

Plasticity in hydraulic architecture of Scots pine across Eurasia

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Abstract Widespread tree species must show physiological and structural plasticity to deal with contrasting water balance conditions. To investigate these plasticity mechanisms, a meta-analysis of *Pinus sylvestris* L. sap flow and its response to environmental variables was conducted using datasets from across its whole geographical range. For each site, a Jarvis-type, multiplicative model was used to fit the relationship between sap flow and photosynthetically active radiation, vapour pressure deficit (D) and soil moisture deficit (SMD); and a logarithmic function was

used to characterize the response of stomatal conductance (G_s) to D . The fitted parameters of those models were regressed against climatic variables to study the acclimation of Scots pine to dry/warm conditions. The absolute value of sap flow and its sensitivity to D and SMD increased with the average summer evaporative demand. However, relative sensitivity of G_s to D ($m/G_{s,ref}$, where m is the slope and $G_{s,ref}$ is reference G_s at $D = 1$ kPa) did not increase with evaporative demand across populations, and transpiration per unit leaf area at a given D increased accordingly in drier/warmer climates. This physiological plasticity was

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linked to the previously reported climate- and size-related structural acclimation of leaf to sapwood area ratios. $G_{s,ref}$ and its absolute sensitivity to D (m), tended to decrease with age/height of the trees as previously reported for other pine species. It is unclear why Scots pines have higher transpiration rates at drier/warmer sites, at the expense of lower water-use efficiency. In any case, our results suggest that these structural adjustments may not be enough to prevent lower xylem tensions at the driest sites.

Keywords Hydraulic plasticity · Leaf area:sapwood area · *Pinus sylvestris* · Sap flow · Stomatal conductance

Introduction

Tree species with extensive geographical distributions or living in contrasting habitats must develop a set of strategies that allow them to cope with a variety of climatic conditions, of which, atmospheric and soil moisture deficits (SMDs) are among the most relevant in determining plant growth and survival. Long-term changes in physiological responses and structural traits operate at different levels in the soil–plant–atmosphere continuum to maintain the hydraulic functioning of the tree under drought conditions (Larcher 2003; Mencuccini 2003). In the case of widely distributed and abundant tree species at the global scale, these intraspecific climate-driven adjustments must be considered in order to understand the spatial variations in water and carbon exchange of forest ecosystems and the further implications of plastic responses to drought under climate change (DeLucia et al. 2000).

Short-term physiological regulation of tree transpiration by reducing stomatal conductance (G_s) prevents excessive losses of water and maintains water potentials within an operative range (Meinzer 2002), avoiding critical losses of xylem conductivity due to embolism (Tyree and Sperry 1989). Thus, with the remaining hydraulic conditions being equal, trees growing in drier sites would benefit from an enhanced stomatal control of transpiration. Long-term structural changes in the transpiration pathway also contribute to maintain a favorable hydric status under water-limiting conditions. A simple steady-state hydraulic model (Whitehead et al. 1984a) predicts three mechanisms (in addition to stomatal regulation) to prevent the development of potentially damaging tensions in the xylem: (1) increased biomass allocation to conducting tissue, (2) higher sapwood conductivity, or (3) an enhanced resistance to embolism (Maherali and DeLucia 2000). Recent models also include an enhanced allocation to belowground biomass in response to hydraulic constraints (Magnani 2000; Magnani et al. 2002). All these adjustments bear costs in terms of carbon gain and growth through reduced photo-

synthetic tissue, increased respiration (Ryan and Waring 1992) or increased construction costs (Hacke et al. 2001).

A reduction in the amount of leaf area relative to the conducting sapwood area with increasing evaporative demand has been shown to occur in several conifers, particularly pines (DeLucia et al. 2000), such as *Pinus ponderosa* Laws. (Callaway et al. 1994) or *Pinus sylvestris* L. (Mencucini and Grace, 1995; Mencuccini and Bonosi 2001). In addition, higher allocation to roots has been reported in trees growing in soil water-limited environments for *Pinus taeda* L. (Hacke et al. 2000) and *Pinus palustris* Mill. (Addington et al. 2006). Apart from climatic and soil factors, tree size (McDowell et al. 2002) and stand-level traits, such as tree density (Whitehead et al. 1984b), can also influence hydraulic architecture.

The phenomenological response of canopy-averaged G_s to vapour pressure deficit (D), can be described by the equation (Oren et al. 1999),

$$G_s = G_{s,ref} - m \cdot \ln D \quad (1)$$

in which G_s sensitivity to D (slope m in Eq. 1) tends to be linearly related to the magnitude of the reference conductance at $D = 1$ kPa ($G_{s,ref}$) (Oren et al. 1999). Hence, if populations growing in warmer/drier climates showed an enhanced stomatal closure with increasing D , this response would be proportional to the value of $G_{s,ref}$. In this case, one would predict an increase in *absolute* sensitivity to D accompanied by an increase in $G_{s,ref}$ under warmer/drier conditions (Fig. 1, black triangles compared to black dots). It is important to distinguish between absolute and relative stomatal sensitivity. The former is defined here as the absolute decline in G_s per relative increase in D (that is, the value of the parameter m ; see first derivative of G_s with respect to D in Eq. 1), whereas the relative stomatal sensitivity is defined in this study as the relative decline in G_s per relative increase in D (that is: $m/G_{s,ref}$). If there were a disproportional increase, with respect to $G_{s,ref}$, in the rate of stomatal closure with D , then an enhanced *relative* sensitivity would occur (Fig. 1, white circles). Alternatively, some authors (Mäkelä et al. 1996) predict a decrease in G_s with increasing evaporative demand, which implies low values of G_s at low D and low sensitivity to D (Fig. 1, white diamonds). In fact, this response has been shown in some acclimation experiments with plants grown under different levels of D (Leuschner 2002). Finally, one could predict a disproportional increase in $G_{s,ref}$ (with regards to m) at high D , which would correspond to a decline in relative stomatal sensitivity at high D (Fig. 1, inverted triangles). Note that these different scenarios have fundamental implications on transpiration rates per unit of leaf area (E_L) (Fig. 1a) and on calculated leaf water potentials. Compared to the baseline scenario, reductions in E_L at a

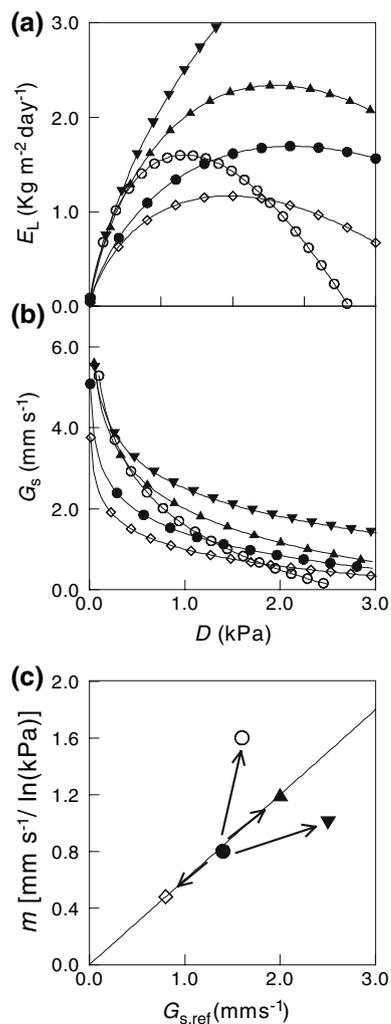


Fig. 1 **a** Theoretical relationship between transpiration per unit leaf area (E_L) and vapour pressure deficit (D) according to stomatal responses described in **b** and **c**. The notional baseline scenario is represented by the *black dots*. Phenotypic plasticity in stomatal responses to water deficits at warmer/drier sites is represented by two alternative cases, whereby stomatal conductance (G_s) at a reference $D = 1$ kPa ($G_{s,\text{ref}}$) and slope (m) either decrease (*diamonds*) or increase (*triangle*) proportionally in warmer/drier sites. The proportionality between $G_{s,\text{ref}}$ and m is ca. 0.6 (Oren et al. 1999). Alternatively, $G_{s,\text{ref}}$ can increase more than proportionately (*inverted triangles*) or less than proportionately (*white dots*) with respect to m

given D are only obtained by either reducing $G_{s,\text{ref}}$ or by increasing the relative stomatal sensitivity (i.e., $m/G_{s,\text{ref}}$).

Experimental evidence based on analysis of carbon isotope discrimination supports that indeed, stomatal control increases with aridity for different conifers (Warren et al. 2001) and more specifically, within the Pinaceae family (Martínez-Vilalta et al. 2004). In addition, pines show little plasticity in their vulnerability to embolism (Maherali and DeLucia 2000; Piñol and Sala 2000; Stout and Sala 2003), suggesting a dominant role of the above-mentioned hydraulic adjustments and stomatal regulation

as principal mechanisms of drought acclimation. This structural plasticity may allow trees from xeric habitats to show similar (Addington et al. 2006) or even much higher (Maherali and DeLucia 2001) maximum G_s s than trees in more mesic areas.

Scots pine (*P. sylvestris* L.) is one of the most widely distributed tree species on earth, spanning a vast climatic gradient from eastern Siberia to southern Spain (Fig. 2). Hence, it has been the object of several studies on geographical variation of different functional traits, including structural plasticity in response to drought (Berninger and Nikinmaa 1997; Mencuccini and Bonosi 2001; Mencuccini and Grace 1995; Palmroth et al. 1999). A recent study has also reviewed plot-scale water and carbon fluxes of Scots pine across some European sites (Ceulemans et al. 2003). Scots pine maintains the integrity of the hydraulic continuum under atmospheric and edaphic drought by closing stomata (Irvine et al. 1998), but its low resistance to embolism (Cochard et al. 1992; Jackson et al. 1995) makes it vulnerable to extremely dry periods in southernmost populations (Martínez-Vilalta and Piñol 2002).

In this study we conducted a meta-analysis of Scots pine sap flow data from several sites across Eurasia, including a climatic gradient in Western Europe, to compare the response of sap flow rates and canopy G_s to the main environmental variables. Regarding physiological plasticity in response to drought, we hypothesized that populations growing in warmer/drier sites should have: (1) higher E_L at a reference D , and (2) an enhanced *absolute* stomatal sensitivity to atmospheric drought, proportional to the value of conductance at low D (Oren et al. 1999). Finally, we hypothesized that: (3) physiological and structural plasticity combine to maintain homeostasis in plant water transport across different climates.

Materials and methods

Study sites: climatic and stand parameters

The eight studied sites were highly representative of the broad range of climatic conditions across the geographical distribution of Scots pine (Table 1), from Siberian boreal forests to montane populations in the north-eastern Iberian Peninsula (Fig. 2). Mean annual temperature, mean annual precipitation, elevation and geographical coordinates were available for all sites from the literature. Potential evapotranspiration (PET), calculated with a modified Thornthwaite method, was taken from a global dataset with a resolution of 0.5° (UNEP 1992), in order to make it comparable across the sites. Summer vapour pressure deficit ($\text{VPD}_{\text{summer}}$) and summer air temperature (T_{summer}) were calculated by averaging the monthly VPD and air tem-

Fig. 2 Geographical distribution of Scots pine (Critchfield and Little 1966) and location of study sites. See Table 2 for site abbreviations

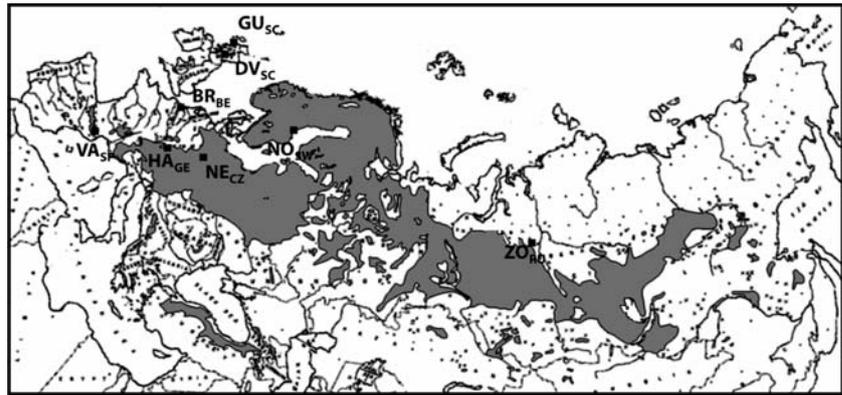


Table 1 Climatic characteristics of the studied sites (arranged from north to south). *MAT* Mean annual temperature, *MAP* mean annual precipitation, *VPD_{summer}* summer vapour pressure deficit, *PET*

potential evapotranspiration, *P/PET* precipitation to potential evapotranspiration ratio, *Cb* Marine, *Cs* Mediterranean, *Dc* Subarctic, *Db* Continental

Site	Latitude	Longitude	Elevation (m)	MAT ^a (°C)	MAP ^a (mm year ⁻¹)	VPD _{summer} ^b (kPa)	PET ^c (mm year ⁻¹)	P/PET	Climate ^d
Zotino	60°43'N	89°08'E	100	-3.7	493	1.36	218	2.3	Dc
Norunda	60°05'N	17°29'E	41	5.5	527	0.99	311	1.7	Dc
Guisachan	57°16'N	4°49'W	300	6.5	1,215	0.66	231	5.3	Cb
Devilla	56°02'N	3°43'W	75	8.5	820	0.80	249	3.3	Cb
Brasschaat	51°18'N	4°31'E	16	9.8	767	1.16	412	1.9	Cb
Nedamov	50°28'N	14°35'E	300	8.5	456	1.45	482	0.9	Db
Hartheim	47°56'N	7°37'E	201	9.8	647	1.17	489	1.3	Db
Vallcebre	42°12'N	1°49'E	1,260	8.6	924	1.35	733	1.3	Cs

^a Taken from the respective publications

^b Taken from the climatic database in New et al. (1999)

^c Taken from the climatic database in UNEP (1992)

^d Climate codes according to Köppen climate classification

peratures, respectively of June, July and August, which were also taken from a global dataset (New et al. 1999). Note that for vapour pressure deficit the symbol *D* will be used for the daytime averages and *VPD* for the climatic parameter referring to the long-term evaporative demand.

The stands at the different sites, most of which were monospecific, varied in their structure and age (Table 2). The stands in Brasschaat and Hartheim were thinned during the measurement period. In the analyses we lumped together pre- and post-thinning data for each of those sites, as we were interested in an average response of each site. Treating separately data before and after the thinning meant, for example, having two different periods with contrasting meteorological conditions for a given site, missing what would be an average response to climate. Leaf to sapwood area ratios ($A_L:A_S$) were always related to sapwood measured at breast height. Leaf area (projected, summertime values) was either measured on harvested trees or calculated from reported values of stand sapwood basal area and maximum leaf area index (LAI) of each site.

Dataset preparation

The main meteorological factors which determine transpiration from forest canopies (i.e. radiation, vapour pressure deficit and soil moisture) were first converted into comparable quantities. Global shortwave radiation (R_g) was converted into photosynthetically active radiation (PAR, $\mu\text{mol m}^{-2} \text{s}^{-1}$) wherever necessary. PAR was estimated as 45% of R_g (González and Calbó 2002), and the coefficient used to convert energy to photon flux units was $4.6 \mu\text{E J}^{-1}$. If two different measurements of radiation were taken simultaneously [i.e. PAR and net radiation (R_n)] linear regressions were performed to fill in the gaps in PAR or R_g data. We used 24-h averages of PAR, thus taking into account differences in daylength across the sites.

Vapour pressure deficit was present in all datasets, except for the dataset from Nedamov for the year 1980, where only radiation, temperature and PET data were available. In this specific case, we used meteorological data from the same site during the previous year, when relative humidity measurements were available, to perform a multiple regression

Table 2 Main biometric characteristics of the studied stands. A_B Basal area, LAI_{max} maximum leaf area index, DBH mean diameter at breast height, $A_L:A_S$ leaf-to-sapwood area ratio, *Man.* management

Site	Code ^a	Age (years)	A_B^b (m ² ha ⁻¹)	Density ^b (trees/ha)	LAI_{max} (m ² m ⁻²)	DBH (cm)	Height (m)	$A_L:A_S$ (m ² cm ⁻²)	Man.
Zotino	ZO _{RU}	138	28.2 ^c	1,110	1.3	18.0	15.2	0.226	Natural
Norunda	NO#1 _{SW}	50	28.9 (64%)	872 (59%)	5.0	20.0	17.4	0.141	Natural
Norunda	NO#2 _{SW}	100	39.1 (63%)	735 (42%)	4.5	32.2	26.8	0.099	Natural
Guisachan ^d	GU#1 _{SC}	28	20	316	0.9	10.8	7.2	0.188	Natural
Guisachan ^d	GU#2 _{SC}	49	20	316	0.9	37.6	11.7	0.180	Natural
Guisachan ^d	GU#3 _{SC}	195	20	316	1.6	83.1	17.6	0.153	Natural
Devilla	DV _{SC}	40	28.0	836	2.3	20.1	15.0	0.108	Plantation
Brasschaat ^e	BR _{BE}	70	31.4/27.0	560/376 (80%)	3/3	26.8/30.0	20.6/21.3	0.160	Plantation
Nedamov	NE _{CZ}	100	17.2	266	2.0	42.1	13.8	–	Plantation
Hartheim ^e	HA _{GE}	36	36.7/24.1	3,753/1,754	3/1.9	10.8/12.9	11.5/12.3	0.090	Plantation
Vallcebre	VA _{SP}	40	44.7	2165	2.4	15.2	11	0.065	Natural

^a Site abbreviations and geographical subscripts (*RU* Russia, *SW* Sweden, *SC* Scotland, *BE* Belgium, *CZ* Czech Republic, *GE* Germany, *SP* Spain)

^b In multispecific stands, the total is given, with the corresponding percentage of Scots pine *in parentheses*, where available

^c Calculated from mean tree diameter and tree density

^d Trees belonging to the different age classes are growing in the same stand. Basal area and density are given for the whole stand

^e Pre/post thinning values

analysis including air temperature (T_a), R_g and PET. This regression, which explained 84% of the variance in D , was used to estimate D for the Nedamov dataset in 1980.

SMD in the upper soil was calculated as (Granier and Loustau 1994):

$$SMD = \frac{\theta_{max} - \theta}{\theta_{max} - \theta_{min}}, \quad (2)$$

where θ is volumetric soil moisture (cm³ cm⁻³), and θ_{min} and θ_{max} represent minimum and maximum values measured at each site during the period of study. Soil moisture was measured in the first 20 to 30 cm below the surface for all sites, except Brasschaat, where it was measured in the top 50 cm.

Sap flow measurements

Sap flow was measured on dominant trees at breast height in all locations using thermal methods (Table 3). Heat dissipation (HD) (Granier 1985, 1987) and tissue heat balance (Čermák et al. 1973, 2004) were the most employed methods, whereas the heat pulse velocity (Marshall 1958; Swanson and Whitfield 1974) and heat field deformation (Nadezhdina et al. 1998) techniques were used in only one site each. Tissue heat balance and HD methods have already been cross-compared for Scots pine (Köstner et al. 1996; Lundblad et al. 2001), and some disagreement in the quantitative estimates of sap flow density was found. Based on these studies we concluded that these methods

obtain similar qualitative responses to environmental variables, but that there may be a degree of uncertainty in the absolute sap flow density values and in the subsequent scaling-up to whole-stem sap flow which should be taken into account when interpreting our results. This will be considered further in the Discussion.

Tree sap flow per unit leaf area (Q_L) of dominant trees was obtained from sap flow measurements and tree leaf area, except for Devilla, where a stand-level leaf $A_L:A_S$ was used to convert sapwood area-related sap flow to Q_L . E_L for each site was obtained averaging the individual values of Q_L , except for Nedamov, where it was derived from stand transpiration (E_c) divided by LAI. The number of trees measured in each stand varied between 3 and 11 (Table 3), and only days with a minimum number of two trees measured simultaneously were considered in the analyses. Measurement periods began in April/May and finished in September/November for all the stands, except for Vallcebre, which included winter and early spring data. When the measurement period extended to early spring, autumn or winter, data were corrected for seasonal changes in leaf area, as supplied by the authors of the studies or estimated from data published elsewhere (Beadle et al. 1982; Gond et al. 1999; Wang et al. 2005).

Analysis of sap flow responses to environmental variables across the sites

We used a Jarvis-type, multiplicative model, originally developed for G_s (Jarvis 1976), but also applicable to sap

Table 3 Years of measurement, number of measured trees (n) and sap flow method used in each stand. Relevant bibliographic references are also shown. *HD* Heat dissipation, *HFD* heat field

deformation, *HPV* heat pulse velocity, *THB* tissue heat balance. See Table 2 for site abbreviations

Dataset	Years	n	Sap flow method	References
ZO _{RU}	1995–1997	3	HD	Zimmermann et al. (2000)
NO#1 _{SW}	1998–2000	7	THB	Lagergren and Lindroth (2002, 2004)
NO#2 _{SW}	2001–2002	6	THB	Lindroth et al. (unpublished results)
GU#1 _{SC}	2003	12	HD	Martínez-Vilalta et al. (2007)
GU#2 _{SC}	2003	5	HD	Martínez-Vilalta et al. (2007)
GU#3 _{SC}	2003	14	HD	Martínez-Vilalta et al. (2007)
DV _{SC}	1995 ^a	4	HPV	Irvine et al. (1998)
BR _{BE}	1997, 2000	7	HFD	Meiresonne et al. (2003)
NE _{CZ}	1980	– ^b	THB	Čermák et al. (unpublished results)
HA _{GE}	1993–1994	10	HD, THB	Granier et al. (1996), Köstner et al. (1996)
VA _{SP}	2003–2004	11	HD	Poyatos et al. (2005)

^a Only data from the control treatment were used

^b Stand-level data

flow (Oren and Pataki 2001), in which daily totals of sap flow per unit leaf area (i.e. E_L ; $\text{kg m}^{-2} \text{day}^{-1}$) were related to daytime averages (daily averages in the case of PAR) of the main meteorological variables:

$$E_L = \frac{\text{PAR}}{\text{PAR}_{\text{max}}} (a + b \cdot \ln D) (1 - k \cdot \exp(\text{SMD})). \quad (3)$$

In the following sections the parameters a , b and k (Eq. 3) fitted for each dataset are related to the main climatic (Table 1) and structural (Table 2) parameters of each site. Each of these parameters bears a physiological meaning: a is related to the absolute value of sap flow; whereas b and k measure the sensitivity to changes in D and SMD, respectively. Model fitting was carried out using the Levenberg-Marquardt algorithm in SPSS v.13.0 (SPSS, Chicago, Ill.), after discarding data with average daytime $D < 0.05$ kPa.

Analysis of canopy G_s

As Scots pine stands are well coupled to the atmosphere (Whitehead and Jarvis 1981), canopy G_s (mm s^{-1}) was calculated from daily values of sap flow and meteorological variables using the inversion of the Penman–Monteith equation for well-coupled canopies (Phillips and Oren 1998):

$$G_s = \frac{\gamma \cdot \lambda \cdot E_L}{\rho_a \cdot c_p \cdot D} \cdot 10^3, \quad (4)$$

where E_L is in $\text{kg m}^{-2} \text{s}^{-1}$, γ is the psychrometric constant (kPa K^{-1}), λ is the latent heat of vaporisation of water (J kg^{-1}), ρ_a is air density (kg m^{-3}), c_p is specific heat of air

($\text{J K}^{-1} \text{kg}^{-1}$), D is daytime average vapour pressure deficit (kPa) and the 10^3 factor is used to convert conductance units from m s^{-1} to mm s^{-1} .

The relationship between G_s and D was analyzed using boundary-line analysis performed with quantile regression (Cade and Noon 2003; Koenker and Basset 1978), using the statistical package R (R version 2.0.1; R Development Core, Vienna). Regression results for the upper quantiles yield optimal relationships between a pair of variables when other factors not included in the model may also be affecting the response of the dependent variable. We assumed that the linear regression results for the 95th quantile were representative of the boundary-line relationship between G_s and $\ln D$ (Eq. 1), and identified the obtained intercept and slope with the model parameters $G_{s,\text{ref}}$ and m , respectively (Eq. 1).

Analysis of sap flow-related parameters with respect to climatic and structural variables across sites

The variability of sap flow and canopy G_s across sites was related to factors indicative of climatic dryness, tree level structural adjustments and stand characteristics. These three factors were calculated as follows. Two separate principal component analysis (PCA) were run, one with climatic variables [$\text{VPD}_{\text{summer}}$, T_{summer} , mean annual precipitation, and precipitation to PET ratio (P/PET)] and the other with stand characteristics (age, density, basal area and LAI). The scores of the first PC axis from the first and second PCAs (90 and 52% of the variance explained, respectively) were used as proxies to climatic (CLIM) and stand-level (STAND) factors, respectively. Leaf to sapwood area ratio multiplied by tree height (h) ($A_L:A_S \cdot h$)

accounted for the tree-level structural adjustments (STR). Stepwise multiple regressions were carried out between the parameters from the sap flow and conductance analyses and the predictors CLIM, STAND and STR. All the analyses were carried out using SPSS v.13.0 (SPSS).

CLIM and STR factors explained most of the variance in sap flow-related parameters (see Results). However STAND factors were also relevant for some of the response variables. Since we interpreted the first axis of the PCA on stand characteristics as a measure of stand development, we selected the sites with a relatively homogeneous age (35–50 years old, $n = 5$) to conduct a detailed climatic analysis. In this way we also avoided the problem of pseudoreplicating stands, as different-aged stands within the same site had identical climate but potentially different sap flow dynamics. All datasets were used when analyzing structural effects. We accepted that correlations were marginally significant when $0.05 < P < 0.1$.

As mentioned above, some studies have found quantitative discrepancies between the HD and other sap flow methods (Köstner et al. 1996; Lundblad et al. 2001). To test the sensitivity of the relationships we found to the variability posed by the use of different sap flow methods, we carried out a sensitivity analysis based on a Monte Carlo approach as follows. For each detected significant correlation ($P < 0.05$) we repeated the analysis 1,000 times after multiplying the (sap flow-related) value for each site by a coefficient. This coefficient was equal to 1 for the sites that used the HD method. For the rest of the sites the coefficient was sampled randomly and independently for each realization from a log-normal distribution of numbers with $\mu = 0$ and $\sigma = 0.2$, so that 99% of values were between ca. 0.6 and 1.7. A log-normal distribution was used to account for the fact that the limited amount of experimental data available suggests that the HD method may consistently underestimate sap flow compared to other methods. We then analyzed the percentage of realizations in which the original relationship was retained. Dataset coding for each site, years of measurement and relevant references for each study are summarized in Table 3.

Results

Sap flow multiplicative model

The sap flow multiplicative model explained 42–78 % of the variance in E_L across the different datasets. Parameter a was found to be highly significant in all the datasets; sensitivity to D and SMD (parameters b and k) was non-significant only in Devilla and for one age class in Guisachan, respectively (Table 4). When fitted to the Brasschaat data, the model yielded negative values for parameters b and k ,

meaning that sap flow would decrease with D and increase with SMD (data not shown). Similar results were obtained when using alternative nonlinear regression algorithms. The reason for this problem lies in the similarity in the relationships between sap flow and both PAR and D for the range of D values of the Brasschaat dataset, together with the high correlation between these two variables. This has likely led to collinearity problems and the inability to fit this particular model. Since negative values of b and k are not consistent with our present understanding of sap flow responses to meteorological variables and are likely artefactual, this dataset was excluded from some of the analyses.

Analysis of sap flow-related parameters against climatic, structural and stand factors

All the parameters of the sap flow multiplicative model were related to climate drivers (Table 5). Parameter a , in addition, depended on structural adjustments at the tree level (i.e. $A_L:A_S \cdot h$), which was the only detected influence on the sensitivity of G_s to $\ln D$ (parameter m). $G_{s,ref}$ was the only parameter primarily related to stand characteristics according to our results (Table 5). Overall this analysis suggests a major influence of climate, together with structural adjustments at the tree level on sap flow and stomatal control. In the following section we will investigate in detail the relationships between the sap flow-related parameters and the main climatic and structural factors.

Physiological plasticity with respect to evaporative demand and water availability

When the analysis was restricted to the five sites included in the climatic dataset, all the sap flow-related parameters

Table 4 Model parameters (\pm SE), valid n (number of valid date points) and R^2 for the sap flow multiplicative model. For site abbreviations and datasets see Table 3

Dataset	n	R^2	Parameter values		
			a	b	K
NO#1 _{SW}	377	0.54	1.47 \pm 0.12**	0.16 \pm 0.07*	0.13 \pm 0.03**
NO#2 _{SW}	155	0.42	0.65 \pm 0.08**	0.39 \pm 0.08**	0.12 \pm 0.05*
GU#1 _{SC}	80	0.75	1.01 \pm 0.09**	0.16 \pm 0.04**	0.06 \pm 0.04 ^{n.s.}
GU#2 _{SC}	90	0.78	0.65 \pm 0.07**	0.14 \pm 0.03**	0.10 \pm 0.04*
GU#3 _{SC}	82	0.71	0.74 \pm 0.06**	0.14 \pm 0.03**	0.13 \pm 0.03**
DV _{SC}	177	0.49	1.46 \pm 0.10**	0.09 \pm 0.05 ^{n.s.}	0.16 \pm 0.02**
BR _{BE}	298	–	–	–	–
NE _{CZ}	117	0.71	0.67 \pm 0.08**	0.16 \pm 0.04**	0.23 \pm 0.04**
HA _{GE}	178	0.54	2.12 \pm 0.12**	0.32 \pm 0.10**	0.33 \pm 0.01**
VA _{SP}	382	0.67	2.38 \pm 0.09**	0.46 \pm 0.06**	0.24 \pm 0.01**

* $0.01 < P < 0.05$, ** $P < 0.01$, ^{n.s.} $P > 0.05$

Table 5 Results of the stepwise multiple regression of sap flow-related parameters with respect to climate (*CLIM*), tree-level structural adjustments (*STR*) and stand characteristics (*STAND*). These variables^a have been derived from PCA analysis of individual characteristics of each dataset (see text for details). Coefficients of the regression equation (\pm SE), n and statistical significance, R^2 and adjusted R^2 are shown

Parameter	Predictor	Coefficient	n	P	R^{2b}	Adjusted R^{2b}
a	Constant	2.52 ± 0.28	8	0.000	0.88	0.83
	STR	-0.60 ± 0.15		0.001		
	CLIM	0.33 ± 0.12		0.035		
b	Constant	0.27 ± 0.04	8	0.001	0.50	0.41
	CLIM	0.10 ± 0.04		0.052		
k	Constant	0.18 ± 0.02	8	0.000	0.49	0.41
	CLIM	0.062 ± 0.03		0.052		
$G_{s,ref}$	Constant	1.32 ± 0.15	10	0.000	0.49	0.43
	STAND	0.43 ± 0.15		0.024		
m	Constant	2.53 ± 0.49	10	0.001	0.51	0.45
	STR	-0.611 ± 0.21		0.021		

^a The criteria for variable acceptance and rejection were $F \leq 0.10$ and $F \geq 0.15$, respectively

^b Whole model R^2

showed clear relationships with all climatic variables related to evaporative demand (VPD_{summer} , P/PET , PET and T_{summer}). Since these four variables were highly correlated and showed very similar trends, in the following we only present the results with regard to VPD_{summer} and P/PET . In the sap flow multiplicative model, parameter a , related to the absolute magnitude of sap flow, was positively correlated with VPD_{summer} (Fig. 3a) and negatively correlated with P/PET ($R^2 = 0.83$, $P = 0.030$). Sensitivity to D and SMD increased with VPD_{summer} (Fig. 3c, e) and showed declining trends with P/PET ; the latter patterns, however were not statistically significant (parameter b , $R^2 = 0.59$, $P = 0.129$; parameter k , $R^2 = 0.65$, $P = 0.100$). Multiplying parameter a by $A_L:A_S$ (representing a value of reference sap flow per unit sapwood) did not result in any significant relationship with VPD_{summer} or P/PET . The observed relationships with P/PET did not change when PET was obtained with alternative methods (Turc, Penman–Monteith reference evapotranspiration) (data not shown).

Reference canopy G_s ($G_{s,ref}$) was positively correlated with VPD_{summer} (Fig. 4a) and negatively correlated with P/PET ($R^2 = 0.88$, $P = 0.019$). Canopy G_s also showed an increased sensitivity to D at lower P/PET values ($R^2 = 0.83$, $P = 0.030$) and an increasing trend with VPD_{summer} , which was not statistically significant (Fig. 4c). In fact, except for Guisachan, all locations showed very similar values of parameter m (ca. $2 \text{ mm s}^{-1} \text{ ln kPa}^{-1}$). Overall, the general trends in stomatal param-

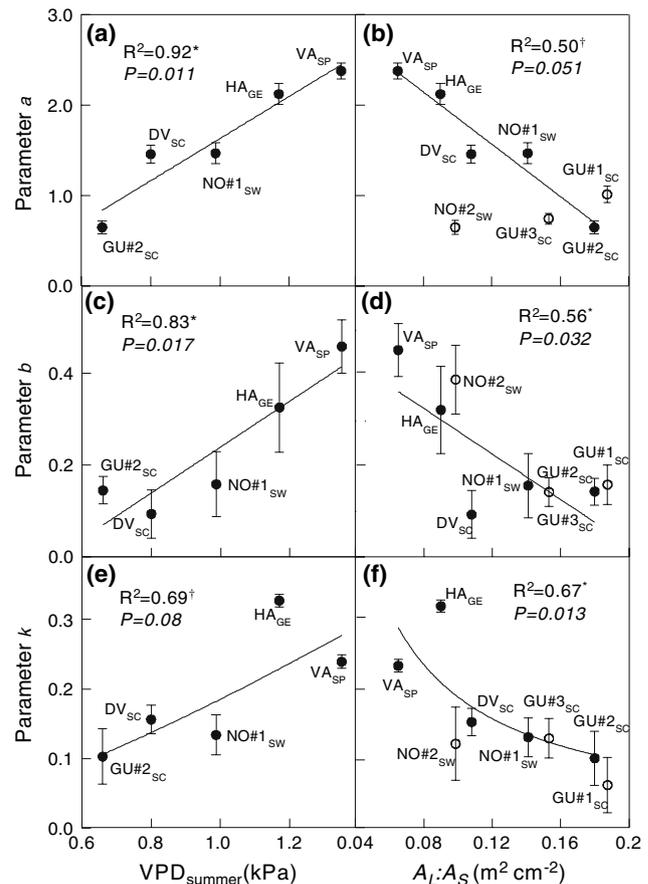


Fig. 3 Relationships between the three parameters (\pm SE) of the sap flow multiplicative model (Eq. 3) and long-term summer vapour pressure deficit (VPD_{summer}) and leaf to sapwood area ratios ($A_L:A_S$) for the different study sites. The sites included in the climatic analysis are represented by closed symbols. Statistically significant relationships are marked with full lines. \dagger $0.05 < P < 0.1$, $*$ $0.01 < P < 0.05$. See Table 2 for site abbreviations

ters with respect to climate were consistent with the results of the sap flow model.

The parameters $G_{s,ref}$ and m were positively related in a logarithmic fashion, showing a tendency for sensitivity to D to plateau at high $G_{s,ref}$ (Fig. 5). No significant trend was found between the ratio of these two parameters ($m/G_{s,ref}$) and VPD_{summer} ($R^2 = 0.30$, $P = 0.342$) or P/PET ($R^2 = 0.06$, $P = 0.680$) for the stands in the climatic analysis.

Structural and physiological plasticity

$A_L:A_S$ showed a marginally significant increase with decreasing VPD_{summer} ($R^2 = 0.75$, $P = 0.056$) and a non-significant decline with increasing P/PET ($R^2 = 0.62$, $P = 0.111$) for the sites included in the climatic analysis. However, the inclusion of the stands reviewed in Mencuccini and Bonosi (2001) which lay within the age range of our climatic analysis, resulted in a much stronger linear trend of $A_L:A_S$, with VPD_{summer} ($R^2 = 0.60$, $P = 0.003$).

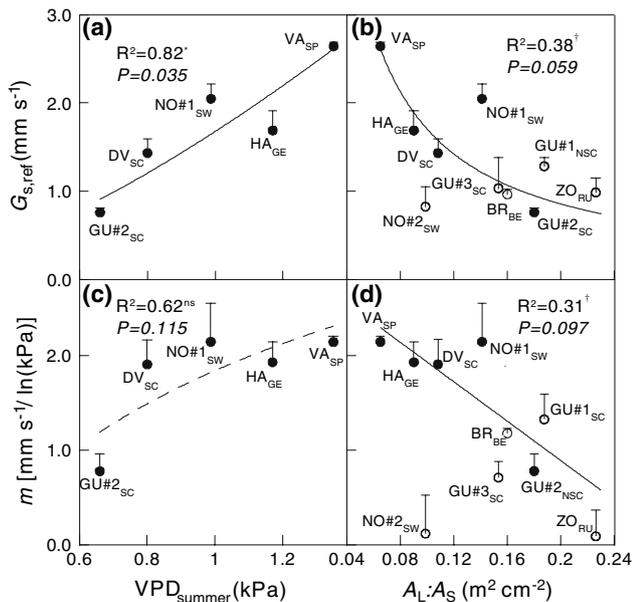


Fig. 4 Relationship of $G_{s,ref}$ (Eq. 1) and absolute sensitivity of G_s with respect to $\ln D$ (m ; Eq. 1) with long-term VPD_{summer} and $A_L:A_S$ for the different study sites. The sites included in the climatic analysis are represented by closed symbols. Error bars are +ve SE. † $0.05 < P < 0.1$, * $0.01 < P < 0.05$, ns $P > 0.1$. See Table 2 for site abbreviations and Figs. 1 and 3 for other abbreviations

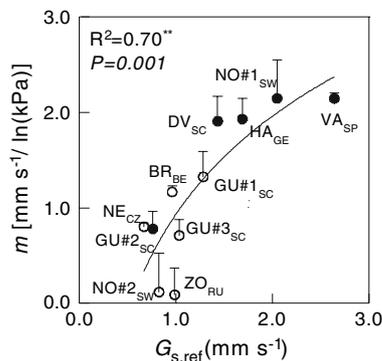


Fig. 5 Relationship between $G_{s,ref}$ (Eq. 1) and absolute sensitivity of G_s with respect to $\ln D$ (m ; Eq. 1). The sites included in the climatic analysis are represented by closed symbols. Error bars are +ve SE. See Table 2 for site abbreviations and Fig. 1 for other abbreviations

We examined the relationships between the parameters describing the stomatal behavior of Scots pine (a , b , k , $G_{s,ref}$ and m) and $A_L:A_S$, pooling all the datasets regardless of stand age (Figs. 3, 4). All the parameters of the multiplicative model showed significant decreasing trends with increasing $A_L:A_S$, except parameter a , for which the correlation was very close to being significant at the 0.05 level (Fig. 3b, d, f). Similarly, $G_{s,ref}$ and m also decreased with $A_L:A_S$, although in both cases the relationships were only marginally significant (Fig. 4b, d). However, multiplying $A_L:A_S$ by h ($A_L:A_S \cdot h$, see Discussion for further infor-

mation regarding the rationale for such an analysis), resulted in significant linear correlations for parameter a ($R^2 = 0.54$, $P = 0.010$), $G_{s,ref}$ ($R^2 = 0.67$, $P = 0.013$) and m ($R^2 = 0.51$, $P = 0.014$). For parameters b and k , the relationships with $A_L:A_S \cdot h$ were better described by a second-order polynomial fit (parameter b , $R^2 = 0.69$, $P = 0.011$; parameter k , $R^2 = 0.45$, $P = 0.069$). These results and the departure of the parameter m for $NO\#2_{SW}$ from the observed general relationship between $G_{s,ref}$ and $A_L:A_S$ (Fig. 4d), suggest a decrease in stomatal sensitivity to D with increasing mean height of the trees, as trees in this stand were appreciably taller than in any other site (Table 2). This was supported by our results for parameter m (Fig. 7c), although a parallel decrease of $G_{s,ref}$ with height was not found (Fig. 7a) and therefore, h cannot be regarded as the only factor affecting stomatal conductance parameters. Both $G_{s,ref}$ and m decreased with age of the stand (Fig. 7b, d), showing tighter relationships than those observed for h . The ratio between these two parameters, $m/G_{s,ref}$, was found to decrease with age, but the trend was only marginally significant ($R^2 = 0.32$, $P = 0.07$). We did not observe a clear pattern in the relationships between $A_L:A_S$ and h or tree age (Fig. 7e, f).

The results of the Monte Carlo sensitivity analysis performed to test the robustness of the observed relationships showed that in seven out of nine cases the original significant relationship was retained in more than 50% of the realizations. The relationship between the parameter a and P/PET was retained in 45% of the realizations, and the one between m and P/PET was retained only in 38% of the realizations. In these two cases, the relationship remained at least marginally significant in >60% of the realizations. Overall (considering the nine tested relationships) in 75% of the realizations the result was consistent with the original conclusion (significance at the 0.05 level). Considering the high degree of variability across methods that we allowed in the sensitivity analysis we concluded that our main results are robust with regard to potential quantitative differences between sap flow methods.

Discussion

Methodological issues

The range of environmental conditions in this meta-analysis is greater than in previous studies concerning structural adaptation to drought in *P. sylvestris* (Palmroth et al. 1999; Mencuccini and Bonosi 2001), although our climatic analysis is limited to sites within the climatic limits of the mentioned studies. To our knowledge, this is the first study comparing the physiological responses of sap flow across

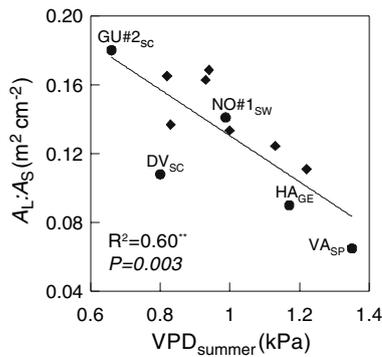


Fig. 6 Relationship between $A_L:A_S$ and long-term VPD_{summer} including stands in the climatic analysis of the present study (dots) and stands reviewed in Mencuccini and Bonosi (2001) within the age range of our climatic analysis (diamonds). See Table 2 for site abbreviations, and Fig. 3 for other abbreviations

the range of a tree species, although a review of E_c across Europe for *Fagus sylvatica* has recently been published (Schipka et al. 2005).

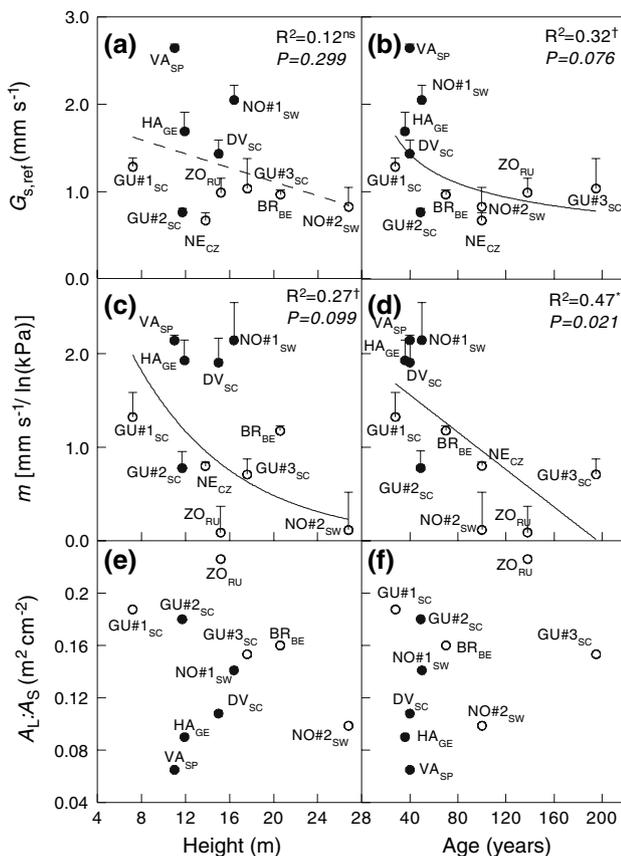


Fig. 7 Variation of $G_{s,ref}$ (Eq. 1), absolute sensitivity of G_s with respect to $\ln D$ (m ; Eq. 1), and $A_L:A_S$ with respect to age and height of the stand. The sites included in the climatic analysis are represented by closed symbols. Error bars are +ve SE. See Table 2 for site abbreviations and Figs. 1 and 3 for other abbreviations

Except for the Brasschaat site, the performance of the multiplicative sap flow model in describing sap flow responses to environmental variables was reasonably good, given the fact that a common model was used for all sites (Table 4). Because of the methodological differences in sap flow techniques across sites, the possibility exists that some or all of our trends are artefactual. We think this is unlikely. First, because of the form of our model, one would expect that parameter a would be most affected, and yet significant correlations were also found for parameters b and k , and always in the directions expected from our physiological understanding of these processes. Second, the extreme sites with regard to VPD_{summer} and P/PET , within the European climatic gradient (Vallcebre and Guisachan), whose values strongly affect the regressions, were both obtained using the HD method. Hence, it is more likely that the diversity of employed methods resulted in random noise around the regression line. Besides, the results of the sensitivity analysis confirm that the observed relationships are robust with regards to potential systematic differences between methods, including also to some degree the uncertainties derived from the radial and azimuthal variability of sap flow.

Climate-driven physiological plasticity

Our results showed that E_L at reference conditions increased with long-term evaporative demand (Fig. 3a) and that the increase in E_L with vapour pressure deficit was steeper in warmer/drier sites (Fig. 3c). As a result, E_L area at a given D was always greater in warmer/drier sites. Indeed, $G_{s,ref}$ was shown to increase in warmer/drier sites whereas absolute sensitivity to D (parameter m) was very similar across stands in the climatic analysis, with the exception of Guisachan (Fig. 4). Therefore, although the variation in absolute sensitivity of G_s to D with respect to evaporative demand is not clear, we have found evidence that relative sensitivity ($m/G_{s,ref}$) decreases in warmer/drier climates. The observed relationship between m and $G_{s,ref}$ (Fig. 5) confirms that m does not increase proportionally at high $G_{s,ref}$. Although this relationship is unlikely to be affected by boundary-layer conductance effects, due to the high coupling between Scots pine canopies and the atmosphere (Whitehead and Jarvis 1981), the observed departure from proportionality might be due, at least partially, to the different ranges of D occurring across sites (Oren et al. 1999).

Although stomatal control has been reported to increase with aridity within the Pinaceae family (Martínez-Vilalta et al. 2004), studies on ponderosa pine showed that maximum G_s estimated using sap flow techniques was about 4 times larger in desert than montane populations (Maherali and DeLucia 2001). Slightly higher conductances were also

found in longleaf pines from xeric habitats in comparison to those from mesic habitats; a slight increase in absolute sensitivity of G_s to D in the drier stand was also reported (Addington et al. 2006). A recent experimental study at the leaf level, has shown that absolute stomatal sensitivity to D increases with maximum summer VPD of the geographic origin of the species for several seedlings of temperate and rainforest trees (Cunningham 2004). However, in the same study, seedlings grown under experimentally manipulated low VPD showed an increase in parameter m , which we did not find in our results.

Our observations of higher G_s in drier/warmer climates are not supported by an increase in stomatal density with aridity (Luoma 1997). However, maximum stomatal aperture also depends on other physical and biological properties of guard cells and stomata (Lushnikov et al. 1995), which were not reported in the Luoma (1997) study.

Climate-driven structural plasticity

Scots pine has been shown to reduce the ratio between transpiring and conducting area when evaporative demand increases (Mencuccini and Bonosi 2001; Mencuccini and Grace 1995), a pattern that has also been observed for other pine species but not for all conifers (DeLucia et al. 2000). Indeed, using a smaller sample size than Mencuccini and Bonosi (2001), this trend was also observed in our stands, but merging both data sets, a much tighter relationship emerged (Fig. 6). In addition, there was a close link between this structural plasticity and the physiological behaviour of Scots pine across the studied climatic gradient (Figs. 3, 4). These findings are consistent with the observed patterns in stomatal behavior if we analyze the results within the framework of the hydraulic model proposed by Whitehead et al. (1984a):

$$A_L : A_S = \frac{cK_s \Delta\psi}{G_s D h}, \quad (5)$$

where K_s is sapwood conductivity, $\Delta\psi$ is the leaf-to-soil difference in water potential including the gravity effect, h is the height of the tree, and c groups a series of variables with a weak dependence on temperature. Although E_L at reference conditions was only marginally related to $A_L:A_S$ (Fig. 3b) the strength of the correlation improved considerably when, as suggested by Eq. 5, $A_L:A_S$ was multiplied by mean stand height. Because of the relevance of size-related structural changes, $A_L:A_S \cdot h$ is a better descriptor of the hydraulics of a developing stand than $A_L:A_S$ on its own. Overall, our results show that in warmer/drier climates, the product $G_s D$ was larger than in cooler/more humid conditions for any value of D . Therefore, climate-driven adjustments in $A_L:A_S$ are

necessary for homeostatic regulation of $\Delta\psi$ within the operative limits for Scots pine.

Notwithstanding this, homeostasis in water transport may not hold across the whole geographic distribution of Scots pine, as the abovementioned hydraulic adjustments may still not be enough to entirely regulate water potentials, leading to lower values of ψ in more arid environments. Although minimum ψ in *P. sylvestris* has been set at ca. -1.4 MPa in a recent review (Magnani et al. 2002), water potentials below -2 MPa have been measured in the Vallcebre site (R. Poyatos et al., unpublished results). This confirms previous observations in other populations from NE Spain (Martínez-Vilalta and Piñol 2002). Consequently, one would expect some degree of plasticity to occur across populations with respect to vulnerability to embolism. Water potentials causing 50% loss of conductivity at these Mediterranean sites (Martínez-Vilalta and Piñol 2002) are indeed much lower than the values reported in Magnani (2000).

Alternatively, according to Eq. 5, increasing sapwood K_s with evaporative demand, as found for *P. ponderosa* (Maherali and DeLucia 2000), would also increase the conducting efficiency and allow higher transpiration rates in drier climates. In that particular case, an elevated K_s was not associated with an increase in vulnerability to embolism. Indeed, high vapour pressure deficits have been related to larger tracheid lumen diameter in *P. radiata* Don seedlings (Whitehead et al. 1983) and higher temperatures have also been positively correlated with larger lumen diameter in Scots pine (Antonova and Stasova 1993). Moreover, latewood proportion has been shown to be higher in cooler climates, and lower in sites with reduced water availability (Rigling et al. 2002). These changes in latewood proportion agree with the abovementioned higher K_s in warmer/drier climates.

Soil- and stand-level factors

Comparing the responses of sap flow and G_s to SMDs across climates is much more difficult than observing patterns in the responses to atmospheric drought, due to our comparatively limited knowledge of the soil characteristics relevant to processes in the soil–plant–atmosphere hydraulic continuum (soil depths, vertical and horizontal extent of root systems, hydrodynamic properties of the soil) and the inherent heterogeneity of the soil matrix. Therefore, it is possible that the observed relationships with climate are due to differences in root systems across sites. For example, considerably higher root-to-leaf area ratios have been found in trees growing in soils with less available water (Hacke et al. 2000; Addington et al. 2006). Nevertheless, if we examine the values obtained for the parameter k in the climatic analysis (Fig. 3), the highest

value corresponds to Hartheim forest, where shallow soils and restricted access to groundwater makes it prone to intense soil water deficits (Sturm et al. 1998), and the lowest are found for the clayey and organic-rich soils in Guisachan.

A further complication of our analysis arises from the necessarily limited number of sites and the fact that the climatic variables were in some cases correlated with stand-level characteristics (Tables 1, 2). Despite the fact that our multivariate analysis of the effects of climatic, structural and stand variables on sap flow suggests a major role of climatic factors (Table 5), we cannot completely rule out the possibility that some of the correlations with climate are in fact due to changes in hydraulic architecture with stand density and/or basal area (Whitehead et al. 1984b).

Size/age-related hydraulic constraints

Apart from climate-driven hydraulic constraints, the increase of path length in tall trees poses an additional limitation on long-distance water transport. For most conifer species, $A_L:A_S$ is inversely related to h (McDowell et al. 2002), but these reductions in $A_L:A_S$ with h do not fully compensate for the size-related hydraulic constrictions and hence canopy conductance still declines with h (McDowell et al. 2002; Schäfer et al. 2000). Despite not finding a direct relationship between $G_{s,ref}$ and h (Fig. 7), we demonstrated the influence of climate- and size-induced changes in hydraulic architecture ($A_L:A_S-h$) on whole-plant transpiration. Relationships between physiological parameters and age were generally better than those found for size (Fig. 7). We reported a decline in $G_{s,ref}$ (Fig. 7b) and $m/G_{s,ref}$ with tree age, consistent with the results for other pine species (Ewers et al. 2005; Irvine et al. 2004). It must be noted that, unlike Scots pine, other conifer species with higher $A_L:A_S$, such as *Picea abies* (L.) Karst. or *Abies balsamea* (L.) Mill., tend to increase their $A_L:A_S$ with height (McDowell et al. 2002) or age (Köstner et al. 2002).

Acclimation or adaptation?

It seems obvious that the studied populations belong to different ecotypes of the same species (Molotkov and Patlaj 1991), but whether the observed gradient in stomatal responses to air humidity is due to ecotypic differentiation must be investigated. It has recently been shown that the contrasting hydraulic traits observed in montane and desert stands of *P. ponderosa* are not a result of ecotypic divergence (Maherali et al. 2002). Hacke et al. (2000) also found a high degree of plasticity in root systems of genetically identical *P. taeda* trees. Structural acclimation to high

evaporative demands in Scots pine was first shown in two stands with identical genetic origin (Mencuccini and Grace 1995). These findings are consistent with the general view that pioneer species such as Scots pine show high phenotypic plasticity (Sultan 2000).

Effects of the observed patterns on carbon and nutrient dynamics

One of the consequences of maintaining high G_s s in dry climates is the decline in water use efficiency due to the non-linear relationship between G_s and carbon assimilation (Cowan 1982). Our results contradict theoretical models of optimal gas exchange, which predict a decrease in G_s with increasing evaporative demand (Farquhar et al. 2002; Mäkelä et al. 1996), and agree with the results of gradient and common garden experiments showing that Scots pine intrinsic water use efficiency is not higher under warmer/drier conditions (Palmroth et al. 1999). Maintaining a high stomatal aperture could be beneficial for trees growing in warmer/drier sites, because photosynthetic rates under full light conditions would not be limited by CO₂ diffusion into the stomata.

Other processes are likely to influence the patterns observed in this study, for example the increased needle retention in cooler climates (Oleksyn et al. 2003). A full understanding of the mechanisms underlying the observed patterns may require the consideration of the complex interactions that determine the resource economy of trees, taking into account explicitly that plants are likely to be regulated in order to maximize carbon gain, and that photosynthesis may be limited by hydraulic factors, but also by other resources, such as nitrogen and light (Buckley and Roberts 2006).

Implications at the global scale

The observed variability in stomatal behavior across climates in Scots pine undoubtedly influences the spatial variation of canopy fluxes of water vapour and carbon dioxide, and has further implications in the parameterization of ecosystem models, because this intraspecific variation is not usually considered (but see Berninger 1997).

The results of this study are consistent with the observed vulnerability of southernmost populations to extreme drought events (Martínez-Vilalta and Piñol 2002). As previously shown, structural adjustments of Scots pine at these latitudes are not able to compensate for the higher transpiration rates, and thus lower water potentials are likely to be reached at warmer/drier sites, with the corresponding increase in the risk of embolism. The predicted increase in the intensity and frequency of acute droughts

can seriously threaten Scots pine stands in the Mediterranean basin. Extensive drought-induced mortality has been identified elsewhere as the cause of drastic vegetation changes at the landscape level (Allen and Breshears 1998; Mueller et al. 2005).

Conclusion

In conclusion, we have found that relative sensitivity of G_s to D (either relative or absolute) does not increase with evaporative demand across Scots pine populations, and that leaf area-related transpiration rates at a given D increase in drier/warmer climates. These patterns were associated with a decline in $A_L:A_S$ values in drier/warmer sites, as previously reported. We could not entirely explain why trees in warmer/drier climates reduced leaf area and kept high G_s s, and not the opposite. Clearly, this topic deserves further attention, probably using a fully coupled photosynthesis–water transport model. More comprehensive surveys of climate-driven variation in hydraulic traits are also needed to complete the picture of physiological and structural hydraulic plasticity in Scots pine, and thus predict how climate change will affect this species across Eurasia.

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