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Drought and frost resistance of trees: a comparison of four species at different sites and altitudes

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Abstract

• **Context** Drought and frost resistances are key factors for the survival and distribution of tree species.
• **Aims** In this study, the vulnerability to drought-induced embolism and frost resistance of four species were analysed, whereby different sites and altitudes were compared and seasonal variation was considered.
• **Methods** *Fagus sylvatica* L., *Sorbus aucuparia* L., *Picea abies* L. Karst and *Larix decidua* Mill samples were harvested at high and low altitude sites in France and Austria, respectively, and sampling occurred in winter and summer. Pressure at 50% loss of conductivity (P_{50}),

specific hydraulic conductivity (k_s) and temperature lethal for 50% of cells (LT_{50}) were determined, and soluble carbohydrate and starch content were quantified.

• **Results** No site-, altitude- or season-specific trend in P_{50} was observed, except for *S. aucuparia*, which showed P_{50} to decrease with altitude. Within regions, k_s tended to decrease with altitudes. LT_{50} was between -48.4°C (winter) and -9.4°C (summer) and more negative in Tyrolean trees. Starch content was overall lower and carbohydrate content higher in winter than in summer, no site-specific or altitudinal trend was observed.

• **Conclusion** Studied species obviously differed in their strategies to withstand to frost and drought, so that site-related, altitudinal and seasonal patterns varied.

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Katline Charra-Vaskou and Guillaume Charrier have contributed equally to this work.

Contribution of the co-authors Katline Charra-Vaskou and Guillaume Charrier have contributed equally and largely to this publication from experiments to correction of the manuscript. Rémi Wortemann, Barbara Beikircher and Hervé Cochard participated in cavitron measurements as well as manuscript preparation. Thierry Améglio was the projectleader of the French part of the Amadee cooperation. Stefan Mayr supervised the Austrian part of the project and prepared important parts of the manuscript.

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1 Introduction

Plant survival and distribution are often determined by frost and drought stress tolerance (Sakai and Larcher 1987; Mayr et al. 2006). Drought and frost stress vary widely with latitude, longitude and altitude as well as with season so that plant life depends on adaptation and acclimation to different environmental conditions. For example, at low altitude, drought often is a summer phenomenon, when precipitation is insufficient and evaporative forces are high. At the timberline, conditions are rather humid during summer, whereas plants are subjected to drought during winter. Water uptake is then blocked by the frozen soil and stem while overheating of the crown and low air humidity increase evaporation and thus cause “frost drought” (Larcher 1972; Tranquillini 1980).

Drought condition can affect the water transport system of plants by formation of embolism (Sperry and Tyree 1990): conduits are obstructed by gas bubbles, which decrease the hydraulic conductivity (Sperry et al. 1998). Drought-induced embolism occurs when the water potential (P) in conduits falls below xylem-specific thresholds, at which air enters from adjacent, already air-filled spaces (“air seeding” theory; Tyree and Zimmermann 2002). These thresholds are determined by the structure of pit membranes in the xylem wall because embolism formation is caused by entry of an air bubble through these membranes. In conifers, cavitation resistance depends on the geometry of the valve-like pits (Sperry and Tyree 1990; Domec et al. 2006; Cochard et al. 2009; Delzon et al. 2010), in angiosperms, on the size of the largest pores in the pit membranes. Vulnerability to drought-induced cavitation differs largely between tree species (Cochard 2006), and it is thought to correlate well with a species’ overall drought resistance. Although Maherali and DeLucia (2000) did not find cavitation resistance variations of ponderosa pine growing in contrasting climates, some studies demonstrated acclimation in drought resistance. At an intra-specific level, Beikircher and Mayr (2009) showed that cavitation vulnerability varied with humidity conditions during growth, and Mayr et al. (2002) found cavitation vulnerability to vary with altitude.

Frost is also a major limiting factor for tree life (George et al. 1974; Sakai and Larcher 1987), particularly at the altitudinal and latitudinal range limits (Gusta et al. 1983). For living cells, damage and subsequent death may occur when intracellular water freezes or when cells dehydrate due to extracellular freezing (Sakai and Larcher 1987; Améglio et al. 2001). Plants from temperate regions show changes in their resistance to freezing temperature in the circle of the year (acclimation; Bower and Aitken 2006), although cold hardening is under genetic control (Xin and Browse 2000). Environmental cues lead to physiological and biochemical changes in the plant, like starch to soluble sugars inter-conversion, inducing greater tolerance (Sakai 1966; Sakai and Larcher 1987; Morin et al. 2007; Poirier et al. 2010). Furthermore, a decrease in water content is involved in cold hardening to decrease the freezing temperature of cytosol (Chen and Li 1976; Gusta et al. 2004; Charrier and Améglio 2011). Species show large variations in frost resistance. Bower and Aitken (2006) found that at an intra-specific level, frost resistance is a well-acclimated trait with variations between summer and winter as well as a plastic trait with variation between regions.

Despite the wide spread importance of drought and frost stress for tree life, only a few datasets on the intra-specific variation of frost and drought resistance of axes tissues are available (Améglio et al. 2001; Mayr et al. 2002; Bower and

Aitken 2006; Morin et al. 2007; Beikircher and Mayr 2009). Although frost and drought resistances are strongly correlated (Medeiros and Pockman 2011), simultaneous information on acclimation and plasticity of these two traits is lacking. In this study, we analysed key resistance parameters of four species, including two angiosperms, one evergreen and one deciduous conifer growing at two different sites and at different altitudes. Measurements of pressure at 50% loss of conductivity (P_{50}), specific hydraulic conductivity (k_s) and lethal temperature for 50% cells (LT_{50}) as well as starch and soluble carbohydrate content were made in summer and winter. Variations of these traits were compared between France and Austria and high vs. low altitude to estimate the species’ plasticity, and summer vs. winter to estimate their acclimation potential. We expected differences in resistance variability between species due to differences in life forms and strategies (e.g. evergreen versus deciduous), but overall higher frost and drought resistance at higher altitudes and at alpine sites. Frost resistance should be higher in winter, and xylem vulnerability might be influenced by the production of new wood in summer.

2 Materials and methods

Studies were carried out on two deciduous angiosperms (*Fagus sylvatica* L. and *Sorbus aucuparia* L.), one evergreen conifer (*Picea abies* L. Karst) and one deciduous conifer (*Larix decidua* Mill.). Trees analysed in this study were growing in Auvergne (France) and Tyrol (Austria). In each region, trees of two altitudes were used. Low-altitude sites were located at 725 (Royat, Auvergne), 850 (Natters, Tyrol) and 575 m (Innsbruck, Tyrol) while high-altitude sites were at 1,300 (Guéry, Auvergne), 1,440 (Hinterhornalm, Tyrol) and 1,850 m (Birgitz Köpfl, Tyrol). See Table 1 for information on study sites and climate. Climate data were from nearby weather stations (Central Institute for Meteorology and Geodynamics ZAMG). Note that the high-altitude sites were about 200 m below the timberline so that also in winter no natural embolism had to be expected (Mayr et al. 2002).

At sampling days, south exposed twigs were harvested, immediately enclosed in dark plastic bags and transported to the laboratory. For the analysis of embolism resistance, summer samples were harvested from April to July 2009 as well as in September 2006 and 2009 whereas winter samples were harvested in March and April 2009 and from February to April 2010. April was within the winter season at the timberline but start of the summer season at low altitude. For the analysis of frost resistance, summer samples were harvested in June 2009 and winter samples in February 2010, except winter samples at the Tyrol timberline which were harvested in March 2010.

Table 1 Study sites and climate

Site	Location	Altitude (m)	Minimum temperature (°C)	Maximum temperature (°C)	Mean annual temperature (°C)	Mean annual precipitation (mm)
France						
Low altitude						
Royat	45°46' N, 3°05' E	725	−13.0	31.1	8.6	777.6
Timberline						
Guéry	45°36' N, 2°49' E	1,300	−11.8	29.2	7.8	1,071.4
Austria						
Low altitude						
Innsbruck	47°16' N, 11°22' E	575	−13.9	33.7	10.3	911.3
Natters	47°13' N, 11°22' E	850				
Timberline						
Hinterhornalm	47°20' N, 11°33' E	1,440	−20.4	19.1	1.0	822.0
Birgitz Köpfl	47°11' N, 11°19' E	1,850				

Mean of annual minimum, average and maximum temperatures as well as mean annual precipitation from 1999 to 2009. Climate data from Central Institute for Meteorology and Geodynamics (ZAMG, Regionalstelle für Tirol und Vorarlberg)

2.1 Vulnerability to drought induced embolism and specific hydraulic conductivity

At the laboratory, branches were re-cut under water several times, their stems put in water-filled plastic bottles and the branches covered with a plastic bag for complete rehydration over night. Vulnerability curves were analysed on three to five samples for each species with the cavitron technique (Cochard et al. 2005).

This technique is based on the centrifugal force to increase water tension in a xylem segment while simultaneously loss of conductance is measured. For measurements, stem segments were fixed in a custom-built rotor with the sample ends positioned in upstream and downstream reservoirs, which were filled with distilled, filtered (0.22 µm) and degassed water containing CaCl₂ (1 mmol) and KCl (10 mmol). The rotational speed was set to the target pressure and maintained constant for one minute. Then, the moving water meniscus was observed using a high resolution camera (Motic MC 2000, Motic China group Co., Ltd.) to measure the flow rate and calculate the hydraulic conductance. For cavitron measurements of conifers, the “conifer method” (Beikircher et al. 2010) was used to avoid pit aspiration.

Samples lengths were 145 (150 mm rotor) or 275 mm (280 mm rotor). While conifer tracheids are only few millimetres in length, maximum vessel length of *F. sylvatica* and *S. aucuparia* were about 330 and 220 mm (data not shown). To minimise the influence of vessels cut open at both ends, samples were flushed with air before conductance measurements according to Cochard et al. (2010).

Vulnerability curves were obtained by plotting the fractional loss of conductivity (%) versus the xylem pressure (Pa).

Curves were fitted using an exponential sigmoidal equation given in Parammenter and Vander Willigen (1998):

$$PLC = 100 / (1 + \exp(a(P - P_{50}))) \quad (1)$$

where PLC is the percent loss of conductivity, P is the corresponding xylem pressure (Pa) and a is related to the slope of the curve. P_{50} is the P value corresponding to 50% loss of conductivity. PLC was calculated from the ratio of actual (after inducing a given P) to the maximum (i.e. first measurement at −0.25 MPa) hydraulic conductance. Specific hydraulic conductivity values at −0.25 MPa were similar in summer and winter indicating that samples had no native embolism. Furthermore, re-hydration over night should lead to re-filling of embolised sections. However, it is not possible to completely rule out native embolism by use of the centrifuge method so that (small) effects on P_{50} may be possible.

Specific hydraulic conductivity (k_s , m² s^{−1} MPa^{−1}) was computed from cavitron flow measurements (at moderate P) related to sample length and axes cross-sectional area.

2.2 Vulnerability to frost for living cells

Species cold hardiness from each site was analysed by the electrolyte leakage test (Zhang and Willison 1987), which determines frost damage to the plasma membrane by measuring electrolyte leakage from the symplast to the apoplast.

The main axis of twigs was cut into six segments (length 5 cm), washed in distilled and deionised water, placed in a moistened tissue and wrapped in aluminum foil. Shoot segments were then cooled to sub-zero temperatures in

temperature-controlled boxes. Inside, temperatures were recorded with a data logger (Campbell, Logan, USA) as one-minute means and averaged at five minute intervals. Sample temperatures were monitored using copper-constantan thermocouples inserted into the foil pouch. For temperature-controlled boxes, the cooling and warming phase was computer-controlled by a circulator bath (Ministat Huber, Germany) connected to a Pt 100 thermocouple situated in the chamber. Cooling and thawing rates were 5 K h^{-1} . Cooling cycles started at $+5^{\circ}\text{C}$ and minimum temperatures were -5°C , -10°C , -20°C or -30°C . Before thawing, the temperature in cooling boxes was maintained during 1 h to the freeze temperature. In addition, there was an unfrozen control in a cold room at $+5^{\circ}\text{C}$ (control) and a lethal control at -80°C . For control and lethal control, samples were cooled at the rate of ca. 7 K h^{-1} in pre-chilled vacuum flasks.

After temperature treatment, segments were cut into 5-mm-discs and placed in glass vials with 15 ml of distilled and deionised water. The vials were shaken for 24 h at $+5^{\circ}\text{C}$ on a horizontal gravity shaker (ST5, CAT, Germany). The conductivity of the water, in which the stem segments were immersed, was then measured (C1) at room temperature with a conductimeter (Held Meter LF340, TeterCon[®] 325, Germany).

Afterwards, samples were autoclaved at $+120^{\circ}\text{C}$ for 30 min, cooled to room temperature, and a second conductivity measurement (C2) was done. Relative electrolytic leakage was calculated as $(C1/C2)*100$ according to Zhang and Willison (1987). Frost hardness (LT_{50}) was estimated as the temperature where we observed the inflection point (C) of the logistic sigmoid function (Repo and Lappi 1989):

$$y = \left[A / \left(1 + e^{B(C-x)} \right) \right] + D \quad (2)$$

where y is the relative electrolyte leakage, x is the exposure temperature, parameters A and D define the asymptotes of the function and B is the slope at the inflection point C. The parameter estimation of Eq. 2 was performed by nonlinear regression using ExcelStat ver. 7.5.2. Mean LT_{50} was calculated from the individual LT_{50} values.

2.3 Quantification of soluble carbohydrates

For each site and species, five replicates were used for the quantification of soluble carbohydrates. Lyophilized samples ($m > 2\text{ g}$) were ground into powder. Fifty milligrammes were shaken in 1 ml of mannitol (5 g l^{-1}), diluted in ethanol 80% for 30 min at 80°C and then centrifuged for 10 min at $15,775\text{ g}$ (SR2000, Prolabo, France). The supernatant was filtered in a cartridge containing AGX-1 anion-exchange resin (150 μl), polyvinylpyrrolidone (100 μl) and activated carbon (200 μl). The solid was melted three more times with 80% (1 ml), 50% (0.5 ml) and 80% ethanol

(0.5 ml), before the cartridge was rinsed with 1-ml 80% ethanol. The liquid fraction was SpeedVac-dried for carbohydrates analysis and the solid was SpeedVac-dried for starch analysis. For carbohydrate analysis, dried samples were made soluble in 0.5 ml of water and separated on an Aminex-HPX87C column with a refractometer (R12000, Sopares).

To measure starch content, solid was melted with NaOH 0.02 N and autoclaved (2 h, 120°C , 1 bar). Samples were then incubated with amyloglucosidase (1 h 30', 52°C) in a microplate well, where each well contained 12- μl ATP ($5 \cdot 10^{-4}\text{ mol l}^{-1}$), 12- μl NADP ($1.4 \cdot 10^{-4}\text{ mol l}^{-1}$), 60- μl triethanolamine buffer (triethanolamine, 0.48 mol l^{-1} ; magnesium sulfate, $1 \cdot 10^{-2}\text{ mol l}^{-1}$; pH=7.6), 96 μl of water and 12 μl of sample supernatant. A spectrophotometric measuring was made at 340 nm (Power Wave 200, BioTek instruments) before (blank) and after incubation with 10 μl of hexokinase/glucose-6-phosphate dehydrogenase (EC 1.1.1.49) for 40 min under shaking.

3 Results

3.1 Vulnerability to drought induced embolism and specific hydraulic conductivity

The pressure at 50% loss of conductivity (P_{50}) was about -3 MPa in *L. decidua* and *P. abies* and slightly higher in *F. sylvatica* (Fig. 1). Only *S. aucuparia* was clearly more resistant (P_{50} between -3.4 and -5.12 MPa) than the other species. No site-specific trend in P_{50} was observed, and, an altitudinal trend was lacking too, except in *S. aucuparia*. In this species, P_{50} decreased with altitude (except for summer high altitude values in Tyrol). In most samples, no significant differences were observed between summer and winter.

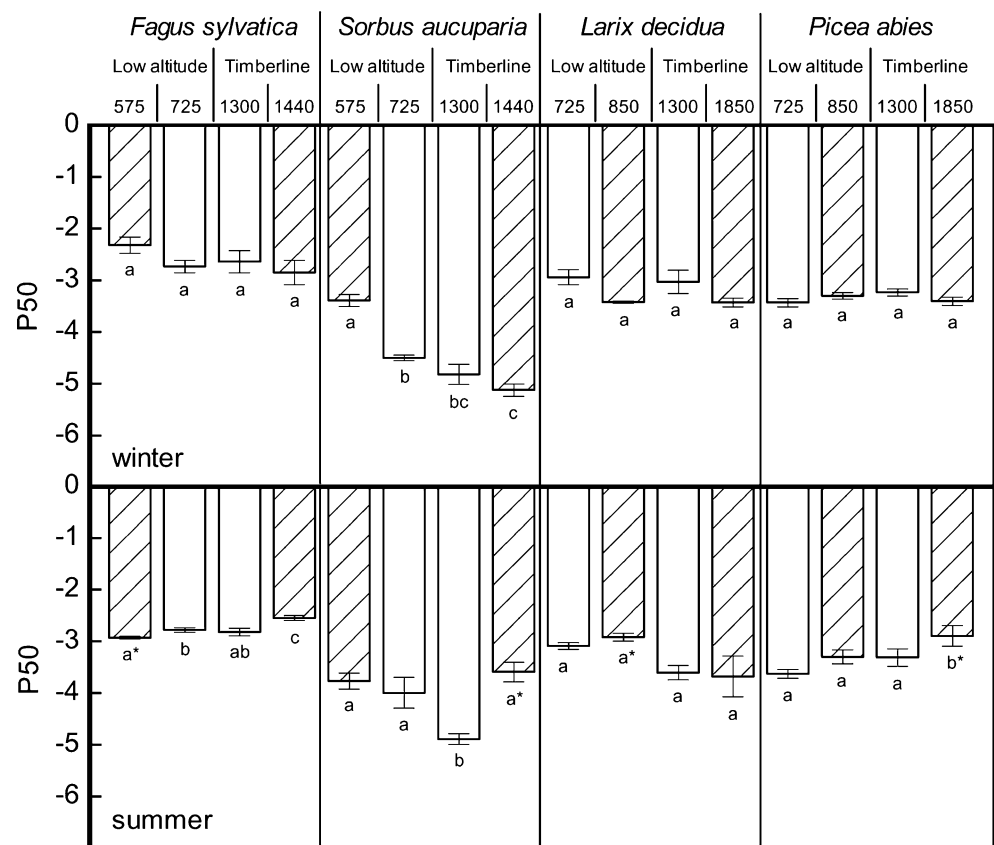
The hydraulic conductivities in *F. sylvatica* (k_s between $2.60 \cdot 10^{-4}$ and $5.76 \cdot 10^{-4}\text{ m}^2\text{ s}^{-1}\text{ MPa}^{-1}$) were higher than in the other species (Fig. 2). In both regions, k_s tended to decrease with altitude in all species (except *F. sylvatica* in Tyrol).

3.2 Vulnerability of living cells to frost and soluble carbohydrate content

Austrian trees were over all more frost resistant (temperature lethal for 50% of cells between -36.7°C and -48.4°C) than French ones (LT_{50} between -28.7°C and -38.5°C). Frost resistance was significantly higher in winter (LT_{50} between -28.7°C and -48.4°C) than in summer (LT_{50} between -9.4°C and -19.7°C) for each site and species. No consistent altitudinal trend was observed (Fig. 3).

Soluble carbohydrate contents in nearly all species were higher in winter (17.2 to 68.9 mg g^{-1}) compared to summer while starch contents were higher in summer (1.8 to 69.93 mg g^{-1}) (Fig. 4).

Fig. 1 Pressure at 50% loss of conductivity (mean $P_{50} \pm SE$) in *F. sylvatica*, *S. aucuparia*, *L. decidua* and *P. abies* at four sites (described in Table 1) in winter and in summer. Hatched bars show Tyrolean sites; open bars, Auvergne sites. A part of P_{50} values of *P. abies* at low altitude (summer) are from Beikircher et al. (2010). Different letters indicate significant differences ($P < 0.05$) between different sites per species, respectively. Stars indicate significant differences between winter and summer



Trees growing at Austrian sites had overall lower total carbohydrate content than French ones (summer) while a general altitudinal trend was lacking.

4 Discussion

4.1 Drought resistance

Studied species represent a broad variety of different eco-physiological strategies including angiosperms as well as

conifers and deciduous as well as evergreen trees. Accordingly, consistent trends were found to be rare in our dataset. The situation is especially complicated by the fact that altitudinal effects on climate conditions (Table 1) differ substantially between winter and summer, which has major effects on drought and frost stress intensities.

The pressure at 50% loss of conductivity (P_{50}) is an important index for a species' drought resistance and therefore used in numerous studies on inter-specific (e.g. Sperry and Tyree 1990; Sperry and Sullivan 1992; Cochard 2006; Mayr et al. 2006), as well as on intra-specific drought

Fig. 2 Specific hydraulic conductivity (mean $k_s \pm SE$) in *F. sylvatica*, *S. aucuparia*, *L. decidua* and *P. abies* at four sites (described in Table 1) in summer. Hatched bars show Tyrolean sites; open bars, Auvergne sites. Different letters indicate significant differences ($P < 0.05$) between different sites per species, respectively

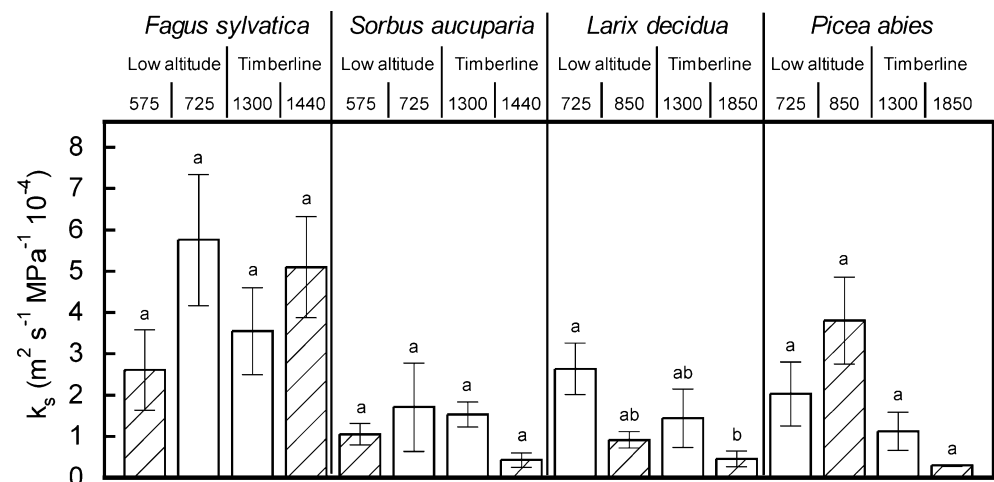
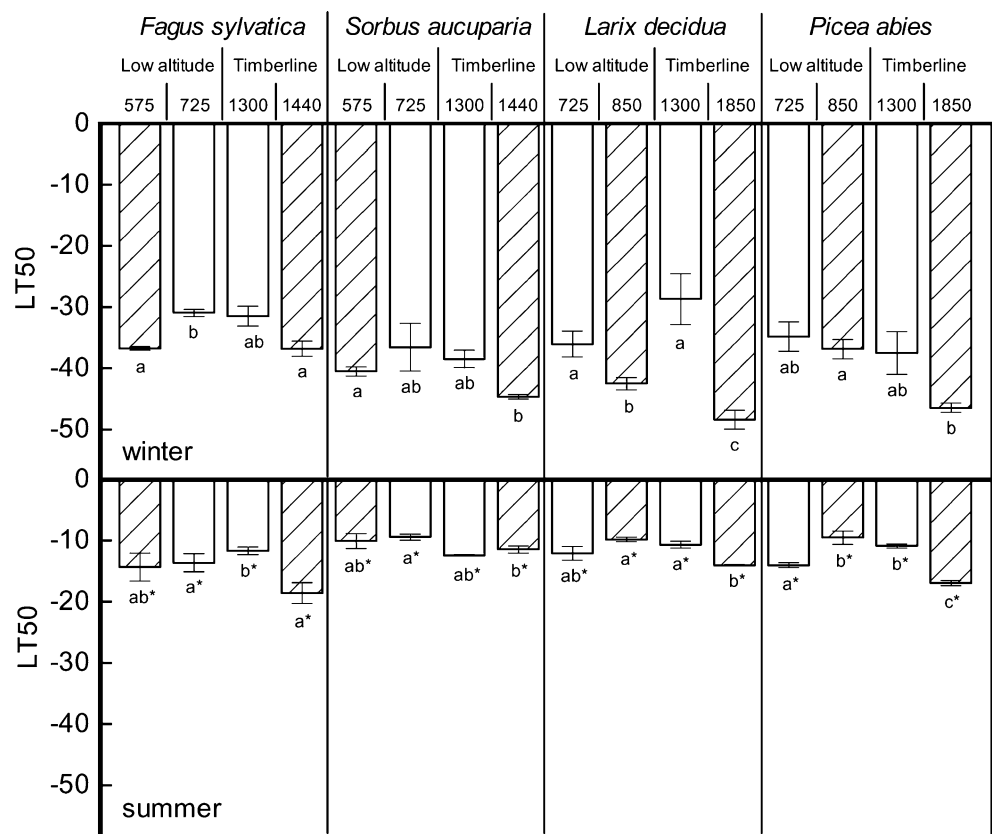


Fig. 3 Temperature at 50% cell mortality (mean $LT_{50} \pm SE$) in *F. sylvatica*, *S. aucuparia*, *L. decidua* and *P. abies* at four sites (described in Table 1) in winter and in summer. Hatched bars show Tyrolean sites; open bars, Auvergne sites. Different letters indicate significant differences ($P < 0.05$) between different sites per species, respectively. Stars indicate significant differences between winter and summer



resistance (Maherali and DeLucia 2000; Beikircher and Mayr 2009).

A clear altitudinal trend in P_{50} of *P. abies* was reported by Mayr et al. (2002), but the situation is complex (Mayr et al. 2006). In summer, trees growing at higher altitudes are normally not subjected to drought stress because of higher precipitations and lower temperatures. In contrast, trees at the timberline are subjected to frost-drought during winter (Larcher 1972; Tranquillini 1980). It thus is not surprising that no general trend in P_{50} was observed (Fig. 1). It also has to be considered that in both seasons, harvesting dates were spread over some months and that other site-specific differences may have masked altitudinal effects (Körner 2007). In the case of *P. abies*, a closed forest stand was used at low altitude, while open stands were analysed in Mayr et al. (2002). Only *S. aucuparia* showed a clear decrease in P_{50} with altitude. We hypothesise that low P_{50} at higher altitude may be advantageous to withstand drought and heat stress in summer and especially frost-drought in winter. *S. aucuparia* was found to exhibit low P and embolism in winter as reported by Vogt (2001) and to close stomata not until -5 MPa (unpublished). Peridermal transpiration might cause low water potentials even in deciduous species. However, it remains unclear, why this trend was only found in *S. aucuparia*. In winter and summer, P_{50} values were similar because the xylem, compound of dead conducting elements, had just one ring latewood more than in summer.

This small portion of new wood probably did not affect P_{50} , although late wood is known to have higher vulnerability. The lack of clear inter- and intra-specific variation in P_{50} may indicate that drought resistance in trees is mostly genetically fixed (according to Maherali and De Lucia 2000).

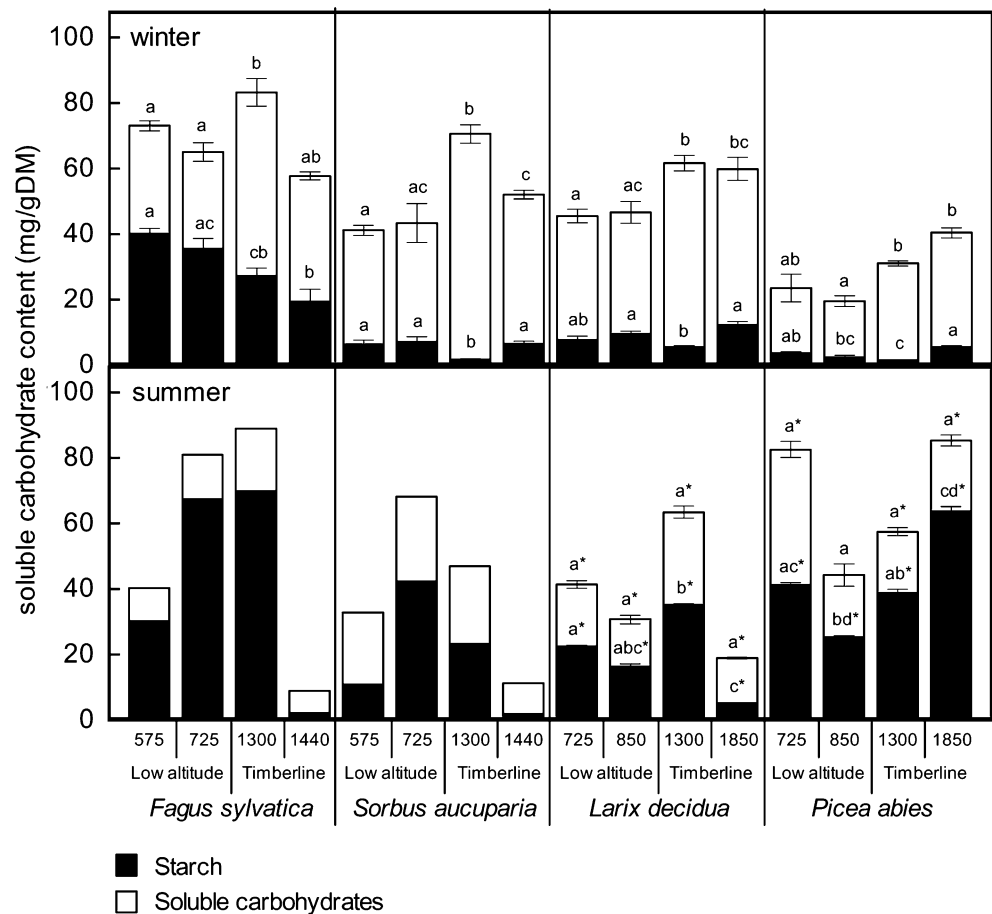
Several field studies showed low P for species analysed in this study. For instance, minimum P in *S. aucuparia* was -4.2 MPa (Vogt 2001), and *L. decidua* and *P. abies* reached -3.2 and -4.3 MPa (Mayr et al. 2006). Thus, field P obviously can reach values sufficient to induce embolism, indicating that adaptations in P_{50} are very important for plant hydraulics. Only *F. sylvatica* does not show very low water potentials (-1.2 MPa; Cochard et al. 1999), probably due to a strictly isohydric behaviour and restrictive stomata closure.

In contrast to P_{50} values, hydraulic conductivities showed an overall uniform altitudinal trend within the study regions (Fig. 2). Nearly all species exhibited lower k_s at higher altitudes, probably caused by growth restrictions and thus smaller or shorter conduits.

4.2 Frost resistance

Frost hardiness was higher in winter compared to summer in all studied species (Fig. 3), trees were thus well acclimated to environmental conditions. This is in accordance to several other studies on frost acclimation such as Sakai (1962), Sakai and Larcher (1987) or Bower and

Fig. 4 Soluble carbohydrate (open bars) and starch (solid bars) content in *F. sylvatica*, *S. aucuparia*, *L. decidua* and *P. abies* at four sites (described in Table 1) in winter and in summer. Hatched bars show Tyrolean sites; open bars, Auvergne sites. Different letters indicate significant differences ($P < 0.05$) between different sites per species, respectively. Stars indicate significant differences between winter and summer. For *F. sylvatica* and *S. aucuparia*, only one replicate was available



Aitken (2006). Charrier et al. (unpublished) describe LT_{50} variations from -11°C in May to -30°C in February in *Acer pseudoplatanus*, *Coryllus avellana*, *Pinus sylvestris*, *Betula pendula* as well as in *F. sylvatica*, and Morin et al. (2007) showed LT_{50} variations between -10°C in October and -60°C in January for oak species.

Trees showed more negative LT_{50} in Tyrol than in Auvergne. Since variation and minimum of frost resistances are genetically determined, these differences could reveal two different ecotypes in France and in Austria, due to the plasticity of this trait. Lowest LT_{50} and coldest annual minimal temperatures were reached at the timberline (Tyrol; Table 1, Fig. 3). For a given climate, Bower and Aitken (2006), Poirier et al. (2010) and Charrier et al. (2011) reported that differences in cold hardiness at the intra-specific level were observed principally in autumn and spring, but not in winter. Indeed, the rate of cold acclimation, related to differences in phenology (which is a highly plastic trait; Larcher 1980), may vary independently of the maximum hardiness attained, which is more genetically fixed.

In contrast to many studies (Sakai and Wardle 1978; Alberdi et al. 1985), no altitudinal trend was observed. In this study, altitudinal effects may have been masked by many other effects which were not taken into account.

LT_{50} variations are mostly related to soluble carbohydrate, starch and water content which follow typical seasonal courses (Sakai and Larcher 1987; Linden 2002). Also in studied trees, higher soluble carbohydrate and lower starch content (Fig. 4) were observed in winter. It is well known that starch-to-sugar conversion is a prerequisite for hardening (Sakai 1962; Sakai 1966; Sakai and Larcher 1987; Ameglio et al. 2004; Morin et al. 2007; Poirier et al. 2010) during autumn months. Hardening is also associated with a decrease in water content as described by Larcher (1972), Chen and Li (1976), Gusta et al. (2004) and Charrier and Améglio (2011).

Interestingly, *F. sylvatica*, *S. aucuparia* and *L. decidua* showed low starch content at highest altitude during summer. At this time, leaf formation probably caused sinks reducing reservoirs in the axis system. In contrast, the evergreen *P. abies* had to produce just a small part of the whole foliage and moreover the photosynthesis and starch accumulation could start earlier.

5 Conclusions

Observed differences in hydraulic aspects and frost resistance are based on variations in numerous physiological

parameters at phenotypic and genotypic level. Frost resistance thereby relatively clearly reflects temperature conditions in spatial and temporal patterns. In contrast, drought stress depends on a complex interplay of climatic and edaphic factors and a broad spectrum of plant reactions, such as rooting depth or transpiration control. This is probably the reason why intra-specific trends in P_{50} were rare, although trends in hydraulic conductivity were observed. Our data also indicate that site-specific conditions can dominate over altitudinal variations and thus always should be considered in studies on altitudinal trends. Use of genetically identical plant material might be imported in future studies to analyse acclimation trends.

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