

Drought effects on hydraulic conductivity and xylem vulnerability to embolism in diverse species and provenances of Mediterranean cedars

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Summary We studied hydraulic traits of young plants of the Mediterranean cedar species *Cedrus atlantica* (Endl.) G. Manetti ex Carrière (Luberon, France), *C. brevifolia* (Hook. f.) Henry (Cyprus), *C. libani* A. Rich (Hadeth El Jebbe, Lebanon) and *C. libani* (Armut Alani, Turkey). With an optimum water supply, no major differences were observed among species or provenances in either stem hydraulic conductivity (K_s) or leaf specific conductivity (K_l) measured on the main shoot. A moderate soil drought applied for 10 weeks induced marked acclimation through a reduction in K_s , particularly in the Lebanese provenance of *C. libani*, and a decrease in tracheid lumen size in all species. *Cedrus atlantica*, which had the smallest tracheids, was the species most vulnerable to embolism: a 50% loss in hydraulic conductivity (Ψ_{PLC50}) occurred at a water potential of -4.4 MPa in the well-watered treatment, and at -6.0 MPa in the moderate drought treatment. In the other species, Ψ_{PLC50} was unaffected by moderate soil drought, and only declined sharply at water potentials between -6.4 and -7.5 MPa in both irrigation treatments. During severe drought, K_s of twigs and stomatal conductance (g_s) were measured simultaneously as leaf water potential declined. For all species, lower vulnerability to embolism based on loss of K_s was recorded on current-year twigs. The threshold for stomatal closure (10% of maximum g_s) was reached at a predawn water potential (Ψ_{pd}) of -2.5 MPa in *C. atlantica* (Luberon) and at -3.1 MPa in *C. libani* (Lebanon), whereas the other provenance and species had intermediate Ψ_{pd} values. *Cedrus brevifolia*, with a Ψ_{pd} (-3.0 MPa) close to that of *C. libani* (Lebanon), had the highest stomatal conductance of the study species. The importance of a margin of safety between water potential causing stomatal closure and that causing xylem embolism induction is discussed.

Keywords: *Cedrus atlantica*, *Cedrus brevifolia*, *Cedrus libani*, drought acclimation, stomatal conductance.

Introduction

The long summer drought that characterizes the Mediterranean climate (Daget 1977, 1983) affects the transport of water in trees. In particular, rapid transpiration during drought increases water tensions in the xylem, increasing the risk of em-

bolism and dysfunction of the conducting system (Crombie et al. 1985, Sperry and Tyree 1988, Sobrado 1997).

Plants may respond to seasonal drought by changes in hydraulic traits that tend to maintain a favorable water balance (Tyree and Ewers 1991). For example, prolonged soil drying can cause an increase in stem conductivity relative to transpiring leaf area (i.e., an increase in leaf specific conductivity, K_l ; $\text{mmol m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$) (Shumway et al. 1991, Hacke et al. 2000, Cinnirella et al. 2002). An increase in K_l lowers the soil-to-leaf pressure gradient (Tyree and Ewers 1991, Mencuccini and Grace 1995), thus reducing the risk of xylem embolism. Ecotypic variation in the response of either K_l or stem hydraulic conductivity (K_s ; $\text{mol m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$) to soil water availability has been reported in several species (Shumway et al. 1991, Cinnirella et al. 2002, Mencuccini 2003). A relationship has also been established between habitat preferences and xylem vulnerability in several species (Brodribb and Hill 1999, Piñol and Sala 2000, Maherali et al. 2004, Tossier et al. 2004). Plasticity of the response of K_s and xylem vulnerability to long-lasting drought are less well understood. Covariation in acclimation of K_s and xylem vulnerability provides an opportunity to analyze the potential trade-off between these adaptive traits (Linton et al. 1998, Hacke et al. 2000, Piñol and Sala 2000).

An additional aspect of the regulation of water transfer is the synchronization of stomatal closure and loss of hydraulic conductivity during drought. Several studies have demonstrated a relationship between the water potential that induces stomatal closure and that which triggers cavitation of stem xylem (Jones and Sutherland 1991, Sperry and Saliendra 1994, Brodribb and Hill 1999). In some species, stomatal closure is directly correlated with loss of leaf hydraulic conductivity (Cochard 2002, Brodribb et al. 2003, Nardini et al. 2003). A small safety margin may ensure that, in species with low vulnerability to xylem embolism, CO_2 assimilation is maintained for as long as possible during a drought (Tyree and Sperry 1988). In species experiencing wide variation in water availability, however, earlier stomatal closure provides a larger margin of safety against embolism formation (Pockman and Sperry 2000, Martinez-Vilalta et al. 2002, Froux et al. 2005).

Mediterranean cedars (*Cedrus atlantica* (Endl.) G. Manetti

ex Carrière, *C. libani* A. Rich and *C. brevifolia* (Hook. f.) Henry) are valuable forestry species in their regions of origin (Bariteau and Ferrandès 1992). They show high phenotypic plasticity (Aussenac and Finkelstein 1983, Aussenac 1984). These species are well adapted to drought (Ducrey 1994), surviving in semi-arid regions of Algeria (Derridj 1990), Lebanon and in Turkey (Khouzami and Nahal 1983). Studies with *C. atlantica* have shown a 50% loss of hydraulic conductivity at water potentials between -6.5 (Cochard 1992) and -5.1 MPa (Froux et al. 2002). Inter- and intra-specific variation in the response of net assimilation rate to soil drying has been shown in cedars (Aussenac and Finkelstein 1983), and is probably indicative of differences in stomatal response. If stomata normally close before the onset of embolism (Tyree and Sperry 1988), diversity in xylem vulnerability to embolism likely overlays diversity of stomatal response in cedar species.

We studied variation in hydraulic traits in relation to soil drought in greenhouse-grown seedlings of three Mediterranean cedar species. The aims were to: (1) determine if there is phenotypic plasticity and acclimation of hydraulic traits to drought; (2) examine the links between hydraulic traits and the anatomy of the stem xylem; (3) analyze possible trade-offs between xylem transport capacity and vulnerability to embolism; (4) compare changes in hydraulic conductivity and stomatal conductance as leaf water potential declines; and (5) investigate the margin of safety against embolism formation in stems and its variability among species.

Materials and methods

Plant material and water treatments

We studied three species of Mediterranean cedars: *C. atlantica* (Luberon, France), *C. brevifolia* (Cyprus) and two provenances of *C. libani*: Hadeth El Jebbe, Lebanon (L), and Armut Alani, Turkey (T). About 150 plants per species or provenance were grown in 0.4-l plastic pots in a nursery beginning in March 1996. In January 1997, the plants were transplanted to 3-l pots containing a 3:2:1 (v/v) mixture of soil:peat:bark in the nursery. In December 1997, the plants were transferred to an air-conditioned greenhouse.

At the beginning of April 1998, the 3-year-old plants were separated into two groups: the first received a normal supply of water (predawn water potential maintained between -0.5 and -0.7 MPa; well-watered treatment); the other group was subjected to moderate drought for 10 weeks (predawn water potential varying between -0.7 and -1.1 MPa; moderate drought treatment). Hydraulic conductivity and xylem vulnerability to cavitation were measured in May 1998, 4 weeks after the beginning of the water treatments, and in February 1999 during the winter resting period.

In August 1999, plants from the 1998 well-watered treatment were subjected to severe drought by withholding irrigation completely (severe drought treatment).

Xylem hydraulic conductivity and Huber value

Stem hydraulic conductivity K_h ($\text{mmol m}^{-1} \text{MPa}^{-1}$) was mea-

sured as described by Sperry and Tyree (1988). Stem segments were perfused at a pressure of 6.5 kPa with a degassed solution of water acidified with HCl (pH 2) filtered to $0.1 \mu\text{m}$. The following hydraulic properties were determined for each segment: (1) stem hydraulic conductivity (K_s ; $\text{mol m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$): $K_s = K_h/S_a$, where S_a is the cross-sectional area (m^2) of the segment; and (2) leaf specific conductivity (K_l ; $\text{mmol m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$): $K_l = K_h/L_a$, where L_a is the leaf area (m^2) of foliage located downstream from the segment. The Huber value (HV; $\text{m}^2 \text{m}^{-2}$), $HV = S_a/L_a$ (or K_l/K_s), which is the cross-sectional area of the segment related to the supplied leaf area, was calculated to give a direct measure of shoot morphology.

Xylem vulnerability to embolism

For each set of measurements, six plants of each species or provenance and water treatment were randomly selected. Each plant was cut at its base and the cut base was recut under water. Subsequent handling of the samples was carried out under water. A 10-cm-long segment of the stem was excised and debarked. The segments measured in 1998 belonged to the 1997 growth unit. Measurements made in February 1999 were on stem segments produced during the 1998 growing season, comprising xylem formed during the irrigation treatments.

Each segment was placed in a small cylindrical pressure chamber with an opening at each end. The segment was sealed in the chamber with its ends extending about 15 mm from either end of the chamber. The first conductivity measurement (K_h) was made, as described above, at a chamber pressure of 0.05 MPa, which prevents water escaping through scars on the branches and needles. This measurement corresponded to maximum conductivity ($K_{h\text{max}}$). The samples were subsequently subjected to increasing air pressure (increments of 1 MPa). After each 5-min period of pressurization, the pressure was released for at least 30 min; hydraulic conductivity was then measured at a chamber pressure of 0.05 MPa. Applied air pressures ranged from 3–9 MPa in May 1998 and from 1–9 MPa in February 1999.

The percentage loss in conductivity (PLC), following the application of different air pressures relative to the initial measurement at 0.05 MPa, was calculated according to the equation:

$$\text{PLC}_i = 100 \left(1 - \frac{K_{hi}}{K_{h\text{max}}} \right) \quad (1)$$

where PLC_i is percentage loss in conductivity at a given pressure i , $K_{h\text{max}}$ is maximum conductivity at 0.05 MPa and K_{hi} is conductivity measured at the given pressure i . Vulnerability curves were obtained by plotting PLC against air pressure.

The vulnerability curves were fitted using a nonlinear regression model (procedure NLIN of SAS statistical software, SAS Institute, Cary, NC) described by Pammenter and Vander Willigen (1998):

$$\text{PLC} = \frac{100}{1 + \exp(a(\Psi - b))} \quad (2)$$

where Ψ is the water potential of xylem corresponding to the opposite of the pressure applied, a is a coefficient linked to the slope of the curve at the inflection point and b corresponds to the pressure inducing a 50% loss in conductivity.

Tracheid dimensions and theoretical hydraulic conductivity

Tracheid length, lumen diameter and wall thickness were measured on the samples used for the xylem vulnerability measurements in February 1999. Lumen diameter and wall thickness measurements were made on cross sections cut with a freezing microtome. Tracheid lengths were measured after macerating xylem fragments for 4 h in Jeffrey solution (Spicer and Gartner 1998). Sixty tracheids from each of six plants per category were measured (species or provenance \times treatment).

Tracheid lumen values were classified in diameter classes of 2 μm . The contribution of each diameter class to the theoretical hydraulic conductivity was calculated by the Hagen-Poiseuille law (Tyree and Ewers 1991) according to the method described by Froux et al. (2002).

Hydraulic conductivity, stomatal conductance and drought

From August to September 1999, eight plants of each species or provenance were simultaneously subjected to a severe drought treatment (irrigation was withheld). Predawn water potential (Ψ_{pd}) and minimum water potential (Ψ_{min}) were monitored with a Scholander pressure chamber. The mean critical predawn water potential ($\Psi_{\text{cpd}} = \Psi_{\text{pd}} - \Psi_{\text{min}}$ (Aussenac and Granier 1978)) was calculated.

Stem hydraulic conductivity (K_h) was measured on lateral twigs as described above. However, in the severe drought study, the samples were not pressurized or subjected to chamber air pressure during measurements. At the beginning of the experiment, conductivity was monitored in the same plants every day. Subsequently, measurements were made every 2 or 3 days and continued until there was complete loss of conductivity. For each plant, the maximal value of K_s was calculated. A curve of PLC was plotted as K_s (as a percent of the maximal value per plant) versus Ψ_{min} .

Stomatal conductance (g_s) was measured each day on one small branch per plant, between 1000 and 1130 h with a portable closed-circuit gas exchange measurement system (LI-6200, Li-Cor, Lincoln, NE), until the measured value reached zero. Mean microclimatic conditions in the measuring chamber were: photosynthetic photon flux = 578.2 $\mu\text{mol m}^{-2} \text{s}^{-1}$; temperature = 27.6 $^{\circ}\text{C}$; CO_2 concentration = 368.6 ppm, relative air humidity = 55.6%; and vapor pressure deficit = 16.7 hPa.

Results

Characteristics of hydraulic conductivity

In May 1998, we observed no effect of the moderate drought treatment on either the overall mean or the species or provenance values of K_s or K_1 (Table 1). In 1998, there were no significant differences between species in the well-watered treatment, although *C. atlantica* and *C. brevifolia* had lower K_s than *C. libani*. An effect of species on K_s appeared at the beginning of the moderate drought treatment.

Table 1. Effects of a moderate drought on stem hydraulic conductivity (K_s ; $\text{mol m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$), leaf specific conductivity (K_1 ; $\text{mmol m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$) and Huber value (HV; $10^3 \text{m}^2 \text{m}^{-2}$) of the main shoot of 3-year-old cedars during May 1998 and February 1999. Different letters denote significant differences among species and provenances for a given parameter, treatment and year ($n = 6$, $P = 0.05$). Significance of the treatment effect by date and species for each parameter is shown by an asterisk ($n = 6$, $P = 0.05$, Duncan test). Abbreviations: Wet and Dry = well-watered and moderate drought treatments, respectively.

Parameter	1998		1999	
	Wet	Dry	Wet	Dry
K_s				
<i>C. atlantica</i>	19.2 a	19.7 b	21.6 a	17.0 b
<i>C. brevifolia</i>	20.8 a	19.4 b	25.7 a	25.0 a
<i>C. libani</i> (L)	26.6 a	22.0 ab	24.5 a	17.5 ab*
<i>C. libani</i> (T)	26.4 a	27.8 a	26.7 a	22.8 ab
Mean	23.2	22.4	25.0	20.6 *
K_1				
<i>C. atlantica</i>	9.95 b	15.1 a*	18.1 a	17.9 b
<i>C. brevifolia</i>	16.0 a	19.3 a	20.3 a	28.4 a
<i>C. libani</i> (L)	12.8 ab	14.4 a	22.6 a	22.2 ab
<i>C. libani</i> (T)	14.6 a	19.5 a	19.4 a	28.1 a
Mean	13.4	17.0 *	20.2	24.1
HV				
<i>C. atlantica</i>	0.526 b	0.768 ab*	0.854 a	1.067 a*
<i>C. brevifolia</i>	0.757 a	0.999 a*	0.791 a	1.130 a*
<i>C. libani</i> (L)	0.496 b	0.661 b	0.932 a	1.279 a
<i>C. libani</i> (T)	0.575 b	0.709 b	0.728 a	1.315 a*
Mean	0.595	0.775 *	0.819	1.198 *

In February 1999, in all species, the moderate drought treatment had a significant effect on K_s , the mean value of which dropped from 25.0 to 20.6 $\text{mol m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$. The moderate drought treatment effect was particularly marked in *C. libani* (L), but not significant in *C. brevifolia*. The reduction in K_s was linked to a substantial increase in Huber value, whereas K_1 remained more or less constant.

In 1999, a species effect on K_s and K_1 was observed only in the moderate drought treatment. Significant differences in K_s were observed between *C. brevifolia* and *C. atlantica* (25.0 and 17.0 $\text{mol m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$, respectively). The lowest value for K_1 in the moderate drought treatment (17.9 $\text{mmol m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$) was observed in *C. atlantica*, and the highest (28.1 $\text{mmol m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$) in *C. libani* (T).

Vulnerability to embolism

In May 1998, there was no effect of irrigation treatment or species on the applied pressure required to cause 50% loss of conductivity (Ψ_{PLC50}) (Table 2). Mean values ranged from 5.9 (*C. libani* (L)) to 7.0 MPa (*C. brevifolia*).

Measurements made in February 1999 (Table 2) showed a greater tendency to embolism at a lower water potential in plants in the moderate drought treatment ($\Psi_{\text{PLC50}} = -6.9$ MPa) than in the well-watered treatment ($\Psi_{\text{PLC50}} = -6.2$ MPa); however, the difference (-4.4 MPa in the well-watered treatment versus -6.0 MPa in the moderate drought treatment) was sig-

Table 2. Parameters calculated from percent loss of conductivity (PLC) curves for species of *Cedrus* in the well-watered and moderate drought treatments during May 1998 and February 1999: xylem water potential at 50% loss of conductivity (Ψ_{PLC50}) and 10% loss of conductivity (Ψ_{PLC10}). Different letters denote significant differences among species or provenances for a given parameter and treatment ($n = 6$, $P = 0.05$). Significance of the treatment effect for each parameter and species is shown by an asterisk ($n = 6$, $P = 0.05$, Duncan test). Significance of the date effect for each parameter, species and treatment is shown by an "S" ($n = 6$, $P = 0.05$, Duncan test). Abbreviations: Wet and Dry = well-watered and moderate drought treatments, respectively.

Parameter	1998		1999	
	Wet	Dry	Wet	Dry
Ψ_{PLC50}				
<i>C. atlantica</i>	-6.9 a	-6.7 a	-4.4 bS	-6.0 b*
<i>C. brevifolia</i>	-7.0 a	-7.0 a	-6.7 a	-7.0 ab
<i>C. libani</i> (L)	-5.9 a	-6.6 a	-6.4 a	-7.5 a
<i>C. libani</i> (T)	-6.4 a	-6.3 a	-6.8 a	-7.1 ab
Mean	-6.6	-6.6	-6.2	-6.9
Ψ_{PLC10}				
<i>C. atlantica</i>	-3.2 a	-3.2 a	-2.4 a	-3.9 a
<i>C. brevifolia</i>	-4.4 a	-3.0 a	-4.7 a	-3.8 a
<i>C. libani</i> (L)	-3.4 a	-4.0 a	-4.7 a	-3.9 a
<i>C. libani</i> (T)	-3.5 a	-3.0 a	-4.8 a	-4.4 a
Mean	-3.7	-3.3	-4.3	-4.0

nificant only in *C. atlantica*. Treatment effects on Ψ_{PLC10} were similar, with cavitation occurring at water potentials less than -3.0 to -4.0 MPa (Table 2). For all species, the decrease in vulnerability caused by moderate drought treatment was associated with a decrease in K_s (Figure 1).

Xylem anatomy and hydraulic characteristics

Tracheid length and lumen diameter were significantly smaller in the moderate drought treatment than in the well-watered treatment, with the exception of tracheid length in *C. libani* (T) (Table 3). There were species differences in lumen diameter and tracheid length in both irrigation treatments. The widest tracheids were found in *C. libani* (L), which was the most drought sensitive provenance. The size of the tracheids in *C. libani* (T) and the other two species were similar.

Mean tracheid wall thickness was similar in both irrigation treatments. However, the moderate drought treatment decreased tracheid wall thickness in *C. libani* (T), had no effect on wall thickness in *C. brevifolia* and increased it in *C. libani* (L) and *C. atlantica* compared with the corresponding well-watered control values.

Individual values of K_s and of Ψ_{PLC50} for each plant studied in February 1999 were related to the mean lumen diameter of the tracheids from the same plants (Figure 2). Lower K_s values were in most cases associated with smaller tracheid lumen diameters. For both treatments, *C. libani* (L) displayed the greatest vulnerability to embolism in plants with the largest diameter tracheids. In *C. atlantica* and *C. libani* (T), this relationship was observed only in the well-watered treatment. In *C. brevi-*

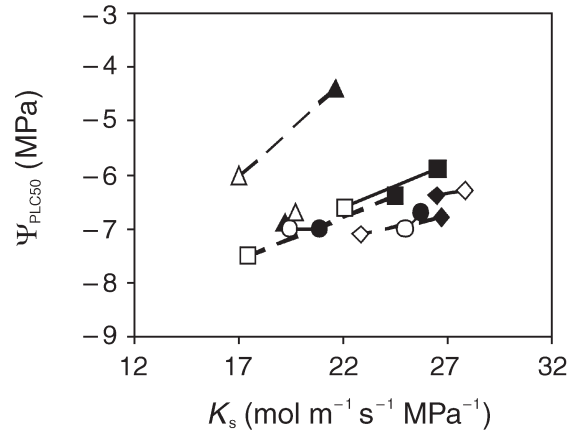


Figure 1. Relationship between xylem water potential inducing 50% loss of conductivity (Ψ_{PLC50}) and specific hydraulic conductivity (K_s) in twigs of *Cedrus* in the well-watered (closed symbols) and moderate drought (open symbols) treatments in 1998 (solid line) and 1999 (dashed line). Values represent the means of six plants each for *Cedrus atlantica* (\triangle and \blacktriangle), *C. brevifolia* (\circ and \bullet), *C. libani* (L) (\square and \blacksquare) and *C. libani* (T) (\diamond and \blacklozenge).

folia, there was little variation in vulnerability, and this was independent of lumen diameter of the tracheids.

The frequency distributions of tracheid lumen diameter (Figure 3) indicated that there were more tracheids with small diameters in plants of all species that had previously acclimated to drought. Figure 3, showing the contribution of each tracheid diameter class to total conductivity, indicates that, because of their large number, tracheids with the smallest diameters (from 7 to 11 μm) contributed most to total theoretical conductivity

Table 3. Effects of a moderate drought on tracheid lumen diameter (LD), length (TL) and wall thickness (WT) in February 1999. Different letters denote significant differences among species or provenances for a given parameter and treatment ($n = 360$, $P = 0.05$). In each case, significance of the treatment effect is shown by an asterisk ($n = 360$, $P = 0.05$, Duncan test).

Parameter	Species	Treatment	
		Well-watered	Moderate drought
LD (μm)	<i>C. atlantica</i>	9.61 d	7.48 d*
	<i>C. brevifolia</i>	10.29 c	8.22 c*
	<i>C. libani</i> (L)	12.32 a	10.03 a*
	<i>C. libani</i> (T)	10.71 b	9.65 b*
	Mean	10.78	8.82 *
TL (mm)	<i>C. atlantica</i>	1.25 b	1.10 c*
	<i>C. brevifolia</i>	1.31 b	1.25 b*
	<i>C. libani</i> (L)	2.50 a	1.69 a*
	<i>C. libani</i> (T)	1.10 c	1.10 c
	Mean	1.51	1.28 *
WT (μm)	<i>C. atlantica</i>	3.72 a	3.79 a*
	<i>C. brevifolia</i>	3.69 a	3.69 b
	<i>C. libani</i> (L)	3.65 a	3.80 a*
	<i>C. libani</i> (T)	3.64 a	3.38 c*
	Mean	3.67	3.67

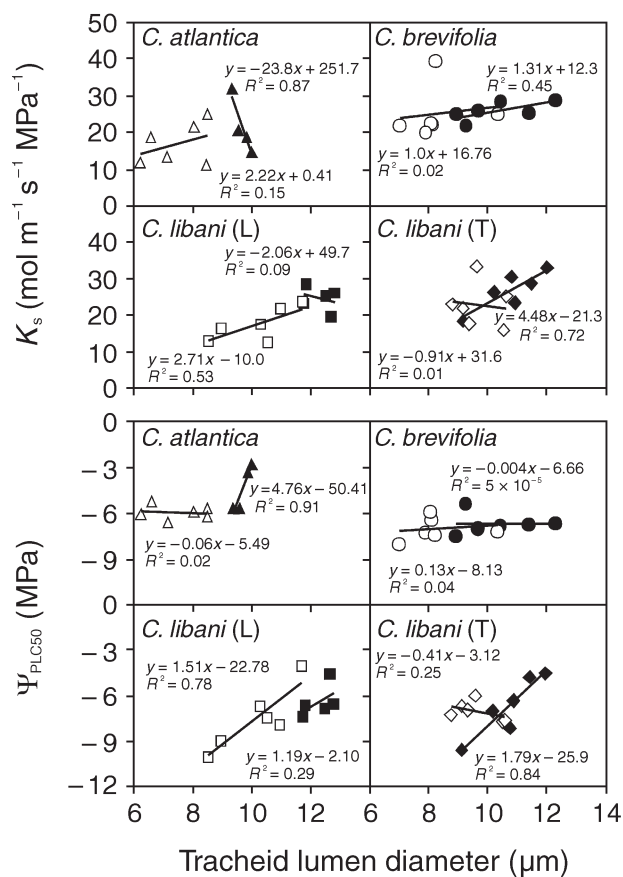


Figure 2. Relationship between specific hydraulic conductivity (K_s) and xylem water potential inducing 50% loss of conductivity (Ψ_{PLC50}) and tracheid diameter in *Cedrus atlantica* (Δ and \blacktriangle), *C. brevifolia* (\circ and \bullet), *C. libani* (L) (\square and \blacksquare) and *C. libani* (T) (\diamond and \blacklozenge) in February 1999 in the well-watered (closed symbols) and moderate drought (open symbols) treatments. Each value represents one plant. The straight lines indicate the fitted linear regressions for the well-watered (solid line) and moderate drought treatments (dashed line).

in the moderate drought treatment. Under well-watered conditions, diameters measuring from 11 to 15 μm contributed the most to conductivity.

Drought hydraulic conductivity and stomatal conductance

The response to severe drought in Ψ_{pd} and in Ψ_{min} during soil drying made it possible to calculate Ψ_{cpd} . The mean Ψ_{cpd} in *C. brevifolia* (-3.0 MPa) was more negative than in *C. atlantica* (-2.5 MPa) and *C. libani* (L and T) (-2.3 MPa) (Table 4, raw data not shown).

Maximal stomatal conductance ranged from 0.135 $\text{mol m}^{-2} \text{s}^{-1}$ in *C. brevifolia* to 0.097 $\text{mol m}^{-2} \text{s}^{-1}$ in *C. atlantica*. The threshold for stomatal closure, set at 10% of maximum stomatal conductance ($g_{srel} < 10\%$), was reached at a Ψ_{pd} of -3.1 MPa in *C. libani* (L) and at -2.5 MPa in *C. atlantica*; the other species showed intermediate values (Table 4).

Changes in K_s during soil drying (Figure 4) showed an initial slight increase in K_s with a decrease in Ψ_{min} , followed by a decrease in K_s from a water potential of around -4 MPa. This

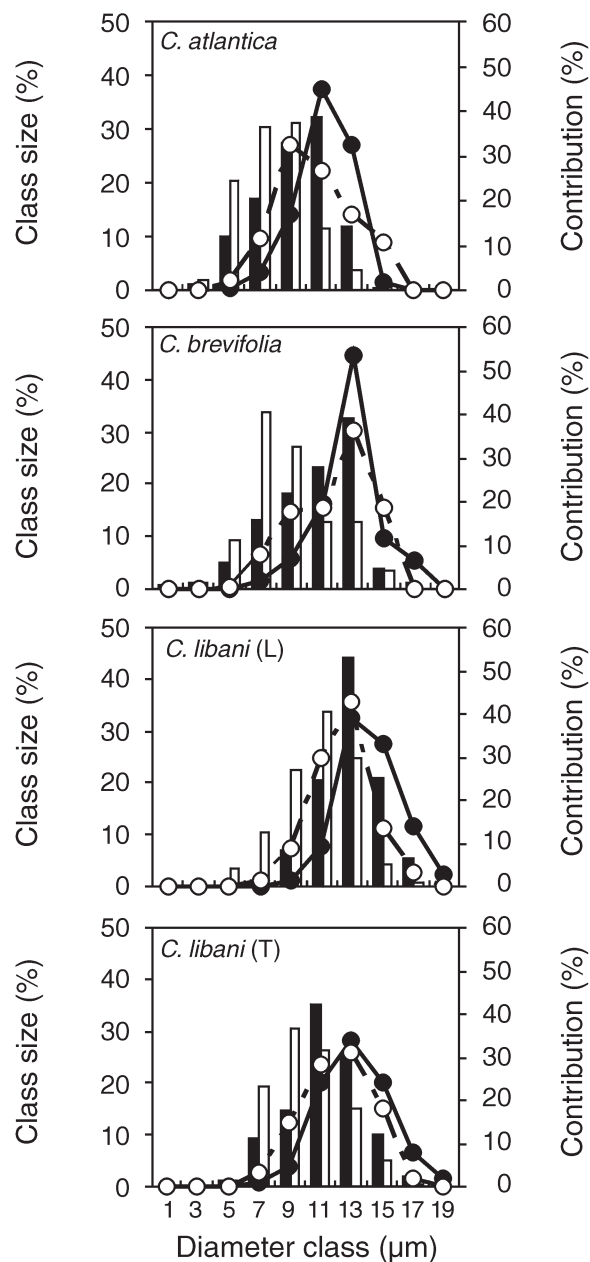


Figure 3. Frequency distribution of lumen diameter of tracheids of *Cedrus* in the well-watered (solid bars) and moderate drought (open bars) treatments. Relative contribution of each lumen diameter class to total calculated conductivity in the well-watered (\bullet) and moderate drought treatments (\circ). The x-axis values correspond to the center of each 2- μm lumen diameter class.

pattern was more marked in *C. libani* (L) than in the other provenance and species. The curve showing loss of conductivity relative to maximum K_s showed induction of embolism (Ψ_{PLC10}) at a xylem water potential close to -5 MPa, and a 50% loss of conductivity (Ψ_{PLC50}) at a water potential close to -8 MPa for all species. The drop in K_s occurred when the stomata were completely closed and the safety margin between the water potential causing stomatal closure and that resulting in embolism induction was close to 2.0 MPa. Figure 4 includes

Table 4. Plant water status during a severe drought treatment in September 1999: critical predawn water potential (Ψ_{cpd} ; MPa); predawn leaf water potential (Ψ_{pd} ; MPa); and minimum leaf water potential (Ψ_{min} ; MPa) for a stomatal conductance (g_s) less than 10% of its maximum value; water potential at maximum conductivity (Ψ_{Kmax} ; MPa); and water potential at 10 and 50% loss of conductivity (Ψ_{PLC10} and Ψ_{PLC50} ; MPa). Different letters denote significant differences among species for a given parameter ($P = 0.05$, Duncan test). The last three parameters are derived directly from Figure 4.

	n	Ψ_{cpd}	Ψ_{pd} ($g_s < 10\%$)	Ψ_{min} ($g_s < 10\%$)	Ψ_{Kmax}	Ψ_{PLC10}	Ψ_{PLC50}
<i>C. atlantica</i>	12	-2.5	-2.5 a	-2.9 a	-4.1	-5.8	-8.0
<i>C. brevifolia</i>	7	-3.0	-3.0 ab	-3.1 ab	-4.0	-4.6	-7.4
<i>C. libani</i> (L)	16	-2.3	-3.1 b	-3.1 b	-3.9	-5.0	-7.8
<i>C. libani</i> (T)	14	-2.3	-2.8 ab	-2.9 ab	-3.8	-4.4	-8.0
Mean		-2.5	-2.85	-3.0	-4.0	-5.0	-7.8

the vulnerability curves constructed in February 1999 for samples in the well-watered treatment. The curves indicate that stomatal closure coincided with embolism induction in the stem, calculated from PLC in *C. libani* (T), *C. libani* (L) and *C. brevifolia*. Conversely, in *C. atlantica*, it was predicted that embolism began before complete closure of the stomata.

Discussion

In the absence of water stress, seedlings of the cedar species investigated displayed little difference in the hydraulic conductance parameters K_s and K_1 . *Cedrus brevifolia*, the species from the driest bioclimate, did not have a higher K_s than *C. atlantica*, originating from the most mesic environment. The

moderate drought treatment resulted in a decrease in K_s . The acclimation of K_s was particularly noticeable in *C. libani* (L). This type of long-term acclimation has been observed in natural populations of *Pinus ponderosa* Dougl. ex P. Laws & C. Laws, where provenances from dry areas have higher K_s values than provenances from moist climates (Maherali and DeLucia 2000). Acclimation of K_s has also been found in *Eucalyptus camaldulensis* Dehnh. seedlings from contrasting bioclimates (Franks et al. 1995). We found a negative effect of drought on the dimensions of conducting elements (Figures 2 and 3), suggesting that the drought-induced decrease in K_s was most likely a result of a reduction in tracheid size. The effect of drought on wood anatomy has been analyzed in several recent studies (Lovisolo and Schubert 1998, Arnold and Mauseth

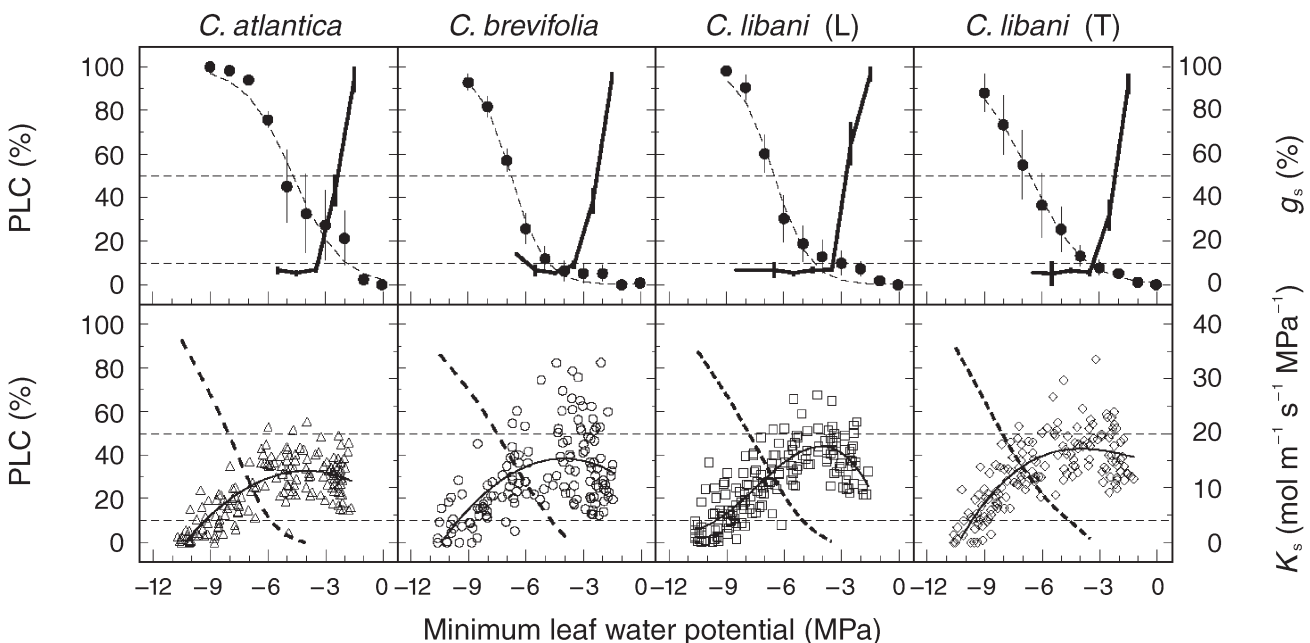


Figure 4. Upper panels: stomatal conductance (g_s , solid lines) as percent of the maximal conductance for each species or provenance of *Cedrus* versus minimum xylem water potential (Ψ_{min}) during severe soil drought (bar = SE, $n = 8$); and vulnerability curves (dotted lines) obtained by pressurization in the well-watered treatment on February 1999. Dashed horizontal lines represent 10 and 50% loss of conductivity. Lower panels: specific hydraulic conductivity (K_s) versus Ψ_{min} during severe soil drying. Solid lines are fitted with a cubic spline (value of smoothing parameter = 60). Percent loss of conductivity (PLC, dashed lines) was obtained from values of K_s expressed as percent of the maximal value of K_s per plant. The PLC curves are fitted with a cubic spline (value of smoothing parameter = 70). Dashed horizontal lines represent 10 and 50% loss of conductivity.

1999, February and Manders 1999, Schume et al. 2004) and found to have a negative effect on tracheid size and change the tracheid size class distribution.

In all species and provenances, there was a general increase in K_1 in the well-watered treatment between the beginning of the growing season (1998) and the winter resting period (1999). This increase may be linked to a different physiological stage from one season to the next. During the moderate drought treatment in February 1999, *C. atlantica* was distinguished by its lower K_1 value. At a given transpiration rate, the lower value will have induced a higher water potential gradient in *C. atlantica* than in the other species. As a consequence, stomatal control will be imposed earlier during a drought, as would be necessary to avoid embolism (Tyree 1989, Tyree and Ewers 1991, Cochard 1992). We also observed that the increase in K_1 in response to the moderate drought treatment was less in *C. atlantica* than in *C. brevifolia* and *C. libani* (T). The moderate drought treatment caused a significant increase in the HV, reflecting preferential biomass allocation to stems at the expense of needle area.

In spring 1998, no differences were observed between species and provenances in vulnerability to embolism (Table 2). Observed values for Ψ_{PLC50} ranged from -7.0 to -5.9 MPa, and were in agreement with those previously reported for *C. atlantica* (around -6 MPa; Cochard 1992). Measurements made in February 1999 on segments of stems formed during the irrigation treatments showed *C. atlantica* to be the most vulnerable to embolism (Table 2). Vulnerability in this species was greater in the well-watered treatment in 1999 than in 1998. Variation in xylem vulnerability among species has been linked by some authors to climate at the plant's origin. Brodribb and Hill (1999) found a positive correlation between vulnerability of xylem to embolism in 10 species of conifers and rainfall during the hottest 3-month period at the origin of the material tested. A similar relationship was established in a subspecies of *Artemisia tridentata* Nutt. (Kolb and Sperry 1999). In its natural habitat, *C. atlantica* is subject to less pronounced summer drought than are the eastern species *C. libani* and *C. brevifolia* because of the greater oceanic influence along the western coast of the Mediterranean basin (Khouzami and Nahal 1983).

We examined whether larger tracheids and higher K_s were achieved at a cost of higher vulnerability to embolism. A relationship between lumen diameter of the conduits and vulnerability was found in *C. libani* (L and T) and in *C. atlantica* in the well-watered treatment, and in *C. libani* (L) in both irrigation treatments (Figure 2), but not in *C. brevifolia* in either treatment. It has been shown that, in addition to influencing hydraulic conductivity, the diameter of the conducting elements influences vulnerability to embolism (Hargrave et al. 1994, Lovisolo and Schubert 1998). At the scale of the plant, the largest vessels or tracheids are the most efficient, but they are also the most vulnerable to cavitation. According to Hargrave et al. (1994), a large conducting element has a statistically greater chance of being located near an embolized element and may have more pores with a large diameter than a smaller element. This would facilitate the propagation of the embolism. Environmental conditions linked to the well-

watered treatment, which favor growth and the formation of large vessels (Figure 3), could trigger earlier embolism. This trade-off between vulnerability and K_s was evident when we examined the effect of irrigation treatment on the relationship between these parameters in the different species or provenances (Figure 1).

During the course of the severe drought, K_s showed a marked increase up to a Ψ_{min} of -4 MPa. This distinctive pattern, which refutes our prediction that conductivity decreases due to progressive embolism during severe drought, suggests a change in the pore size of the membrane pits through adjustment of the network of microfibrils. This could be interpreted as an adaptation to drought because higher K_s may help in the short term to mitigate the increase in xylem water tension.

The PLC determined from the maximum value for K_s differs from the curve of PLC determined by pressurization (Figure 4). In contrast to previous findings (Sparks and Black 1999), there was a large discrepancy between the two methods (2 MPa) for more negative values of Ψ_{PLC50} . Kikuta et al. (2003) also found marked discrepancies between values measured on sections of xylem using the controlled pressurization method and those measured on branches that were excised and dehydrated. According to these authors, one of the reasons for this discrepancy may lie in the different degree of exposure of the tracheids to air. This exposure would be greater in debarked branches in the pressurization method. Differences in the size and the position of the branch used in both methods may also be a factor. Sperry et al. (1988) found during May to late August a significant increase (from 11 to 31%) in embolism in stems and larger branches of *Acer saccharum* Marsh., whereas embolism remained at about 10% in twigs. In *A. saccharum*, the xylem of the current year could withstand higher xylem tensions than older wood (Melcher et al. 2003). Differences of 1 MPa in Ψ_{PLC50} are also reported in sapwood of Douglas-fir trees between node 5 and node 15 (Domec and Gartner 2001). The lower vulnerability of the distal parts of the branch that we observed could be explained on the basis of reduced tracheid size.

Stomatal conductance of our study species became negligible ($< 10\%$ of maximum) at a Ψ_{pd} of between -2.3 and -3.0 MPa (Ψ_{min} of -2.9 and -3.1 MPa, respectively). This is consistent with changes in g_s observed in *C. atlantica* and *C. libani* (Aussenac and Finkelstein 1983), and places Mediterranean cedars in the category of drought-tolerant species (Aussenac 1984, Cochard 1992). We found that *C. brevifolia* had a Ψ_{cpd} that was more negative than the other species. Like *C. libani* (L), *C. brevifolia* did not close its stomata until leaf water potential reached a low value, thereby maintaining a high rate of transpiration. For this reason, *C. brevifolia* is prone to greater water loss than the other species, so that we would expect its conducting system to be less vulnerable to embolism (Tyree 1989, Jones and Sutherland 1991). In our experimental conditions, *C. brevifolia* displayed a vulnerability to xylem embolism that was lower than that of *C. atlantica* and equivalent to that in the two provenances of *C. libani*. Several studies have shown the importance of stomatal regulation in maintaining the integrity of the conducting system (Jones and Sutherland 1991, Cochard et al. 2002). Relating stomatal conduc-

tance and loss of conductivity based on vulnerability curves by pressurization (Figure 4) revealed a limited safety margin between the water potential at onset of embolism and at stomatal closure; however, this safety margin increased at the twig level in all species.

Based on our observations of stomatal conductance and vulnerability during the course of soil drying, we distinguished *C. brevifolia*, a species with delayed stomatal closure, high stomatal conductance and a relatively low xylem vulnerability, from *C. atlantica*, a species with early stomatal closure and xylem more vulnerable to embolization. Taking into account the respective roles of these parameters in the maintenance of hydraulic functioning during drought, *C. atlantica* appears to be more exposed to the risk of embolism than *C. libani* (L and T). Moreover, *C. brevifolia*, like *C. libani* (L), maintained a high stomatal conductance during soil drying, reflecting a substantial degree of drought tolerance. However, because of its vulnerability to xylem embolism, which was equivalent to that of *C. libani* (L and T), we conclude that *C. brevifolia* is at greater risk of drought injury than either provenance of *C. libani*.

In conclusion, this study allowed us to gain insight into the variability of hydraulic traits in several Mediterranean cedars and their possible acclimation to water stress. However, we were unable to identify the biological origin of differences in vulnerability of xylem to embolism, although it is clear that the implications of the differences in stomatal functioning as they affect water-use efficiency and adaptation to drought merit detailed study. Answers to these questions would increase our understanding of the mechanisms of resistance to summer embolism in cedar species and improve our understanding of their current distribution and the future place of cedar forests in the context of global warming.

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