta*. Because ψ s did not reach the vulnerability threshold of drought-induced embolism, the authors also hypothesized that development of winter embolism depends on the shoot ψ during thawing of the tissue and the number of freeze-thaw events during winter. A role of repeated freeze-thaw cycles was also suggested by

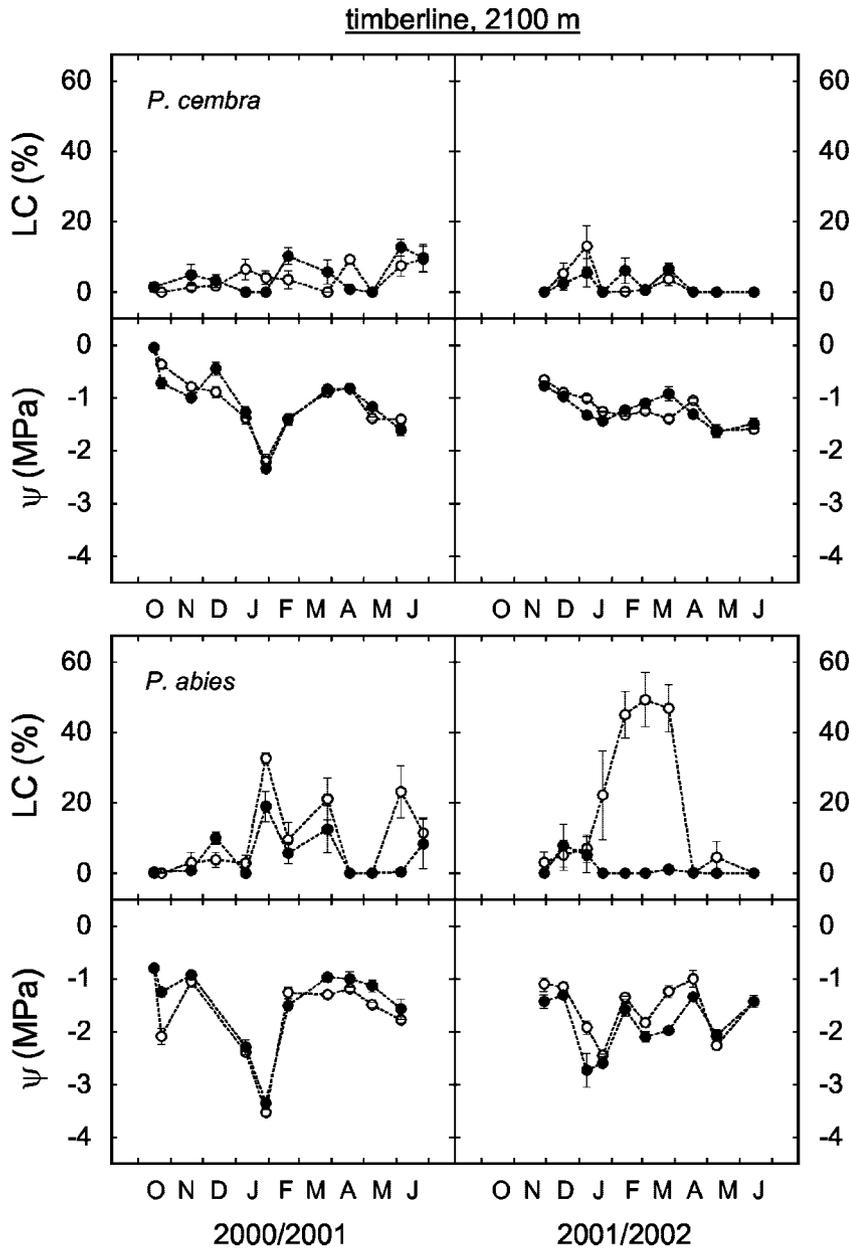


Figure 5. Seasonal courses of embolism and ψ at 2,100 m. Embolism rates (loss of conductivity [LC]) and Ψ in sun-exposed (white symbols) and shaded twigs (black symbols) of stone pine (*P. cembra*) and Norway spruce (*P. abies*) at the timberline (2,100 m) near Praxmar, Tyrol in winter 2000/2001 and 2001/2002 (October–June). ψ s of five and conductivity measurements of at least three samples of each species and exposition. Mean \pm SE.

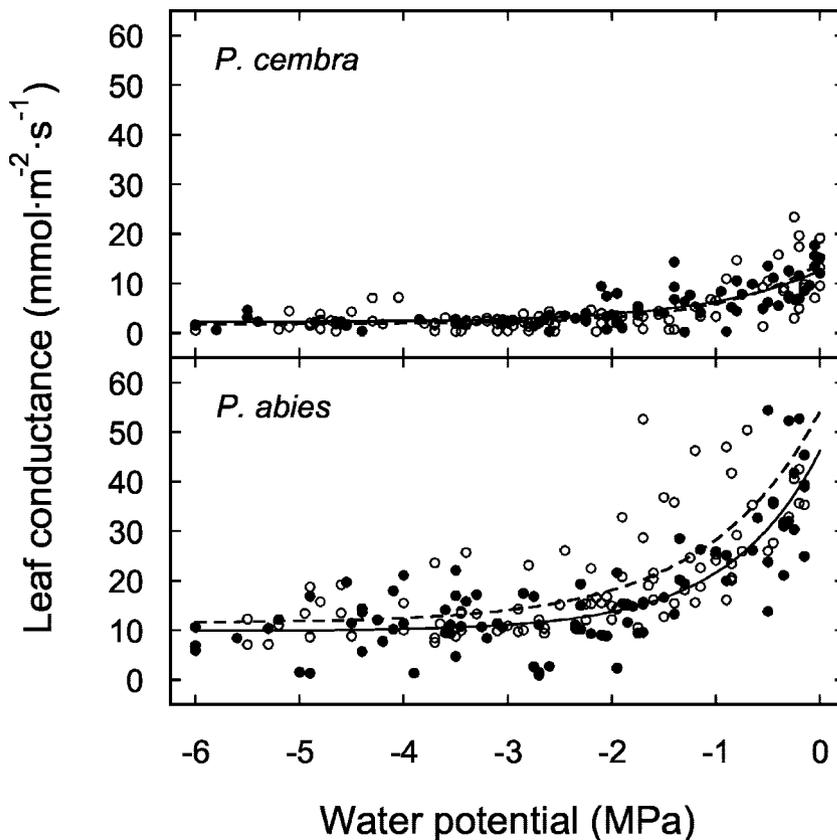
Utsumi et al. (1998) for diffuse-porous trees. In contrast, Sperry et al. (1994) did not find a correlation between the number of freeze-thaw events and loss of conductivity in conifers (*P. glauca*, *L. laricina*, and *A. lasiocarpa*). Sperry and Robson (2001) conclude from inter- and intraspecific comparisons that freeze-thaw-induced cavitation does not occur in small tracheids. Furthermore, in experimental approaches, no indication for freeze-thaw-induced embolism was found either in *P. glauca* (Sperry et al., 1994; one cycle) or in *A. lasiocarpa* or *Juniperus scopulorum* (Sperry and Sullivan, 1992; 11 cycles). Also, Davis et al. (1999) could not detect an increase of conductivity losses after freezing and thawing in *A. lasiocarpa* (one cycle). Nevertheless, because of the low number of temperature cycles, these experiments cannot be

compared with the field situation at the alpine timberline, where more than 100 freeze-thaw events during winter-season may occur (Table I and II).

In any case, the absence of winter embolism in stone pine therefore must be due to relatively high ψ s far above the drought-related vulnerability threshold and even far above the critical range for embolism induced by freeze-thaw cycles. In January 2002, when repeated freeze-thaw events were observed, ψ s were about 1 MPa less negative than in Norway spruce.

But why were ψ s of stone pine always more favorable than those of Norway spruce? Baig et al. (1974) and Tranquillini (1974) have already demonstrated (based on the hypothesis of Michaelis, 1934) the limited cuticular protection of conifers growing at the alpine timberline due to the short vegetation period.

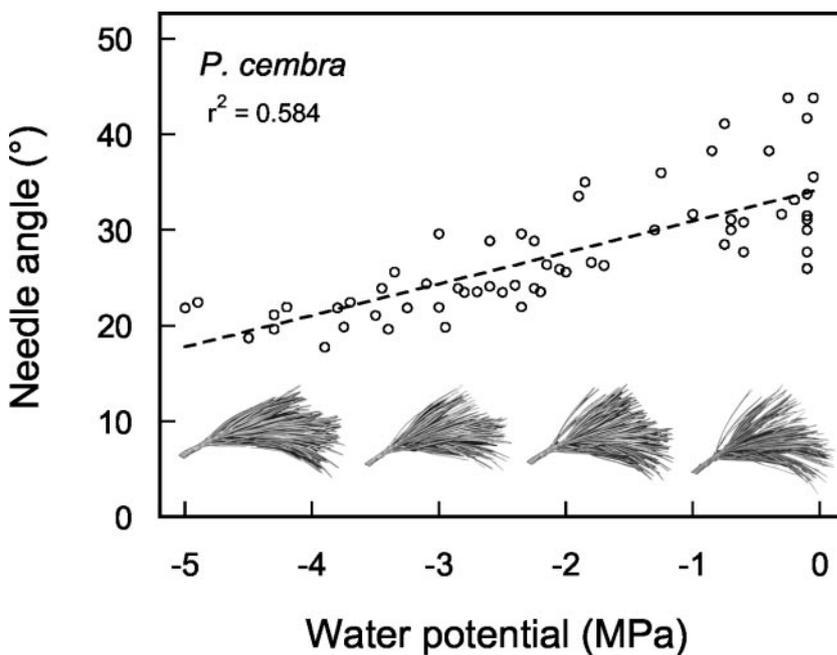
Figure 6. Cuticular leaf conductance. Leaf conductance of current-year shoots of sun-exposed (white symbols) and shaded (black symbols) twigs at different ψ s during dehydration. Twigs were harvested from stone pine (*P. cembra*) and Norway spruce (*P. abies*) growing at the alpine timberline (2,100 m) near Praxmar, Tyrol. Stomatal closure was obtained with abscisic acid solution before measurements. Curves were fit by a monoexponential function. Data of five sun-exposed and five shaded twig samples.



Current-year needles of stone pine thereby were found to exhibit a 2.75- to 4.5-fold lower cuticular transpiration during winter than Norway spruce (Mt. Patscherkofl, Central Austrian Alps; Baig and Tranquillini, 1976). In a recent publication Anfodillo et al. (2002) show a 2.8-fold higher cuticle resistance of

stone pine compared with Norway spruce growing in the Dolomites (NE Italian Alps). Our measurements revealed a similar difference between these species (3.5-fold higher cuticular conductance in Norway spruce), which increased with decreasing ψ s (Fig. 6). Even when ψ s of stone pine were 1.2 MPa

Figure 7. Needle-axes angles. Angle between needles and twig axes versus ψ of dehydrated current year shoots of sun-exposed twigs of stone pine growing at the timberline (2,100 m) near Praxmar, Tyrol. Data of 10 samples. Significant correlation at $P \leq 0.05$.



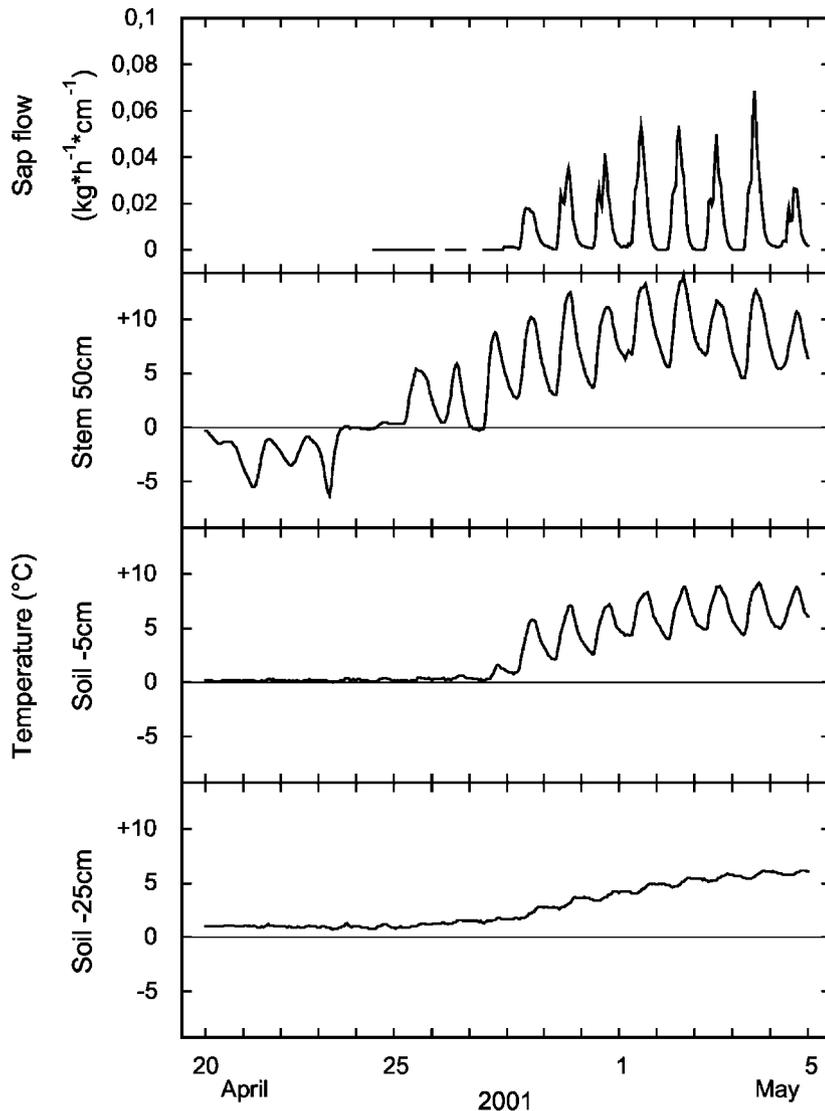


Figure 8. Sap flow in spring. Sap flow (per centimeter stem circumference) and temperatures (soil at 50 and 5 cm depth; stem at 50 cm height) of a stone pine growing at Praxmar, Tyrol (1,700 m) from April 20 to May 5, 2001.

less negative than in Norway spruce (Fig. 5; January 29, 2001), the latter exhibited a more than 3-fold higher cuticular conductance. Leaf conductance of stone pine in winter reported by Wieser (2000) was up to $3 \text{ mmol m}^{-2} \text{ s}^{-1}$ (see also Tranquillini, 1974; Körner, 1994). The higher values of the presented study may be due to an underestimation of the projected leaf area but also reflect stand and exposition differences. Nevertheless, the more effective transpiration protection of stone pine enables (possibly beside other physiological parameters such as hydraulic capacitance) the avoidance of critical ψ_s and therefore of embolism during winter.

We found another mechanism in stone pine probably reducing water losses over needles. This cannot directly be analyzed from transpiration data presented above because measurements were carried out with a fan to prevent boundary layer effects. On the basis of the measurement of the angle between needles and axes, stone pine was found to close its

flushes when dehydrated (Fig. 7). We suppose that this will lead to a further decrease of water losses as the boundary layer is increased. Also during winter, changing needle angles were observed, nevertheless periodic measurements were not possible because of many disturbing factors (e.g. wind and snow). The possibility of flush closure corresponds to the morphology of stone pine (closely packed needles, see Fig. 7) in contrast to Norway spruce, which did not exhibit any changes in needle angles upon decreasing ψ_s (data not shown).

The cuticle protection shield and decrease of needle axes angles enabled stone pine to reduce water losses in winter. Therefore, this species was able to avoid critical ψ_s during the investigated periods and to prevent embolism formation due to drought or to a combination of drought and freeze-thaw events. Despite adaptations in vulnerability Norway spruce is less well protected against embolism so that in both winter seasons conductivity losses were observed in speci-

mens growing at the alpine timberline (see also Mayr et al., 2002). Nevertheless, seasonal courses indicate the existence of refilling mechanisms that enable this species to recover from embolism in late winter. It is especially interesting that this process obviously occurred already in winter when stem and soil are still frozen and ψ s are still negative (although it is not clear whether ψ s are negative at the precise moment of refilling too). Even in February, partial recovery from embolism was observed (Fig. 5, February; see also April 2001 and March/April 2002). Seasonal courses reported by Sperry et al. (1994) and Sperry and Sullivan (1992) also indicated refilling in *L. laricina* and *P. glauca* during spring and in *A. lasiocarpa* during winter. Katz et al. (1989) and Sparks et al. (2001) suggested water uptake by branches or leaves during warm (when snow melts on twigs) or rainy periods. Nevertheless, due to the low temperatures during winter, sufficient metabolic activity of living tissues (ray parenchyma) is hardly probable. Another possibility may be that stem water resources enable water shifts to twigs when ice in the xylem thaws. One of our future goals is to study this process in more detail.

The extent of observed conductivity losses in Norway spruce indicates winter embolism to influence tree life at the alpine timberline. On the other hand, the spectre of avoidance strategies as well as adaptation and recovery mechanisms observed in stone pine as well as in Norway spruce may be essential to withstand these impairments of the water transport system and to explain the higher altitudinal limit of stone pine. The risk of embolism possibly is the reason for the dominance of conifers at the timberline ecotone as suggested for cold habitats by Sperry and Sullivan (1992). As a consequence, winter embolism must be hypothesized to be a relevant factor for tree line formation limiting survival of the plant life form "tree."

MATERIALS AND METHODS

Plant Material and Study Sites

Studies were carried out on sun-exposed and shaded twigs of stone pine (*Pinus cembra*) and Norway spruce (*Picea abies* [L.] Karst.) at Praxmar, Tyrol, Central Alps in winter 2000/2001 and 2001/2002. Investigations were done at 2,100 m and at 1,700 m on twigs of single-standing, 3-m (high elevation) and 7-m (lower elevation) tall trees. Samples for measurements of seasonal courses (October–June, 10–11 sampling dates) were taken from three Norway spruce trees and three stone pines at 2,100 and 1,700 m, respectively.

Water Potential (ψ)

ψ measurements were done on either about 10-cm-long end segments of twigs (Norway spruce) or needles (stone pine, except for measurements of transpiration and needle angle). ψ s were determined with pressure chambers (SKPM1400, Skye Instruments Ltd., Llandrindod Wells, UK, and model 1000 Pressure Chamber, PMS Instrument Company, Corvallis, OR). Samples for seasonal courses were harvested at about 10 AM, immediately sealed in a plastic bag, and transported to the laboratory. It was not necessary to take predawn ψ s, because during winter months (November–April) water supply is permanently blocked (frozen soil) so that ψ s directly corresponded to plant water status (see "Discussion").

Measurements of Xylem Conductivity

For conductivity measurements, twig segments (length 15 cm, diameter <1.0 cm) were harvested in the field (seasonal courses) or prepared from dehydrating twigs (vulnerability analysis). Field samples were cut under water and transported to the laboratory in a water-filled bottle. We used a modified Sperry apparatus (Sperry et al., 1988; Chiu and Ewers, 1993; Vogt, 2001) described by Mayr et al. (2002). Thereby, embolism rates are quantified by determination of the increase in hydraulic conductivity after removal of enclosed air by repeated high pressure flushing. We prepared xylem segments of about 2.5 cm length with diameters between 5 and 8 mm immersed in distilled water by removing the bark before sealing in silicone tubes for measurement of hydraulic conductivity. Measurement pressure was set to 4 kPa (controlled via a 0.4-m glass capillary). Flow rate was measured with a PC-connected balance (Sartorius BP61S, 0.0001g precision, Sartorius AG, Göttingen, Germany) by weight registration every 10 s; flow rate was calculated by linear regression over 200 s. Flushing (0.13 MPa, 20 min) and conductivity measurements were carried out with distilled, filtered (0.22 μ m), and degassed water containing 0.005% (v/v) Micropur (Katadyn Products Inc., Wallisellen, Switzerland) to prevent microbial growth (Sperry et al., 1988). Flushing was repeated until measurements showed no further increase in conductivity. Percent loss of conductivity was calculated from the ratio of initial to maximal conductivity.

Vulnerability Analysis

Vulnerability curves were determined on up to 1.5-m-long twigs of stone pine and Norway spruce. These branches were detached at 2,100 m in December 2001 (before winter embolism occurred) when xylem was frozen, transported to the laboratory in a plastic bag, recut under water while still frozen, and hydrated for 24 h. During different periods of dehydration (up to 10 d), ψ and corresponding embolism rates were measured.

Vulnerability curves were obtained by plotting percent loss of hydraulic conductivity versus ψ . Curves were fitted by the exponential sigmoidal equation (Eq. 1) given in Pammenter and Vander Willigen (1998):

$$PLC = 100 / \{1 + \exp[a(\Psi - b)]\} \quad (1)$$

where PLC is the percent loss of conductivity, Ψ (megapascal) is the corresponding water potential, and a and b are constants.

Similar to experimental vulnerability analysis, conductivity losses versus ψ s were also plotted from field measurements. This was done for sun-exposed Norway spruce twigs growing at 2,100 m (highest embolism rates) for season 2001/2002 (number of days with freeze-thaw events in the xylem available). Only measurements from November to March were used for this analysis because the onset of refilling processes in April would mask effects of freeze-thaw events.

Cuticular Leaf Conductance

Cuticular leaf conductance was measured in January 2002 on end segments of current-year shoots of trees growing at 2,100 m. Twigs were harvested, transported to the laboratory in a plastic bag, and recut under water. To ensure complete stomatal closure, end segments of twigs were placed in a 100 mM abscisic acid solution (2-cis, 4-trans-abscisic acid; Sigma-Aldrich GmbH, Vienna) at daylight for 6 h and subsequently fully hydrated in a plastic bag over night. Weight of saturated twigs was determined (Sartorius BP61S, 0.0001g precision, Sartorius AG, Göttingen, Germany). During the following dehydration (in a darkened room, twigs were exposed on a fine net and ventilated with a fan), fresh weight and corresponding ψ s as well as air humidity, temperature, and atmospheric pressure were measured. After reaching about -5 MPa for each sample dry weight to projected leaf area (measured with a digital video camera, Leaf Area and Analysis System SL 721, Skye Instruments Ltd., Llandrindod Wells, UK) were determined for a representative amount of needles as well as dry weight of all needles of the sample to calculate leaf area. Evapotranspiration (EV [$\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$]) was calculated according to Equation 2

$$EV = (\Delta W) / (\Delta t \cdot LA \cdot 18.015) \quad (2)$$

where ΔW (grams) is the loss in weight during the measurement interval Δt (seconds) and LA (square meters) is the leaf area. The molecular mass of

water is required for the conversion to moles. Leaf conductance, g_L (moles per square meter per second), was calculated as given in Equation 3

$$g_L = EV / [(SVP - VP) / P] \quad (3)$$

where SVP (pascals) is the saturated vapor pressure, VP the actual vapor pressure (pascals), and P (pascals) the atmospheric pressure.

Needle Angle

Needle angles (angle between needles and axes) were measured on saturated (hydrated for 24 h) end segments of sun-exposed stone pine twigs harvested at 2,100 m in January 2002 (transported to the laboratory in a plastic bag and recut under water). During dehydration, ψ and corresponding needle angles were determined. The angle between needles and twig axes was calculated from the maximal diameter of the flush and the needle length.

Micrometeorological Data

At 1,700 m, air temperature and temperatures of xylem of a stone pine (stem at 50 cm height, sun-exposed twig, and shaded twig) and soil (5 and 25 cm depth) were measured every 6 min during both years with thermocouples. Mean values (1-h intervals) were stored with a data logger (Squirrel 1250, Grant Instruments, Cambridge, UK). In addition, xylem temperature data (sun-exposed and shaded twig) of a Norway spruce tree growing at 2,100 m were registered during winter season 2001/2002 (datalogger Grant SQ 1045, Grant Instruments). Sensors were inserted about 2 cm into the xylem of the stem (diameter, 20 cm at 1,700 m and 10 cm at 2,100 m) and about 0.8 cm into twigs (diameter, 1.5–2 cm).

Sap Flow Measurements

Sap flow was measured on a stone pine growing at 1,700 m with a sap flow measurement system (Sap Flow Meter PT 4.1, Environmental Measuring Systems EMS, Brno, Czech Republic). The stem was insulated with a set of 10-cm broad foam stripes connected to each other. None of the numerous side branches had to be cut with this insulation system (Environmental Measuring Systems EMS). Temperature values due to sap flow velocities were registered in 15-min intervals on two opposite points of the stem (diameter 20 cm) at breast height. The system was switched off when xylem temperature of the stem dropped below 4°C to avoid thawing and drying out of branches when heated during cold periods.

Statistics

All values are given as mean \pm SE. Differences were tested with Student's t test at 5% probability level after checking for normal distribution and variance of the data. Correlation analyses was carried out via Pearson's linear correlation coefficient r at 5% probability level.

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