Hydraulic adjustment of Scots pine across Europe

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Summary

• The variability of branch-level hydraulic properties was assessed across 12 Scots pine populations covering a wide range of environmental conditions, including some of the southernmost populations of the species. The aims were to relate this variability to differences in climate, and to study the potential tradeoffs between traits.
• Traits measured included wood density, radial growth, xylem anatomy, sapwood- and leaf-specific hydraulic conductivity (K_S and K_L), vulnerability to embolism, leaf-to-sapwood area ratio ($A_L : A_S$), needle carbon isotope discrimination ($\Delta^{13}C$) and nitrogen content, and specific leaf area.
• Between-population variability was high for most of the hydraulic traits studied, but it was directly associated with climate dryness (defined as a combination of atmospheric moisture demand and availability) only for $A_L : A_S$, K_L and $\Delta^{13}C$. Shoot radial growth and $A_L : A_S$ declined with stand development, which is consistent with a strategy to avoid exceedingly low water potentials as tree size increases. In addition, we did not find evidence at the intraspecific level of some associations between hydraulic traits that have been commonly reported across species.
• The adjustment of Scots pine's hydraulic system to local climatic conditions occurred primarily through modifications of $A_L : A_S$ and direct stomatal control, whereas intraspecific variation in vulnerability to embolism and leaf physiology appears to be limited.

Introduction

Plants exhibit enormous ecophysiological and functional diversity at all organizational levels. This variation provides a critical link between microevolutionary processes within populations and larger patterns of diversification and adaptation (Ackerly *et al.*, 2000) and, generally, it is not independent among traits because of a combination of developmental constraints and adaptive relationships (Preston & Ackerly, 2003). The study of trait correlations in plants has provided much insight into general functional relationships and convergent evolution among different taxa across growth forms, biomes or climatic regions (cf. Reich *et al.*, 1997; Wright *et al.*, 2004; Westoby & Wright, 2006). Water availability is one of the major determinants of plant fitness and is believed to be one of the key factors underlying trait variability among populations and communities (Maherali *et al.*, 2004).

Water loss through leaves must be compensated by the upward water flow through the xylem. In steady-state the water balance of a plant can be expressed in the following terms (Oren *et al.*, 1999):

$$D \cdot g_{wv}(D, \Psi) = \frac{A_S}{A_L} \cdot K_S(\Psi, P_{50}) \cdot \nabla\Psi = K_L(\Psi, P_{50}) \cdot \nabla\Psi$$

Eqn 1

(D is the vapour pressure deficit of the atmosphere; $g_{wv}(D, \Psi)$ is a canopy-weighted conductance for water vapour between leaves and bulk air, as a function of D and water potential (Ψ) in leaves; A_L and A_S are leaf area and cross-sectional sapwood area, respectively; $K_S(\Psi, P_{50})$ and $K_L(\Psi, P_{50})$ are, respectively, sapwood-specific and leaf-specific hydraulic conductivity as a function of xylem water potential and the pressure causing a 50% loss of conductivity in the xylem (a measure of vulnerability to embolism); and $\nabla\Psi$ is the water potential gradient through the system). When there is a strong aerodynamic coupling between the canopy and the atmosphere (e.g. coniferous forests) g_{wv} can be substituted by stomatal conductance (g_s) (Whitehead & Jarvis, 1981).

For a given D and soil water potential, the combination of g_s, $A_L : A_S$ (leaf-to-sapwood area ratio), maximum K_S and vulnerability to xylem embolism (P_{50}) determine, together with water storage in the stem (Zweifel *et al.*, 2007), the leaf water potential and thus the water status of the plant. This seems a convenient framework to study the variation in hydraulic traits as a function of climate. A gradient of increasing climate dryness implies both greater D and, in principle, lower (more negative) soil water potentials. In the absence of any hydraulic adjustment, and assuming no changes in the elasticity of storage tissues, the two previous trends would imply much lower leaf water potentials at drier sites. However, it is normally assumed that minimum seasonal water potential is constrained by the risk of hydraulic failure (Sperry *et al.*, 2002), and previous research has shown that hydraulic adjustments of different types are possible (DeLucia *et al.*, 2000; Addington *et al.*, 2006).

Plant resistance to drought is thus conferred by a complex set of many interacting traits (Piñol & Sala, 2000; Martínez-Vilalta *et al.*, 2002; Chaves *et al.*, 2003; Ackerly, 2004). Much is known about the variability of these hydraulic traits among plant species (Pockman & Sperry, 2000; Maherali *et al.*, 2004; Jacobsen *et al.*, 2007a,b). However, there is much less information on the intraspecific variability of these properties and their limits (but see Hacke *et al.*, 2000; Maherali *et al.*, 2002; Cornwell *et al.*, 2007). In particular, there are few studies comparing hydraulic properties along wide environmental gradients for a given species. Yet, the study of such phenotypic variability, which represents the combination of genetic variability, environmental effects and genotype × environment interactions, is critical to understanding and predicting plant responses to climate change (Parmesan, 2006).

Similarly, our knowledge of the trade-offs and interactions among hydraulic traits is largely based on interspecific comparisons. Species that are resistant to embolism tend to experience lower minimum leaf water potentials and have denser wood (Maherali *et al.*, 2004; Jacobsen *et al.*, 2007b). Several safety versus efficiency trade-offs have been reported at the tissue level in plants (Sperry *et al.*, 2008), although the situation appears to be less clear in conifers because of the role of torus–margo pits in preventing air-seeding (Pittermann *et al.*, 2006; but see Piñol & Sala, 2000). Again, however, much less is known about those relationships at the intraspecific level, despite the fact that functional trade-offs are probably better studied at this level (Futuyma & Moreno, 1988), as the problems associated with the presence of additional characters differing markedly between taxa and confounding the tradeoffs are likely to be minimized.

Scots pine (*Pinus sylvestris*) is one of the most widely distributed trees on Earth. Despite the fact that the largest populations of this species occur in boreal regions, Scots pine also occupies large areas in relatively dry regions within the Mediterranean basin, from the Iberian Peninsula to Turkey. This wide range makes this species particularly suitable for the study of geographic variability in hydraulic properties, not only because of the economic importance of the species, or its importance in the landscape, but also because within its range it inevitably encounters contrasted environmental conditions, and it is thus a candidate for showing high levels of phenotypic variability.

Two additional factors make Scots pine particularly interesting from the point of view of this study. First, and partly because of the previous, we probably know more about the geographical variability of functional properties for Scots pine than for any other tree species (Oleksyn *et al.*, 1999, 2003; Mencuccini & Bonosi, 2001). This knowledge suggests that pines from warmer/drier environments have lower leaf-to-sapwood area ratios (Mencuccini & Grace, 1994; Mencuccini & Bonosi, 2001), but that this structural acclimatization does not go together with a physiological acclimatization to save water through increased stomatal control (cf. Poyatos *et al.*, 2007; but see also Luoma, 1997). Second, many European Scots pine populations have suffered high mortality rates associated with recent drought episodes, including populations in Spain (Martínez-Vilalta & Piñol, 2002; Hódar *et al.*, 2003) and at higher latitudes (Rebetez & Dobbertin, 2004; Bigler *et al.*, 2006).

In this study we compared the branch-level hydraulic properties of 12 Scots pine populations sampled simultaneously along a climatic gradient in Western Europe. The main objective of the study was to characterize the variability in vulnerability to xylem embolism and other relevant hydraulic properties across populations, and to relate it to differences in climate. A secondary objective was to provide increased understanding of the relationships and potential tradeoffs between different hydraulic traits within a species. Specifically, we hypothesized that: vulnerability to embolism would be related to climate, with lower vulnerability at drier sites; branch-level, leaf-specific hydraulic conductivity would be greater at drier sites, owing to lower leaf-to-sapwood area ratios at those sites; and vulnerability to embolism would be inversely related to wood density and positively related to specific hydraulic conductivity, reflecting potential trade-offs between these properties.

Table 1 Climate characteristics of the sites studied (based on the CRU CL 2.0 10′ dataset; see text)

Site	Country	Latitude (°)	Longitude (°)	Elevation (m)	MAT (°C)	MAP (mm yr^{-1})	PET (mm yr^{-1})	P/PET (ad.)	D (kPa)
Granada	Spain	37.367 N	2.850 W	2025	7.86 ± 0.5	783.0 ± 146	958	0.82	2.05
Prades	Spain	41.333 N	1.013 E	970	10.87 ± 0.5	709.0 ± 159	922	0.77	1.73
Potenza	Italy	40.594 N	15.807 E	957	10.70 ± 0.3	684.5 ± 140	811	0.84	1.43
Vallcebre	Spain	42.200 N	1.817 E	1260	7.67 ± 0.5	940.4 ± 183	804	1.17	1.24
Leuvenumse	Netherlands	52.300 N	5.717 E	20	9.22 ± 0.7	785.3 ± 121	715	1.10	1.09
Kootwijk	Netherlands	52.167 N	5.733 E	20	9.22 ± 0.7	785.3 ± 121	715	1.10	1.09
Pfyn	Switzerland	46.306 N	7.614 E	615	8.95 ± 0.4	703.1 ± 115	696	1.01	1.01
Fonfreyde	France	45.704 N	2.981 E	940	7.27 ± 0.8	930.6 ± 139	777	1.20	1.24
Salgesch	Switzerland	46.324 N	7.578 E	975	8.97 ± 0.4	737.6 ± 120	697	1.06	1.01
Jeizinen	Switzerland	46.323 N	7.725 E	1270	7.46 ± 0.4	693.7 ± 113	629	1.10	0.85
Hyytiala	Finland	61.848 N	24.295 E	181	2.84 ± 1.1	584.5 ± 82	554	1.06	1.11
Selm Muir	Scotland	55.868 N	3.457 W	220	7.41 ± 0.5	850.8 ± 86	587	1.45	0.76

MAT, Mean annual temperature (± SD); MAP, mean annual precipitation (± SD); PET, annual potential evapotranspiration; P/PET, ratio of annual precipitation to potential evapotranspiration; D, summer vapour pressure deficit. All climatic values correspond to the period 1961–90. Sites are ordered from drier to wetter according to our climate dryness index (see text).
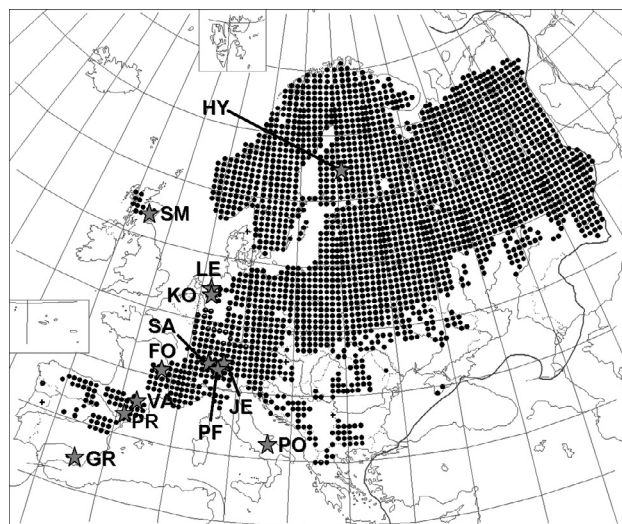
Fig. 1 Map of Europe showing the distribution of Scots pine (reproduced from Jalas & Suominen (1976), by permission of the Committee for Mapping the Flora of Europe and Societas Biologica Fennica Vanamo), and the location of the 12 study sites. See Table 2 for site codes.

Materials and Methods

Study sites: climatic and stand-level data

The 12 sites studied ranged from southern Finland to the southernmost populations of the species in southern Spain (Fig. 1), and covered a broad range of environmental conditions across the distribution of Scots pine (*P. sylvestris* L., Table 1). For each site, average monthly precipitation (P), temperature (T), daily temperature range (DTR), and relative humidity (RH) were extracted from the CRU CL 2.0 10′ global dataset for the period 1961–90 (New *et al.*, 2002). The values of temperature and rainfall were corrected for the altitudinal

difference between the study sites and the average for the corresponding grid cells, the latter taken again from the CRU CL 2.0 10′ dataset. The values of temperature and rainfall lapse rates were set to match the values from local weather stations when available. Temperature lapse rate thus varied between 0.3° 100 m^{-1} and 0.6° 100 m^{-1}, and rainfall lapse rates between −20 mm 100 m^{-1} and −100 mm 100 m^{-1}, depending on the site. When no local weather data were available, temperature and rainfall lapse rates were set to 0.6° 100 m^{-1} and −25 mm 100 m^{-1}, respectively.

Monthly potential evapotranspiration (PET) was calculated from monthly values of mean temperature and daily temperature range using the Hargreaves–Samani method (Hargreaves & Samani, 1982). Monthly vapour pressure deficit (D) was calculated using mean monthly temperature and RH to estimate vapour pressure, and maximum monthly temperature (calculated as T + DTR/2) to estimate saturation vapour pressure. These values were used to compute the following climatic variables used to characterize the sites: annual P (MAP), annual T (MAT), annual PET, annual P/PET, summer P/PET and summer D. Summer values corresponded to June–August averages.

The stands at the different sites, most of which were mono-specific, varied in their structure and age (Table 2). Stand properties were either measured in this study using standard protocols or taken from previous studies at the same sites (see Table 2 for details). Measurements of shoot water potentials during the dry summer period were only available for a subset of five populations (Table 2), and even these values should be considered with caution as they might not represent accurate estimates of (long-term) minimum water potentials.

Sample collections and hydraulic measurements

At each site, terminal shoots from five to nine representative trees were collected in early spring 2007. Sampled shoots

Table 2 Scots pine stand characteristics of the sites studied

Site	Code	Management	Age (yr)	DBH (cm)	Height (m)	Density (trees ha^{-1})	A_{basal} (m^2 ha^{-1})	LAI (m^2 m^{-2})	Ψ_{pd} (MPa)	Ψ_{md} (MPa)
Granada	GR	Natural	90	32.90	7.70	512 (80%)	40	2.24	–	–
Prades	PR	Natural	100	26.50	9.80	351	19	1.13	−1.92	−2.25
Potenza	PO	Plantation	25	17.60	12.60	995	24	4.22	−1.12	–
Vallcebre	VA	Natural	45	15.20	11.00	2165	45	2.40	−0.90	−2.10
Leuvenumse	LE	Plantation	50	25.00	20.00	1894 (39%)	37	2.50	–	–
Kootwijk	KO	Plantation	55	30.00	16.80	731 (65%)	32	2.50	–	–
Pfyn	PF	Natural	94	21.20	11.00	750	25	2.10	–	–
Fonfreyde	FO	Natural	35	12.16	11.40	4012	52	3.00	–	–
Salgesch	SA	Natural	125	16.30	4.00	776 (29%)	10	0.90	−2.00	−2.50
Jeizinen	JE	Natural	150	22.40	12.00	2708 (12%)	30	3.00	–	–
Hyytiala	HY	Plantation	45	13.40	13.80	1371	23	2.13	–	−1.90
Selm Muir	SM	Plantation	30	21.18	8.33	547	21	1.10	−0.50	−1.30

DBH, diameter at breast height; Density, tree density (in parenthesis the proportion corresponding to Scots pine); A_{basal}, basal area; LAI, early summer projected leaf area index; Ψ_{pd}, minimum measured predawn water potential; Ψ_{md}, minimum measured midday water potential. Sites are ordered from drier to wetter according to our climate dryness index (see text). Source of data: Martínez-Vilalta & Piñol (2002) for PR; Poyatos *et al*. (2005, 2008) for VA; Zweifel *et al*. (2007) for SA; Hölttä *et al*. (2005) and the CarboEurope project database for HY; and Korakaki (2007) for SM. For the other sites (GR, PO, LE, KO, PF, FO and JE) measurements were taken specifically for this study during years 2007 or 2008.

were fully exposed, > 40 cm long and 0.5–1 cm in diameter (> 3 yr of age). Sampled branches were wrapped in wet towels and bagged upon collection to prevent dehydration. Once in the laboratory, needles were stripped off to measure the leaf-to-sapwood area ratio ($A_L : A_S$; using projected leaf area) of measured branches, and the specific leaf area (SLA) of current-year needles. Wood samples were bagged again and sent to the University of Edinburgh, UK, for hydraulic analyses.

Vulnerability to xylem cavitation was measured by the Cavitron technique (Cochard *et al.*, 2005) within 2 d of sample collection. The principle of the technique is to lower the pressure in a xylem segment by centrifugal force and, at the same time, measure the variation in its hydraulic conductivity. The per cent loss of xylem conductivity (PLC) versus the xylem pressure represents the sample's vulnerability curve. Samples were cut in the air to obtain 0.28-m long segments (i.e. far longer than the longest tracheids). Bark was removed from all segments and they were placed in the rotor of the centrifuge. Xylem pressure (P_x) was first set to a reference pressure (−1.0 MPa) and maximal conductivity (K_{max}) determined. The xylem pressure was then set to a more negative pressure for 30 s and subsequently returned to the reference pressure to determine the new conductivity (K). Per cent loss of conductivity was then computed as PLC = 100 (1 − K/K_{max}). The procedure was repeated for more negative pressures (−0.5 MPa step increments) until PLC reached at least 90%. Rotor velocity was monitored with an electronic tachymeter (10 rpm resolution) and xylem pressure was adjusted to *c.* ± 0.02 MPa. The following sigmoid function was fitted to each curve (Pammenter & Vander Willigen, 1998):

$$ PLC = \frac{100}{1 + e^{\frac{s}{25} \cdot (P - P_{50})}} \qquad \text{Eqn 2} $$

(P_{50} is the pressure causing 50% loss of conductivity; *s* is a slope parameter). Please note that P_{50} values are reported as negative water potentials throughout the manuscript.

The Cavitron technique does not directly measure water flow through the measured segments, but the flow can be estimated from the velocity at which the meniscus moves in the upstream water reservoir in the centrifuge (Cochard *et al.*, 2005). This flow can be used in turn to estimate the hydraulic conductivity of the sample. Li *et al.* (2007) have recently found that these estimates are consistent with those obtained by employing the more conventional approach of a gravity-induced head to measure conductivity. For each measured segment we calculated its specific hydraulic conductivity (K_S), as the hydraulic conductivity per unit cross-sectional area of wood; and its leaf-specific hydraulic conductivity (K_L), as the conductivity per unit of projected leaf area.

Xylem anatomy and wood density

Microthin sections (*c.* 25 μm thick) were taken from each of the segments used in hydraulic measurements. Samples were soaked in safranine for 3 min to improve contrast, flushed with clean water to avoid safranine diffusion under the light microscope, and embedded in glycerol. Several digital photographs were taken of each cross-section using a Leica DFC 180 camera (×100 magnification). These photographs were combined to get one single image of the whole cross-section of each branch sample. These combined images served as the basis for all anatomical measurements. For each sample, a radius (R_1) was chosen based on visual image inspection, so that it intersected the strongest compression wood formations. This radius, R_1, probably corresponded with the bottom side of the branch. Three additional radii

(R_2, R_3 and R_4) were identified at subsequent 90° angles in the cross-section. Sectors (90°) along radii R_2 and R_4 were selected for analysing the anatomical features, as these radii were most representative for the whole cross-section. Tracheids were counted, and their individual lumen area, together with their minimum and maximum perpendicular lumen diameters, were determined using the software Visilog (NOESIS, Bâtiment Medasys, Saint Aubin, France). Between 500 and 10 000 tracheids were thus measured per sample. Tracheid numbers were scaled to the whole cross-sectional area by correcting for the xylem area in the cross-section. The hydraulic tracheid diameter (D_{tr}) was calculated assuming elliptical tracheid lumens (Lewis & Boose, 1995):

$$D_{tr} = \sqrt[4]{\frac{1}{n} \cdot \sum_{i=1}^{n} \frac{2 \cdot a_i^3 \cdot b_i^3}{a_i^2 + b_i^2}} \qquad \text{Eqn 3}$$

(a_i and b_i are the minimum and maximum lumen diameter, respectively, of the ith elliptical tracheid (m); n is the number of tracheids measured).

For each sample, the fresh wood volume was determined by the water displacement method as described by Olesen (1971). The oven-dry weight was determined after samples were dried at 103°C. Wood density was obtained by dividing the oven-dry weight by the sample volume.

Leaf nitrogen and $\Delta^{13}C$

Carbon isotope discrimination ($\Delta^{13}C$) was used as a proxy of integrated stomatal control and water-use efficiency (Farquhar *et al.*, 1989). For 10 of the 12 populations, subsamples of current-year needles from all trees sampled ($n = 5$–9 trees per site; one subsample per tree) were dried and ground before being analysed at the Cornell University Stable Isotope Laboratory (COIL, Ithaca, NY, USA). These variables could not be measured for the branches sampled at Pfyn and Selm Muir. For the latter, values were taken from a previous study conducted on the exact same trees and analysed at the same laboratory (Korakaki, 2007). Nitrogen and carbon concentrations and carbon isotope composition ($\delta^{13}C$) were measured with a Finnigan MAT Delta Plus isotope ratio mass spectrometer (IRMS) (Bremen, Germany) plumbed to a Carlo Erba NC2500 elemental analyser (Carlo Erba Instruments, Milan, Italy) through a Conflo II open split interface (Finnigan MAT, Bremen, Germany). The relationship between carbon stable isotopes was expressed in relation to a Pee-Dee Belemnite (PDB) standard. The accuracy of the $\delta^{13}C$ measurements was 0.2‰. In the derivation of $\Delta^{13}C$, the $\delta^{13}C$ of air was assumed to be −8‰.

Statistical analyses

All variables were checked for normality and transformed by applying logarithms whenever required. One-way analysis of variance (ANOVA) with site as a factor was used to test for significant differences among populations for a given variable (Table 3). Mantel tests were employed to test whether geographic distance between sites was associated with the differences in the study variables observed among populations. Linear regression analysis and Pearson correlation coefficients were used to quantify association between pairs of continuous variables.

Since both climate and stand descriptors were highly inter-correlated, two separate principal components analyses (PCAs), one with climatic variables and the other with stand variables, were performed to summarize the data. The climate PCA included: annual P, annual T, annual PET, annual P/PET, summer P/PET, and summer D. The two first axes of the climate PCA explained 60.1% and 22.8% of the total variance, respectively. The first axis was interpreted as an index of climate dryness, since variables increasing with atmospheric water demand had highly positive factor loadings and variables increasing with water availability (P/PET) had negative factor loadings (see the Supporting Information Fig. S1). The second axis was mostly related to absolute rainfall (regardless of atmospheric water demand) (Fig. S1).

The PCA of stand variables included tree density, tree age, average tree diameter (DBH), basal area and leaf area index (LAI). The first two axes of the PCA of stand variables explained 67.9% of the variance (42.2% the first and 25.8% the second). The first axis was interpreted as a measure of stand development, as it was positively correlated to stand age and DBH, and negatively correlated to tree density, LAI and basal area (Fig. S2). This first stand axis was uncorrelated to either the first or second axis of the climate PCA ($P > 0.3$ in both cases), suggesting that the differences in forest structure between sites were not related to climatic differences and were instead primarily associated with the temporal development of the stand. However, we cannot rule out the possibility that the differences in stand properties were also related to soil type or other unmeasured variables. The first axes of the climate and stand PCAs were used in further analyses as integrated measures of climate dryness and stand development, respectively.

Finally, path analysis (structural equation modelling with no latent variables) was employed to compare four alternative conceptual models of the way hydraulic traits were affected by stand and climate characteristics. Those models were based on previous studies on the hydraulic acclimatization of Scots pine (see the Introduction) and on the hypothesized hydraulic adjustments to differences in climate dryness. The four models differed on whether climate dryness directly affected $A_L : A_S$ and P_{50} (Model a in Fig. 4), $A_L : A_S$ and $\Delta^{13}C$ (Model b), $A_L : A_S$ and K_S (Model c) or $A_L : A_S$, P_{50} and $\Delta^{13}C$ (Model d).

Although in our case the small sample size limits the complexity of the path models and the strength of the conclusions obtained from the analysis, this technique offers the possibility of analysing indirect effects and structuring the relationships between variables based on previous knowledge, which here complements the use of simple pairwise correlation and linear

Table 3 Hydraulic and other branch-level traits measured in this study

Attribute	Symbol	Units
Wood radial growth (branches)	G_W	$mm^2 \, yr^{-1}$
Wood density (branches)	d_W	$kg \, m^{-3}$
Average tracheid diameter	D_{tr}	μm
Tracheid density	d_{tr}	mm^{-2}
Specific hydraulic conductivity	K_S	$kg \, m^{-1} \, s^{-1} \, MPa^{-1}$
Pressure causing 50% embolism	P_{50}	MPa
Branch leaf-to-sapwood area ratio	$A_L : A_S$	$m^2 \, cm^{-2}$
Leaf-specific hydraulic conductivity	K_L	$kg \, m^{-1} \, s^{-1} \, MPa^{-1}$
Carbon isotope discrimination in needles	$\Delta^{13}C$	‰
Nitrogen content in needles	%N	%
Specific leaf area	SLA	$cm^2 \, g^{-1}$

Table 4 Variation observed within and between populations of Scots pine for all studied variables (expressed as the coefficients of variation within and among sites: CV_{intra} and CV_{inter}, respectively), and results of the one-way ANOVA with site as a factor to test for differences among populations

Attribute	CV_{intra} (%)[a]	CV_{inter} (%)	ANOVA			
			r^2	df[b]	F-value	P-value
G_W	40.63	56.15	0.64	11	11.039	< 0.001
d_W	9.48	7.59	0.38	11	3.77	< 0.001
D_{tr}	7.45	4.82	0.28	11	2.48	0.011
d_{tr}	11.06	7.58	0.30	11	2.63	0.007
K_S	36.79	20.72	0.21	11	1.72	0.088
P_{50}	6.23	8.11	0.63	11	10.49	< 0.001
$A_L : A_S$	32.89	34.29	0.57	11	8.46	< 0.001
K_L	46.95	23.68	0.20	11	1.54	0.137
$\Delta^{13}C$	3.39	5.10	0.65	9	11.94	< 0.001
%N	10.04	17.68	0.74	9	17.72	< 0.001

[a]Weighted by sample size within each population; [b]df, degrees of freedom.

regression. All variables were standardised before fitting the path models. Statistical analyses were carried out with the R software (R 2.6.0; The R Foundation for Statistical Computing). Significance for all statistical tests was accepted at $\alpha = 0.05$.

Results

Variation among populations

The hydraulic properties of Scots pine branches differed markedly among populations (Table S1). Variability across sites was relatively high (CV > 20%, similar or higher than the overall CV = 23% for climatic drivers) for branch-level radial growth, $A_L : A_S$, and K_L and K_S); whereas it was particularly low (CV < 10%) for the pressure causing 50% embolism (P_{50}), average tracheid diameter and density, wood density and $\Delta^{13}C$ discrimination (Table 4). One-way ANOVA showed that the effect of site was significant for all major variables studied except for K_L and K_S (Table 4), implying

that among populations variability was a significant source of variation for most variables.

Relationship between hydraulic properties and climate and stand proxies

Climate dryness (axis one of the climate PCA, Fig. S1) was negatively related to branch $A_L : A_S$ ($r^2 = 0.38$, $P = 0.032$), and positively related to K_L ($r^2 = 0.46$, $P = 0.015$) (Fig. 2), whereas it was unrelated to all other variables studied ($P > 0.05$) (Table 5). The second axis of the climate PCA, mostly related to rainfall (Fig. S1), was unrelated to any of the plant traits studied ($P > 0.1$ in all cases). Predawn and midday water potentials showed decreasing trends with increasing climate dryness, but the trends were not significant ($P > 0.1$ in both cases). Stand development (axis 1 of the stand PCA, Fig. S2) was positively related to tracheid density ($r^2 = 0.43$, $P = 0.021$) and negatively related to shoot radial growth ($r^2 = 0.46$, $P = 0.015$) (Table 5). We also tested whether the variability among populations was associated with their geographic distance, which could be evidence of a genetic effect. Only $A_L : A_S$ and K_S showed a trend towards being more similar in nearer populations (Mantel $r = 0.51$ and Mantel $r = 0.33$, respectively; $P < 0.05$ in both cases).

Relationships among hydraulic properties

Branch hydraulic traits were highly intercorrelated across populations. Out of the 28 possible correlations between the eight measured hydraulic traits (Table 3), 13 (46%) turned out to be significant (Fig. 3). Branch $A_L : A_S$ was the variable showing more correlations with other variables, as it was positively correlated with P_{50}, K_S and tracheid diameter (D_{tr}), and negatively correlated to K_L and tracheid density (d_{tr}) ($P < 0.05$ in all cases). D_{tr} and d_{tr} showed a very high negative correlation between each other ($r^2 = 0.92$, $P < 0.001$) and were correlated to several other variables, including K_S and wood density (d_W) ($P < 0.05$ in all cases). The $\Delta^{13}C$ value was positively correlated to P_{50} ($r^2 = 0.38$, $P = 0.045$) and negatively to K_L ($r^2 = 0.38$, $P = 0.043$). Shoot radial growth
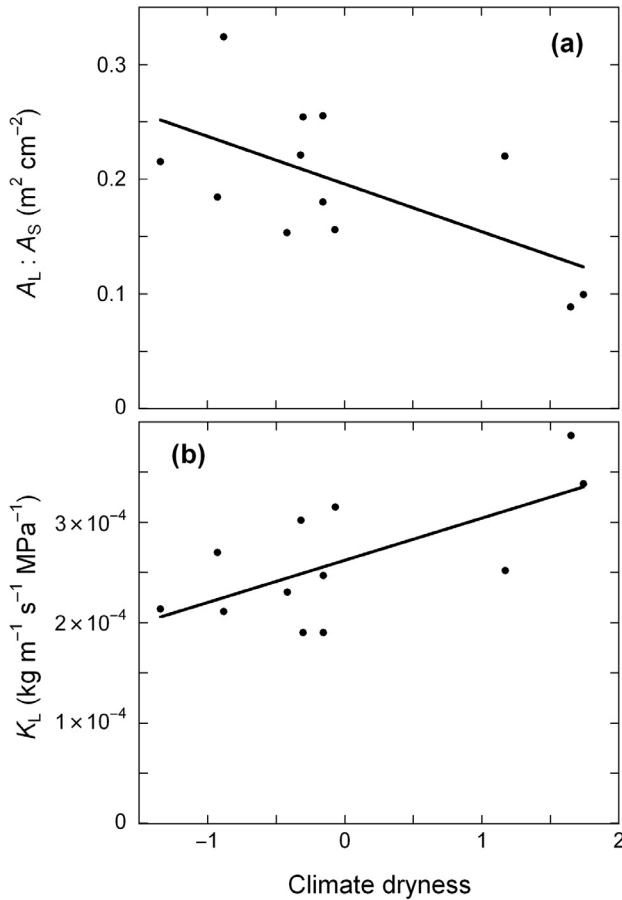
Fig. 2 Relationship between climate dryness (first axis of principal components analysis (PCA) in the Supporting Information, Fig. S1) and (a) leaf-to-sapwood area ratio ($A_L : A_S$), and (b) leaf-specific hydraulic conductivity (K_L) in Scots pine. Both regressions are significant ($P < 0.05$).

(G_W) was positively correlated to tracheid diameter and $A_L : A_S$, and negatively correlated to d_{tr} and K_L ($P < 0.05$ in all cases). Finally, SLA and %N were uncorrelated to each other or to any other attribute, with the exception of the positive relationship between %N and $\Delta^{13}C$, which was marginally significant ($r^2 = 0.36$, $P = 0.051$). The latter relationship completely disappeared if the N content was expressed per unit of leaf area ($r^2 = 0.004$, $P = 0.861$).

The results of the path analysis aided the interpretation of the previously reported relationships, separating the more consistent effects from those likely to correspond to indirect relationships mediated by third variables. The four path models compared provided a reasonable fit to the data (Fig. 4; $P > 0.15$ in all cases; note that because the goal of path analysis is to develop a model that fits the data, a non-significant χ^2 is desired). In most cases, the value of a given coefficient was similar across the four models (Fig. 4), suggesting that the magnitude of the effects detected in the path analyses was not very sensitive to the particular structure of the model. However, two of the models (Models b and d in

Table 5 Linear regression analyses (r^2 and significance) of the relationship between measured branch-level traits and climate dryness and stand development in Scots pine

	G_W	d_W	D_{tr}	d_{tr}	K_S	P_{50}	$A_L : A_S$	K_L	$\Delta^{13}C$	%N	SLA
Climate dryness	0.08 (0.36)	0.24 (0.12)	0.13 (0.25)	0.09 (0.34)	0.17 (0.19)	0.06 (0.43)	0.38* (0.03)	0.46* (0.02)	0.16 (0.16)	0.26 (0.11)	0.00 (0.90)
Stand development	0.46* (0.02)	0.01 (0.76)	0.23 (0.12)	0.44* (0.02)	0.09 (0.34)	0.01 (0.80)	0.19 (0.17)	0.13 (0.25)	0.02 (0.67)	0.04 (0.56)	0.00 (0.91)

P-values are shown in parenthesis ($n = 11$–12). An asterisk indicates significant relationships. Significant, negative relationships are underlined. See Table 3 for definition of symbols.
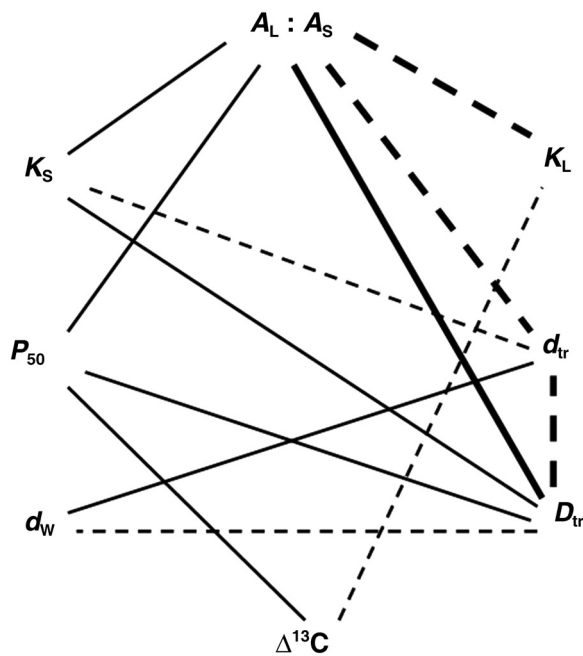
Fig. 3 Pairwise correlations for the eight hydraulic traits measured in this study. Only significant relationships ($P < 0.05$) are depicted. Thick lines correspond to relationships significant at the 0.01 level. Dashed lines indicate negative relationships. See Table 3 for definition of symbols.

Fig. 4) were clearly superior to the rest, both in terms of the P-value of their fit, and their BIC (Bayesian information criterion). The two best-fitting models differed from each other in the presence of a direct effect of climate dryness on P_{50}, but provided identical significant paths, again giving confidence to the robustness of the results. Those significant paths corresponded to the effect of climate drivers on $A_L : A_S$ and $\Delta^{13}C$, the effect of stand characteristics on $A_L : A_S$, and the significant directional effects of $A_L : A_S$ on K_S and of K_S on $\Delta^{13}C$ (Fig. 4b,d).

Discussion

Despite the high between-population variability observed for most of the studied hydraulic traits, this variation was associated with climate dryness in only three of them: branch $A_L : A_S$, K_L and the $\Delta^{13}C$ of needles (Fig. 4). Stand development affected shoot radial growth and, to a lesser extent, $A_L : A_S$. In both cases, the patterns were consistent with a decline in growth rates with tree size and with the corresponding decline in $A_L : A_S$ to avoid exceedingly low water potentials (Mencuccini & Grace, 1994; McDowell *et al.*, 2002). An important consideration is that our sampling design was aimed at standardizing for shoot diameter and age, hence measured wood segments were probably closer to the apex in older trees. Both theoretical analyses and empirical results show that xylem conduits tend to be narrower and denser towards the apex (cf. Petit *et al.*, 2008).

Trait variation in response to climate dryness

Contrary to our initial hypothesis, vulnerability to xylem embolism of the populations studied was unrelated to climate dryness. This lack of adjustment of vulnerability to embolism is consistent with some previous results at the intraspecific level (Mencuccini & Comstock, 1997; Vander Willigen & Pammenter, 1998; Maherali & DeLucia, 2000; Martínez-Vilalta & Piñol, 2002; Cornwell *et al.*, 2007), and with the relatively low plasticity in vulnerability to embolism previously reported for pines (Martínez-Vilalta *et al.*, 2004). However, it contradicts most evidence gathered across species (Maherali *et al.*, 2004; Jacobsen *et al.*, 2007b) and was unexpected here considering the wide range of environmental conditions covered in this study.

The fact that vulnerability to xylem embolism was not directly related to climate dryness in our case supports the concept that minimum tolerable water potentials are not necessarily lower at drier Scots pine populations, consistent with homeostasis of water transport (Magnani *et al.*, 2002; Duursma *et al.*, 2008). This is also in agreement with the fact that we found no significant relationship between minimum (midday) water potential and climate for the five populations that were measured. This relative stability in minimum water potentials has been found in studies comparing Scots pine populations growing under contrasting climates (Magnani & Grace, 2000; Magnani *et al.*, 2002), and within a site over the course of several years (Zweifel *et al.*, 2007).

The results outlined in the previous paragraph imply the presence of hydraulic adjustments. In our case, Scots pine populations responded to lower water availability primarily by structurally reducing their branch $A_L : A_S$. This is consistent with previous studies showing similar results at the tree level (Mencuccini & Grace, 1994; Mencuccini & Bonosi, 2001; Poyatos *et al.*, 2007; but see Palmroth *et al.*, 1999). As hypothesized, lower branch $A_L : A_S$ (and similar K_S) resulted in greater hydraulic capacity per unit of leaf area (K_L) at dry sites. The latter is consistent with the patterns found by Bhaskar *et al.* (2007) when comparing closely related species pairs from sites with contrasted seasonality of precipitation. However, it is opposite to what Panek (1996) reported for *Pseudotsuga menziesii* and, more interestingly, to what Sterck *et al.* (2008) recently found when comparing Scots pines at two neighbouring sites differing only in soil water availability. In this latter study, lower K_L at the dry site was associated with smaller functional sapwood area and narrower tracheids, resulting in a stronger reduction in K_S than in A_L. By contrast, we did not find a consistent reduction of K_S at drier sites. Taken together, these results suggest that hydraulic adjustments in Scots pine may be different depending on whether they respond to soil drought, atmospheric drought or both. In that respect, the interpretation of our results is complicated by the association between temperature and dryness when comparisons are made across wide geographic gradients.
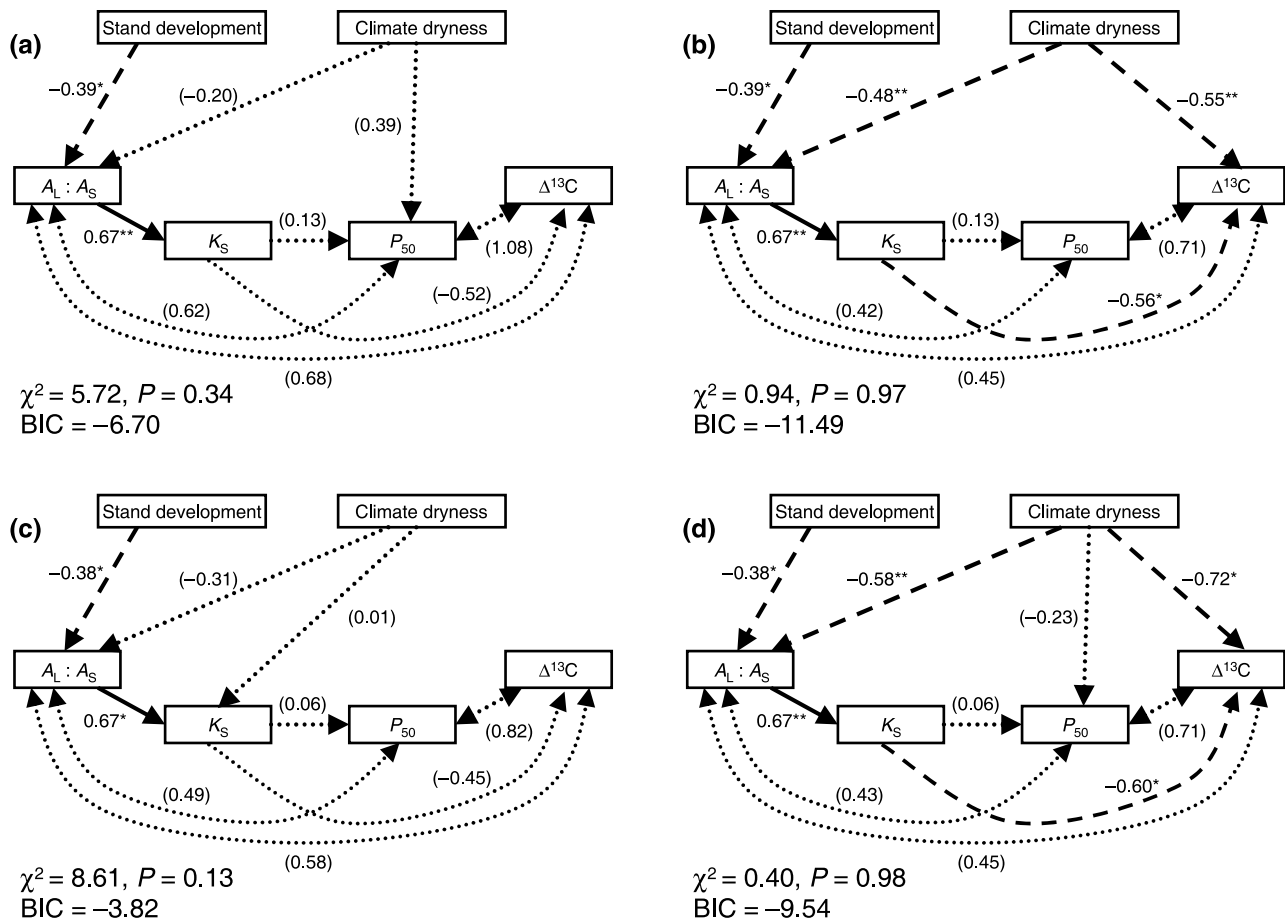
Fig. 4 Path diagrams of the four models relating major hydraulic traits to climate and stand characteristics (see the Materials and Methods section). Arrows indicate the proposed links between variables. Dashed lines indicate negative relationships. Dotted lines indicate non-significant paths. Path coefficients are shown close to the arrows (in parenthesis, $P > 0.05$; *, $0.01 < P < 0.05$; **, $P < 0.01$). See Table 3 for definition of symbols. Overall fit statistics for each path model (χ^2, BIC (Bayesian information criterion)) are shown at the bottom left of each panel.

The nearly universal trend of decreasing A_L with climatic dryness is likely to be related to differences in leaf turnover rates and, ultimately, to water and carbon economy. Leaf lifespan has been shown to decline with increasing aridity in many species as a strategy to maximize carbon gain (Chabot & Hicks, 1982). If leaf and sapwood production are coupled, differences in leaf lifespan would result in different $A_L : A_S$. For Scots pine, needle lifespan increases markedly with increasing latitude and decreasing temperature (Oleksyn *et al.*, 2003). This pattern has been linked to nutrient conservation (Oleksyn *et al.*, 2003), but needle senescence during the dry summer period could also be a contributing factor at the driest edge of the distribution.

The only other hydraulic trait that appeared to vary directly as a function of climate dryness was carbon isotope discrimination in needles ($\Delta^{13}C$; Fig. 4). A decline in $\Delta^{13}C$ with decreasing water availability has been found in previous studies with conifers, both at the intraspecific and interspecific levels (Warren *et al.*, 2001; Martínez-Vilalta *et al.*, 2004), and it is in principle consistent with an increase in stomatal

control over water loss with increasing drought stress. This is particularly so considering that N content in needles did not vary with climate dryness in our case. It should be noted that, as g_s declines with D, stomatal conductance will be lower at drier sites provided that D is high enough, even if relative stomatal sensitivity is lower at those drier sites (Poyatos *et al.*, 2007). Finally, it cannot be ruled out that part of the differences in $\Delta^{13}C$ that we observed might have been caused by biochemical, structural or micrometeorological differences across populations not necessarily related to water availability (Marshall & Zhang, 1994; Hultine & Marshall, 2000).

A limitation of our study is that we characterized water availability using only climatic drivers, as no data were available on stand-level soil characteristics for most sites. Although water availability in the long-term is mostly characterized by the balance between rainfall and evapotranspiration, and soil properties are to an important degree determined by climate, it is possible that two sites with similar climate pose very different hydraulic constraints on trees owing to differences in soil depth or texture. A second limitation is the fact that we

have only looked at branch-level properties, whereas the water balance of a tree is determined by the integration of hydraulic architecture at the whole-tree level. Almost nothing is known, for example, about the variation of belowground properties along climatic gradients in the field (but see Hacke *et al.*, 2000; Addington *et al.*, 2006). In our case, the fact that predawn water potential did not significantly decline with increasing climate dryness suggests some local acclimatization of belowground properties, although sample size ($n = 5$) is probably too low to draw any definitive conclusions.

The question also remains as to what extent the variability in hydraulic traits observed across the studied populations is genetic in origin or, instead, is the result of phenotypic plasticity. Previous evidence suggests that phenotypic plasticity is likely to be an important contributor to the phenotypic differentiation observed in pines along aridity gradients (Mencuccini & Grace, 1994; Maherali *et al.*, 2002). However, other studies have also found evidence of genetic differentiation among populations (Oleksyn *et al.*, 1999; Palmroth *et al.*, 1999). Finally, the contrasted patterns found when comparing hydraulic adjustments within and between populations (see earlier) could also reflect the distinct role of adaptation (genetically controlled) versus acclimatization (environmentally controlled) at different spatial scales. All these issues are key to predicting the response of Scots pine to future climates, and deserve further study.

Hydraulic compensation and trade-offs

According to our results, the major adjustments between hydraulic traits corresponded to an increase in K_S in populations with higher $A_L : A_S$, and a decline in $\Delta^{13}C$ in populations with higher K_S. These associations make hydraulic sense in the framework of Eqn 1, as more leaves are likely to require a greater hydraulic capacity of the conducting system (Whitehead *et al.*, 1984; Shelbourne *et al.*, 1993; Mencuccini & Grace, 1994) and greater stomatal control (but see Panek, 1996). Interestingly, neither of the two previous relationships were found in a study across 11 species of Pinaceae (Martínez-Vilalta *et al.*, 2004). In our case, increased K_S at sites with greater $A_L : A_S$ was achieved by increasing tracheid diameter, while tracheid density was reduced, probably owing to geometrical and/or mechanical constraints (*cf.* Sperry *et al.*, 2008).

We did not find evidence of a trade-off between hydraulic conductivity and resistance to xylem embolism. This result is consistent with the simultaneous increase of K_S and resistance to cavitation within individual Sequoia trees reported by Burgess *et al.* (2006). Similarly, we did not find evidence of several functional relationships and trade-offs that have been previously reported at the between-species level. Wood density, for example, a trait that has been related to resistance to xylem embolism and other major hydraulic traits across species (Hacke *et al.*, 2001; Westoby & Wright, 2006; Jacobsen

et al., 2007b), was unrelated to any hydraulic trait in our dataset, with the exception of the more or less straightforward relationships with wood anatomy. These differences between the intraspecific and interspecific levels are intriguing and deserve further study in order to establish whether some of the negative associations between hydraulic traits that have been reported in the literature correspond to true functional trade-offs or to independent responses of different traits to selection.

Conclusion

Adjustment of the Scots pine hydraulic system to local climatic conditions occurs primarily through modifications of $A_L : A_S$ and direct stomatal control, and not through changes in the vulnerability to xylem embolism or in the sensitivity of leaf physiology to drought. Hydraulic adjustment between populations likely resulted in relatively constant summer needle water potentials despite large differences in water availability. This local acclimatization seems to be effective in buffering the drought stress experienced by the different populations at the leaf level and, in some areas, has the potential to maintain or even increase growth rates under climate change scenarios (Berninger, 1997). However, it has not been enough to prevent episodes of drought-induced dieback at the driest edge of the species' range (Martínez-Vilalta & Piñol, 2002; Bigler *et al.*, 2006). The limited plasticity of its xylem and leaf properties may be behind the high vulnerability of Scots pine to the decline in water availability recently experienced throughout most of its range, and likely to increase in the future.

Acknowledgements

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Factor coordinates of variables on the two first axes of the principal components analysis for climate variables.

Fig. S2 Factor coordinates of variables on the two first axes of the principal components analysis for stand variables.

Table S1 Average values (± SE) for each study site of the branch-level traits measured in this study, and axis scores of each study site on the first axis of the climate and stand principal components analyses

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