ORIGINAL ARTICLE

Fabienne Froux · Michel Ducrey · Erwin Dreyer ·
Roland Huc

Vulnerability to embolism differs in roots and shoots and among three Mediterranean conifers: consequences for stomatal regulation of water loss?

Abstract We investigated the potential links between stomatal control of transpiration and the risk of embolism in root and shoot xylem of seedlings of three Mediterranean conifers (*Cupressus sempervirens*, *Pinus halepensis* and *P. nigra*) grown in a greenhouse under semi-controlled conditions. We measured the intrinsic vulnerability to embolism in roots and current year shoots by the air injection method. Root and shoot segments were subjected to increasing pressures, and the induced loss of hydraulic conductivity recorded. The three species displayed very different vulnerabilities in shoots, with *P. nigra* being much more vulnerable than *P. halepensis* and *C. sempervirens*. Roots were distinctly more vulnerable than shoots in *C. sempervirens* and *P. halepensis* (50% loss of conductivity induced at 3.0 MPa and 1.7 MPa higher xylem water potential in roots vs shoots). In *P. nigra*, no significant difference of vulnerability between shoots and roots was found. Seedlings were subjected to soil drought, and stomatal conductance, twig hydraulic conductivity and needle water potential were measured. The water potential resulting in almost complete stomatal closure (90%) was very close to the threshold water potential inducing loss of conductivity (10%) in twigs in *P nigra*, resulting in a very narrow safety margin between stomatal closure and embolism induction. The safety margin was larger in *P. halepensis* and greatest in *C. sempervirens*. Unexpectedly, this water potential threshold produced a 30–50% loss of conductivity in 3–5 mm diameter roots, depending on the species. The implications of this finding are discussed.

F. Froux · E. Dreyer
Ecologie et Ecophysiologie Forestières, UMR INRA—UHP,
54280 Champenoux, France

F. Froux · M. Ducrey · R. Huc (✉)
Unité de Recherches Forestières Méditerranéennes, INRA,
Avenue A. Vivaldi,
84000 Avignon, France
e-mail: huc@avignon.inra.fr
Fax: +33-4-90135959

Introduction

Water transport in trees under conditions of high potential evaporation is affected by the decrease in soil water content caused by drought. Assuming a conservative water flux in the plant, transpiration may be described as

$$E = \Delta\Psi g_{\mathrm{L}}$$

where $\Delta\Psi$ is the difference in water potential from soil to leaves ($\Psi_{\mathrm{S}} - \Psi_{\mathrm{L}}$) and g_{L} the leaf specific hydraulic conductance of the soil-to-leaf pathway (Sperry and Tyree 1988). When soil moisture declines, unrestrained and elevated midday transpiration rapidly leads to an exceedingly negative xylem water potential (Ψ_{L}) inducing catastrophic embolism (Tyree and Sperry 1988). Plants have to down-regulate their transpiration when soil water potential decreases to stay within the hydraulic limits of the soil-to-leaf conducting system (Sperry et al. 2002).

Even though changes in the leaf/root area ratio may contribute to maintain a favorable water balance, stomatal closure is by far the most efficient response to daily and seasonal decreases in water availability. Therefore, the coordination between stomatal regulation and hydraulic properties of the soil-to-leaf pathway during soil drying is significant for resistance of trees to drought. The role of stomatal closure in avoiding catastrophic xylem dysfunction has been emphasized by several authors (Jones and Sutherland 1991). This closure was often found to occur close to the threshold of xylem water potential that induces embolism in shoots (Tyree and Sperry 1988; Cochard et al. 1996; Lu et al. 1996; Sperry and Pockman 1993). The safety margin between stomatal closure and embolism induction is usually rather small ensuring that CO_2 assimilation is maintained as long as possible during the course of drought (Tyree and Sperry 1988). Nevertheless,

in some species stomatal closure may leave a large security margin before embolism induction. Indeed, species with a high vulnerability often display a narrow safety margin while those presenting a relatively low vulnerability could possibly display a broader safety margin. The occurrence of a narrow security margin in many species has led several authors to the hypothesis that stomatal regulation during drought could be under the control of a hydraulic signal (Sperry and Pockman 1993; Cochard et al. 1996; Salleo et al. 2000). This hypothesis can be tested by quantifying the safety margin of species with low vulnerability to embolism.

Xylem vulnerability to embolism can differ within a tree, and this spatial heterogeneity needs be mapped in order to accurately define safety margins. A gradient from low to high vulnerability has been reported to occur from the main stem to distal branches (Tyree and Ewers 1991; Sperry and Ikeda 1997). The finding that walnut petioles were more vulnerable than twigs led Tyree et al. (1993) to propose that hydraulic segmentation through cavitation may occur in addition to the segmentation by variable resistances. A similar pattern has been found in leaves from the petiole to central vein (Cochard et al. 2002; Nardini et al. 2001). These authors suggested that embolism occurring on distal parts of the trees could cause dehydration which would lead to shedding of leaves and small twigs and result in a reduction of the transpiring area.

Vulnerability to embolism in root xylem has been much less frequently investigated than in stems and petioles despite its importance for water transfer. Root xylem has been found to be more vulnerable than shoot xylem in broadleaved species (Sperry and Saliendra 1994; Alder et al. 1996; Hacke and Sauter 1996; Tsuda and Tyree 1997; Martinez-Vilalta et al. 2002) and in conifers (Sperry and Ikeda 1997) leading to the assumption that gas exchange could be limited by root rather than stem embolism. This assumption cannot be generalized, as roots of *Juglans regia* were found to be less vulnerable than shoots (Cochard et al. 2002). Additional studies are needed to elucidate links between threshold level of embolism in roots and shoots and stomatal closure.

We present the results of an experiment aimed at assessing the width of the safety margin between stomatal closure and embolism induction in different Mediterranean conifer species (*Cupressus sempervirens*, *Pinus halepensis* and *P. nigra*) known to display different vulnerabilities to stem embolism (Froux et al. 2002). We investigated:

1. Differences in vulnerability between roots and shoots, and
2. The stomatal responses to soil drought and associated decline of xylem water potential to assess the width of the safety margin in these species.

Materials and methods

Plants and growth conditions

Seeds from three Mediterranean conifer species (*C. sempervirens* L., *P. halepensis* Mill. and *P. nigra* Arn. ssp. *nigricans* Host. *var. austriaca*) were collected from natural populations in southern France. Seedlings were grown in 0.4-l plastic containers in 1998 in "Les Milles" nursery, near Aix-en-Provence, France. They were transplanted at the end of March 1999 to 7-l containers filled with a mixture of sand/peat/forest soil (1:2:3, v/v/v) and grown during 18 months. The forest soil was an A1 layer collected near Avignon. The pots were watered once or twice a week depending on the weather. A liquid fertilizer (Fertiligène NPK 9:9:9) was added once a week to the irrigation water (1%). Plants were grown in a greenhouse in Avignon, France, under 85% of full sunlight. Temperature minima in winter were kept above 2°C by heating and during the summer the maxima were maintained between 25°C and 32°C by ventilation and cooling.

Experimental design and measurements

Xylem vulnerability to embolism

Xylem vulnerability to embolism was measured in September 2000 in the twigs of well-watered seedlings using the pressurization method. Seedlings were transported to the laboratory and watered to soil capacity. Predawn needle water potential was measured on terminal twigs with a pressure chamber. Eight seedlings of *C. sempervirens*, *P. halepensis* and *P. nigra* were cut just above the collar level and cut again under water to remove embolized tracheids close to the cut end. We established vulnerability curves on current year stems and coarse roots. The current year stem was used to provide one 13-cm long segment. The whole root system was washed and one 10 cm long and about 3–5 mm diameter secondary root just below the collar was severed under water.

Cavitation was induced using the air injection method (Sperry and Saliendra 1994), adapted to our plant material, as described in Froux et al. (2002). Root and shoot segments were debarked and cut under water. They were inserted into a double-ended pressure chamber with both ends protruding to allow direct measurements of hydraulic conductivity (K_h). Samples were not knotched because air entry was enabled through abundant needle scars in twigs and by secondary ramification in roots. Native embolism could not be detected, as flushing under high pressures did not increase K_h. We assumed that because plant water potential was maintained above the water potential inducing xylem embolism during the entire life of the seedlings, the segments displayed no native embolism. Hydraulic conductivity measured before induction of embolism was therefore used as the maximal conductivity (K_{hmax}). Cavitation was induced by successive steps of 10 min of pressurization at pressures ranging from 0.8 MPa to

8 MPa for twigs and from 0.6 MPa to 6 MPa for roots. Each pressurization was followed by a 30 min of relaxation under water at atmospheric pressure and by measurement of K_h. Percent loss of conductivity (PLC) was estimated as

$$\text{PLC} = 100 \frac{(K_{h\,\max} - K_h)}{K_{h\,\max}}$$

Each PLC–applied pressure curve was established on a single twig segment from one seedling. Several PLC–pressure curves were used to assess the mean xylem water potential (assumed to be equal to the applied pressure) at 50% loss of hydraulic conductivity (Froux et al. 2002).

Drought treatment

Seedlings were subjected to a drought treatment at the beginning of August 2000. Three-year-old seedlings were watered to container field capacity for 2 days and then transferred to a controlled climate chamber (night/day: 11/13 h, light: 0/750 μmol m^{-2} s^{-1}, CO$_2$: 400/360 ppm, relative humidity: 96/70%, and temperature: 25/27°C). The top of each pot was covered with a plastic bag to limit soil evaporation. Treatments were applied to six, 1.3 m high, seedlings of *C. sempervirens* and *P. halepensis* (Table 1). The monocyclic *P. nigra* (about 40 cm in height) which produces only one new shoot per year required three sets of six plants to provide enough plant material; one set of plants was measured every third day.

Drought was induced by withholding water in the greenhouse and predawn leaf water potential (Ψ_w) was measured daily with a Scholander pressure chamber. Stomatal conductance to water vapor (g_s) was measured on a twig using a portable photosynthesis system LI-6200 (Li-Cor, Lincoln, Neb.). Percent relative stomatal conductance was computed as the ratio between current and initial value of g_s measured before the drought. The projected needle area was used for gas exchange calculations. Xylem water potential Ψ_x were measured at midday every other day. Determination of Ψ_x in stems was made by wrapping lateral twigs before dawn in aluminum foil to stop transpiration and measuring water potential in the same twigs at midday. A second order polynomial was fitted to the relationship between Ψ_x and Ψ_w for *C. sempervirens*. A linear regression was used for the *Pinus* species (Fig. 1). The fitted equations were

$$\Psi_x = -1.53 + 0.16\Psi_w - 0.11\Psi_w^2 \; (C.\,sempervirens),$$
$$r^2 = 0.98,$$
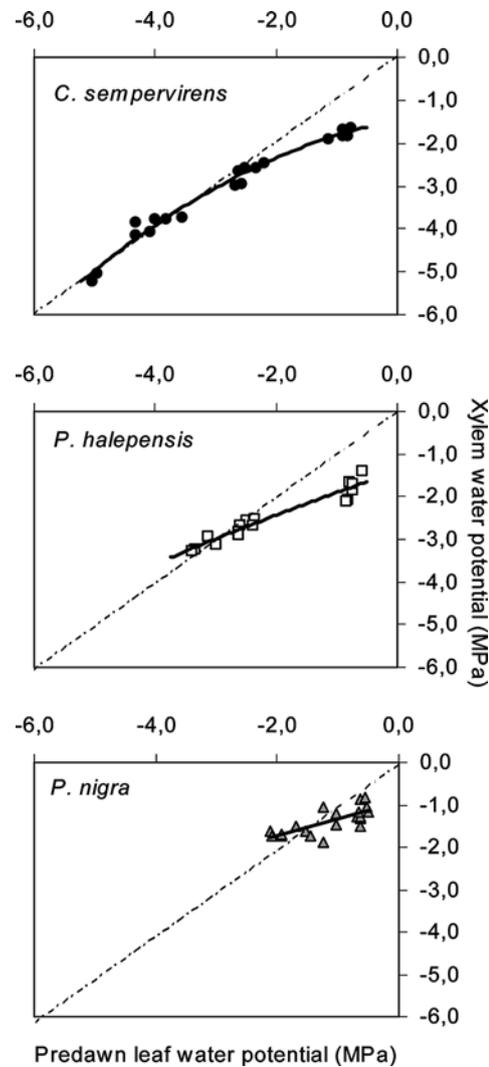
$$\Psi_x = -1.36 + 0.54\Psi_w \; (P.\,halepensis), \; r^2 = 0.93$$

and

$$\Psi_x = -0.94 + 0.39\Psi_w \; (P.\,nigra) \; r^2 = 0.55.$$

The equations were used for interpolating missing values of Ψ_x.

TDR probes (Time Domain Reflectometry, MP917, Moisture Point, Environmental Sensor, Victoria, Canada) were used to measure soil water content. A close

Fig. 1 Relationship between midday xylem water potential and predawn leaf water potential in seedlings of *Cupressus sempervirens*, *Pinus halepensis* (*n*=6 individuals) and *P. nigra* (*n*=18 individuals). Each point represents one measurement. The *fitted lines* are quadratic regression (*C. sempervirens*) and linear regression (*P. halepensis*, *P. nigra*). *Dashed line* 1:1 relationship

Table 1 Height (cm) and diameter at the root collar (mm) of 3-year-old seedlings of *C. sempervirens*, *P. halepensis* and *P. nigra* in September 2000. Values are means and standard errors of the mean (SEM) of six plants per species

Species	Height (cm)	Diameter (mm)
C. sempervirens	137.8 (25.4)	23.57 (3.88)
P. halepensis	132.0 (13.7)	21.21 (7.72)
P. nigra	36.89 (4.81)	13.79 (1.91)

curvilinear relationship was found between soil water content and predawn leaf water potentials in all species.

Hydraulic conductivity of current year twigs

Both Ψ_x and hydraulic conductivity (K_h) were measured on the same twig. Stem segments were cut to a length of 2 cm and debarked under water. Hydraulic conductivity was measured using a low pressure flow meter (Sperry et al. 1988). Segments were perfused at a pressure of 3.5 kPa using a degassed dilute solution of water and HCl (pH=2) filtered with 0.1 μm mesh. For each segment, specific conductivity (K_s) and leaf specific conductivity (K_l) were computed as:

$$K_s = \frac{K_h}{S_a}$$

and

$$K_l = \frac{K_h}{L_a},$$

where S_a is the transverse sapwood area of the segment and L_a the projected area of all the supplied needles.

Statistical analysis

Analysis of variance was used to assess the significance of species and organ effects on vulnerability, and species effects during drought for the measured parameters. The significance of differences between means was assessed with the Duncan test [($P<0.05$), GLM procedure, SAS, Statistical Analysis System, Cary, N.C.].

Results

Hydraulic properties of stems and roots

Root xylem was much more vulnerable to embolism than current year stem xylem in *P. halepensis* and *C. sempervirens*. In contrast, roots and current year stems of *P. nigra* had the same xylem vulnerability (Fig. 2, Table 2). K_s was two times higher in the roots than the stems of *C. sempervirens* and *P. nigra* (Table 3).

Inter-specific differences in hydraulic properties

Pinus nigra xylem was more vulnerable to embolism than *Pinus halepensis* and *C. sempervirens* in both roots and stems (Table 2). *P. halepensis* displayed the highest value of K_l and there was no difference among the two other species (Table 3). The values of K_l for the three Mediterranean conifers were higher than those recorded
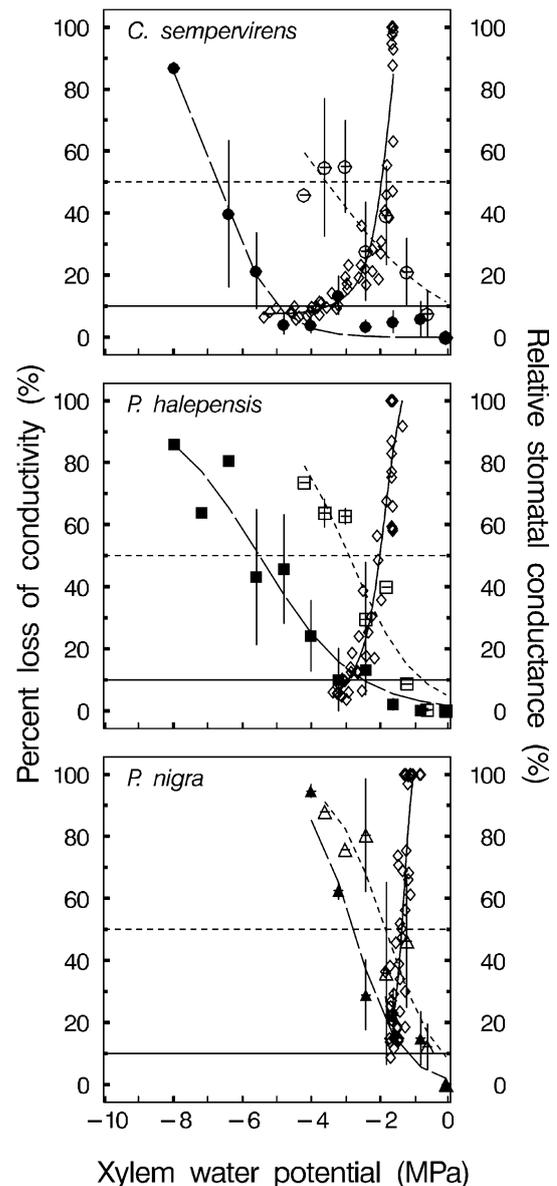
Fig. 2 Vulnerability curves in current year shoots (*long dashed lines*), in fine roots (*short dashed lines*), and stomatal conductance (*solid line*) expressed as percentage of the maximum recorded before dehydration, versus xylem water potential measured in seedlings of *Cupressus sempervirens*, *Pinus halepensis* and *P. nigra*. For vulnerability, the curves are non-linear fits on mean values of 5–6 measurements on 3-year-old seedlings, the symbols are mean values for roots (*open circles*, *squares and triangles*), and for shoots (*closed circles*, *squares and triangles*), and the *errors bars* represent ± SEM. *Horizontal lines* represent the levels of 10% (*solid line*) and 50% (*short dashed line*) loss of conductivity. For stomatal conductance, curves are fitted from individual measurements (*open diamonds*) using a spline routine

in most temperate trees (Tyree and Ewers 1996). There were no differences among species for K_s in roots while the K_s for stems was higher in *P. halepensis* than in the two other species. No trade-off was found between xylem vulnerability and K_s in these species.

Table 2 Xylem water potential inducing 50% loss of conductivity (Ψ_{PLC50}) in fine roots (diameter: 3–5 mm) and in current year stems of *C. sempervirens*, *P. halepensis* and *P. nigra*. Values were estimated from PLC data using the SAS NLIN procedure on individual samples from different seedlings. Mean and SEM of six replicates. Different letters denote significant differences among species for a given plant part (*P*=0.05, Duncan test). Difference between shoots and roots is depicted as *S* (significant) or *NS* (non-significant)

	Species	Roots	Current year shoots	Root–shoot difference	
Ψ_{PLC50} (MPa)	*C. sempervirens*	−2.8 (0.5)[a,b]	−5.8 (0.7)[a]	3.0	S
	P. halepensis	−3.5 (0.6)[a]	−5.2 (0.6)[a]	1.7	S
	P. nigra	−1.7 (0.2)[b]	−2.6 (0.1)[b]	0.9	NS

Table 3 Leaf specific conductivity (K_l, mmol m^{-1} s^{-1} MPa^{-1}) of current year stems and specific (related to segment cross-section) conductivity K_s (mol m^{-1} s^{-1} MPa^{-1}) of stems and roots (diameters of 3–5 mm) of *C. sempervirens*, *P. halepensis* and *P. nigra*. Mean and SEM of six replicates. Different letters denote significant differences among species (*P*=0.05, Duncan test). Organ effect is shown as *S* (significant) or *NS* (not significant)

Species	K_l		K_s	Organ effect
	Shoots	Roots	Shoots	
C. sempervirens	3.90 (1.06)[b]	38.21 (4.61)[a]	19.82 (2.58)[b]	S
P. halepensis	8.07 (2.51)[a]	42.00 (4.89)[a]	32.38 (7.04)[a]	NS
P. nigra	3.10 (1.06)[b]	48.41 (6.01)[a]	20.98 (3.09)[b]	S

Responses to drought

After irrigation cessation, volumetric soil humidity (SW) declined gradually and reached levels below 5% after 6 days in *C. sempervirens* and *P. halepensis* and after 14 days in *P. nigra*. This delay was due to the smaller leaf area in the latter species. The relationship between predawn needle water potential (Ψ_w) and SW was very similar among the three species (data not shown). Detectable effects of SW on Ψ_w became evident when SW dropped below 10%. Values of SW slightly above 0% corresponded to a Ψ_w of −2.5 MPa in all species.

Midday xylem water potential (Ψ_x) were significantly lower in *C. sempervirens* and *P. halepensis* (−1.7 MPa for both species) than in *P. nigra* (−1.2 MPa) at elevated values of predawn needle water potential (Ψ_w≈0.6 MPa in all species) (Fig. 1). Values of Ψ_x close to Ψ_W were reached at lower levels of Ψ_w depending on species: around −1.7 MPa in *P. nigra*, −3.3 MPa in *P. halepensis* and −5.4 MPa in *C. sempervirens*. These observations showed that the down regulation of water losses began at different levels of soil water content among the three species.

In all species, stomatal conductance decreased gradually with xylem water potential (Fig. 2) and was already rather low at −2 MPa. However, complete stomatal closure (g_{sw} close to 0) was reached at significantly different levels of Ψ_W for the three species; the lowest was found in *C sempervirens*. The threshold for stomatal closure was around −2 MPa in *P. nigra*, −3.5 MPa in *P. halepensis* and −4.5 MPa in *C. sempervirens*.

Coordination between stomatal conductance and xylem vulnerability to embolism

The relative stomatal conductance and the loss of xylem conductivity in roots and current year shoots as a function of xylem water potential are shown in Fig. 2. During the course of drought stress, the hydraulic conductivity (K_s) recorded on lateral twigs displayed no detectable decrease even at stress inducing complete stomatal closure (Fig. 3). This observation supported the hypothesis that stomatal closure efficiently protected the stem xylem against drought induced embolism. The safety margin, defined as the range between xylem water potential inducing 90% stomatal closure and 10% loss conductivity in current year stems, differed among species. A larger safety margin was observed in *C. sempervirens* (about 1 MPa) than in *P. halepensis* and *P. nigra* where almost no safety margin was detected. Unexpectedly, these observations were not confirmed when considering root embolism. Xylem water potentials inducing 90% stomatal closure corresponded to a root xylem conductivity of about 50% in all species.

Discussion

Shoot and root xylem vulnerability to embolism

The results clearly showed that the xylem was more vulnerable to cavitation in roots than in shoots in the three Mediterranean conifers tested (*C. sempervirens*, *P. halepensis* and *P. nigra*). This rather unexpected result raises a number of questions. The vulnerability of roots was measured on 3–5 mm diameter lateral roots, and not on the tap root. Within a root system vulnerability may be
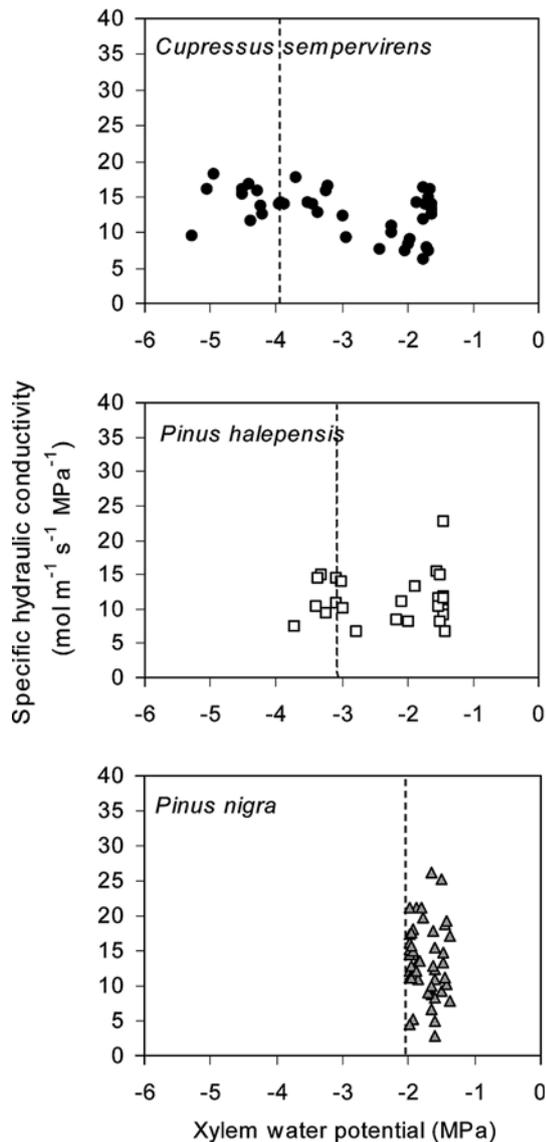
Fig. 3 Specific hydraulic conductivity of current year lateral twigs versus midday xylem water potential in seedlings of *Cupressus sempervirens* (*full circle*), *Pinus halepensis* (*empty square*) and *P. nigra* (*gray triangle*) subjected to drought. The *vertical dashed lines* represent the xylem water potential inducing 90% stomatal closure. Each point represents one measurement per seedling per day (*n*=6 seedlings for *P. halepensis* and *C. sempervirens* and *n*=18 seedlings for *P. nigra*)

dependent on root diameter. For example, fine roots (diameter below 5-mm) of *Pseudotsuga menziesii* were more vulnerable to embolism than coarse roots (Sperry and Ikeda 1997). The present results may therefore only concern the more vulnerable fine roots. Unfortunately due to root system structure it was not possible to investigate coarse roots. The use of pressurization to induce cavitation may also be questioned. We made preliminary tests to compare these results with those produced by a centrifugation technique (Holbrook et al. 1995) on *P. nigra* roots: both approaches yielded similar levels of vulnerability (data not shown). Unfortunately, the centrifugation technique could not be used for *C. sempervirens* nor *P.*

halepensis because the centrifuge was unable to induce the large tensions needed for embolism induction in these species.

This large difference in vulnerability to embolism between roots and shoots was similar to that found in some populations of *P. menziesii* where the difference ranged from 1.0 MPa to 2.2 MPa depending on geographic origin of the seedlings (Kavanagh et al. 1999). Alder et al. (1996) found similar results in *Acer grandidentatum* where there was no genetic variation in stem vulnerability but significant genetic variability in root vulnerability associated with site conditions. It must be stressed that in our inter-specific comparisons, both root and shoot vulnerability differed.

A higher vulnerability to embolism could be related to the occurrence of larger tracheids in roots than in shoots. We did not measure the cross-sections of xylem tracheids in roots and shoots, but significant correlations were found between xylem water potential inducing embolism and mean hydraulic diameter by Sperry and Saliendra (1994) in *Betula occidentalis*. In *Populus balsamifera* there was a strong relation between conduit diameter and water potential inducing embolism when comparing roots (extremely vulnerable) to petioles (low vulnerability) (Hacke and Sauter 1996). In contrast, in *Alnus glutinosa* no such relation was found (Hacke and Sauter 1996). Other anatomical features like differences in inter-tracheid pit structure (Sperry and Tyree 1990; Alder et al. 1996) may be at the origin of the difference in vulnerability between root and shoots. There is still a need for more information on the potential anatomical causes of variation in vulnerability within the plant.

The large range among species in vulnerability to embolism in the xylem of current year stems confirmed earlier findings (Froux et al. 2002) where the ranking by decreasing vulnerability was *P. nigra*, *P. halepensis* and *C. sempervirens*. *Cupressus sempervirens* had a much higher vulnerability in current year twigs of 2-year-old seedlings in an earlier study (Ψ_{PLC50}=−4.8 MPa) than in the current study. Growing conditions were similar in both studies. The question of a seedling age effect on the vulnerability to embolism of current year twigs should be investigated.

Xylem vulnerability and stomatal regulation

Pinus nigra stomata were the most sensitive to drought compared to *P. halepensis* and *C. sempervirens*. Stomatal closure occurred in *P. nigra* at a xylem water potential around −2 MPa. There are several studies that support the important role of stomatal closure as a means to avoid the induction of catastrophic embolism in the xylem (Jones and Sutherland 1991; Sperry et al. 1993; Saliendra et al. 1995; Alder et al. 1996; Cochard et al. 1996; Cochard 2002). Our data confirmed that stomatal closure occurred before the induction of embolism in shoots. Gradual stomatal closure limited water loss and the decrease of xylem water potential during soil water depletion. A 90% stomatal closure corresponded to only 10% loss conduc-

tivity in both *Pinus* species. In *C. sempervirens*, the xylem water potential inducing embolism was 1 MPa below that inducing total stomatal closure. This shows that the width of the safety margin was rather variable among species and supports the earlier finding that the safety margin increases with decreasing vulnerability (Martinez-Vilalta et al. 2002). Pockman and Sperry (2000) also found an increase of the safety margin in the less vulnerable species from the Sonoran desert. Nardini and Salleo (2000) found no safety margin in 11 Mediterranean broad-leaved species for which midday water potential dropped close to and even below the level causing onset of cavitation measured with an acoustic technique. Total stomatal closure may not be sufficient to stop water loss due to cuticular transpiration and the xylem water potential may continue to decrease under extreme drought. A safety margin might be useful in protecting the xylem from this uncontrolled drying. The hypothesis that woody plants operate close to the point of xylem failure (Tyree and Sperry 1988; Sparks and Black 1999) may apply only for species growing in mesic sites submitted to limited variations in soil water availability. Plants that experience only limited drought stress from year to year may be able to survive with a small safety margin and at the other extreme plants experiencing wide variations in water availability may require a large safety margin (Pockman and Sperry 2000).

In addition to the hypothesis of a chemical control of stomatal conductance by an ABA signal from roots (Tardieu and Davies 1993), recent findings suggest the occurrence of some hydraulic control of stomatal closure. A decrease of hydraulic conductivity due to stem embolism lead to a decrease of total hydraulic conductivity and might have been a signal for stomatal closure via xylem tension in *Laurus nobilis* (Salleo et al. 2000). A strong reduction of stomatal conductance was obtained by reducing hydraulic conductivity by 99% without any change in ψ_{leaf} (Hubbart et al. 2001). Results from the current study showed that the threshold for stomatal closure corresponded to the beginning of embolism in stems except for *C. sempervirens*. In contrast, there was significant loss of conductivity in roots before 90% stomatal closure. Thus, the concept of safety margin seems applicable only to shoots. The loss of hydraulic conductivity in roots might stimulate either: (1) a decrease in total hydraulic conductivity of seedlings and stomatal closure through a direct effect in accordance with Ohm's law, or (2) a signal for ABA release to the xylem sap.

Our results showed that stomatal control of transpiration was an effective mechanism to prevent current year twig embolism, because there was no detectable loss of hydraulic conductivity during the course of drought. Unfortunately we have no parallel results for the roots. The levels of vulnerability measured in the laboratory suggested that during drought stress of similar intensity, at least a fraction of the roots might embolize (Alder et al. 1996).

In field conditions, water uptake by trees can be reduced in the upper soil layers, due to soil drying and root embolism, and not in deeper soil (Hacke et al. 2000a). Tap roots maintain high water uptake since they grow in favorable soil moisture conditions but also owing to better resistance to embolism associated to their larger size (Sperry and Ikeda 1997). Early embolism in roots during water depletion can provide some advantages for the plant. During severe soil drought, water potential gradients may lead to reverse fluxes from roots to soil. This reverse flow might be partially avoided due to a suberisation of the apoplastic and the peridermic layers (Steudle 2000) but also due to embolism in lateral roots (Passioura 1988; Ewers et al. 1992; Hacke et al. 2000b).

Nevertheless, the high vulnerability to embolism found in roots raises different questions with respect to seedling survival. First, is this loss at least partly reversible whenever soil water returns to optimum? At least partial refilling of root xylem following intense rain has been detected in *Laurus nobilis* (Salleo et al. 1996) and in *Acer grandidentatum* (Alder et al. 1996). In conifer species, despite the irreversible aspiration of bordered pit during embolism (Sperry and Tyree 1990), it has been shown that stems of *Pinus sylvestris* could be partly refilled (Sobrado et al. 1992; Edwards et al. 1994). Nocturnal root pressures have been described in a few species (Tyree and Yang 1992) and refilling of embolized elements might be possible in roots even at low positive pressures (Ewers et al. 1997; Fisher et al. 1997). Nevertheless, we believe refilling is probably not a major process. Second, another way of restoring root system hydraulic conductance is through the growth of new lateral roots. In desert cacti, new lateral roots develop and establish new hydraulic connections with the stem (Ewers et al. 1992; North and Nobel 1996). Growing regions in roots are hydraulically isolated from the vascular system and may survive even severe stress (Hsiao and Xu 2000). Carbon cost for root elaboration is smaller than that for stem and needles. The replacement of a lateral root is therefore much less costly than that of stem or needles; this observation may be an argument for a more efficient protection from embolism in twigs than roots. The sacrifice of lateral roots may be a strategy to eliminate roots located in dry parts of the soil and favor growth of roots in wet parts.

Conclusion

The three Mediterranean conifers displayed a variety of hydraulic properties ranging from a high vulnerability to xylem embolism in *P. nigra* to a very low vulnerability in *C. sempervirens*. Stomatal closure was coordinated with the hydraulic properties and prevented the onset of severe embolism in the twigs during drought. Roots displayed a much high level of vulnerability than shoots in the three species, and the results suggest that loss of conductivity in lateral roots may be a common occurrence during soil drying.

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References

Alder NN, Sperry JS, Pockman WT (1996) Root and stem xylem embolism, stomatal conductance, and leaf turgor in *Acer grandidentatum* populations along a soil moisture gradient. Oecologia 105:293–301

Cochard H (2002) Xylem embolism and drought-induced stomatal closure in maize. Planta 215:466–471

Cochard H, Bréda N, Granier A (1996) Whole tree hydraulic conductance and water loss regulation in *Quercus* during drought: evidence for stomatal control of embolism? Ann For Sci 53:197–206

Cochard H, Coll L, Le Roux X, Améglio T (2002) Unraveling the effects of plant hydraulics on stomatal closure during water stress in walnut. Plant Physiol 128:282–290

Edwards WRN, Jarvis AJ, Grace J, Moncrieff JB (1994) Reversing cavitation in tracheids of *Pinus sylvestris* L. under negative water potentials. Plant Cell Environ 17:389–397

Ewers FW, North GB, Nobel PS (1992) Root-stem junctions of a desert monocotyledon and a dicotyledon: hydraulic consequences under wet conditions and during drought. New Phytol 121:377–385

Ewers FW, Cochard H, Tyree MT (1997) A survey of root pressures in vines of a tropical lowland forest. Oecologia 110:191–196

Fisher JB, Guillermo AA, Ewers FW, Lopez-Portillo J (1997) Survey of root pressure in tropical vines and woody species. Int J Plant Sci 158:44–50

Froux F, Huc R, Ducrey M, Dreyer E (2002) Xylem hydraulic efficiency versus vulnerability in seedlings of four contrasting Mediterranean tree species (*Cedrus atlantica*, *Cupressus sempervirens*, *Pinus halepensis* and *Pinus nigra*). Ann For Sci 59:409–418

Hacke UG, Sauter JJ (1996) Drought-induced xylem dysfunction in petioles, branches, and roots of *Populus balsamifera* L. and *Alnus glutinosa* (L.) Gaertn. Plant Physiol 111:413–417

Hacke UG, Sperry JS, Ewers BE, Ellsworth DS, Schäfer KVR, Oren R (2000a) Influence of soil porosity on water use in *Pinus taeda*. Oecologia 124:495–505

Hacke UG, Sperry JS, Pittermann J (2000b) Drought experience and cavitation resistance in six shrubs from the Great Basin, Utah. Basic Appl Ecol 1:31–41

Holbrook NM, Burns MJ, Field CB (1995) Negative xylem pressure in plants: a test of balancing pressure technique. Science 270:1193–1194

Hsiao TC, Xu L-K (2000) Sensitivity of growth of roots versus leaves to water stress: biophysical analysis and relation to water transport. J Exp Bot 51:1595–1616

Hubbart RM, Ryan MG, Stiller V, Sperry JS (2001) Stomatal conductance and photosynthesis vary linearly with plant hydraulic conductance in ponderosa pine. Plant Cell Environ 24:113–121

Jones HG, Sutherland RA (1991) Stomatal control of xylem embolism. Plant Cell Environ 14:607–612

Kavanagh KL, Bond BJ, Aitken SN, Gartner BL, Knowe S (1999) Shoot and root vulnerability to xylem cavitation in four populations of Douglas-fir seedlings. Tree Physiol 19:31–37

Lu P, Biron P, Granier A, Cochard H (1996) Water relations of adult Norway spruce (*Picea abies* (L) Karst) under soil drought in the Vosges mountains: whole-tree hydraulic conductance, xylem embolism and water loss regulation. Ann For Sci 53:113–121

Martinez-Vilalta J, Prat E, Oliveras J, Piñol J (2002) Xylem hydraulic properties in roots and stems of nine Mediterranean woody species. Oecologia 133:19–29

Nardini A, Salleo S (2000) Limitation of stomatal conductance by hydraulic traits: sensing or preventing xylem cavitation? Trees 15:14–24

Nardini A, Tyree MT, Salleo S (2001) Xylem cavitation in the leaf of *Prunus laurocerasus* and its impact on leaf hydraulics. Plant Physiol 125:1700–1709

North GB, Nobel PS (1996) Radial hydraulic conductivity of individual root tissues of *Opuntia ficus-indica* (L.) Miller as soil moisture varies. Ann Bot 77:133–142

Passioura JB (1988) Water transport in and to roots. Annu Rev Plant Physiol Plant Mol Biol 39:245–265

Pockman WT, Sperry JS (2000) Vulnerability to xylem cavitation and the distribution of Sonoran Desert vegetation. Am J Bot 87:1297–1299

Saliendra NZ, Sperry JS, Comstock JP (1995) Influence of leaf water status on stomatal response to humidity, hydraulic conductance, and soil drought in *Betula occidentalis*. Planta 196:357–366

Salleo S, Lo Gullo MA, De Paoli D, Zippo M (1996) Xylem recovery from cavitation-induced embolism in young plants of *Laurus nobilis*: a possible mechanism. New Phytol 132:47–56

Salleo S, Nardini A, Pitt F, Lo Gullo MA (2000) Xylem cavitation and hydraulic control of stomatal conductance in Laurel (*Laurus nobilis* L.). Plant Cell Environ 23:71–79

Sobrado MA, Grace J, Jarvis PG (1992) The limits to xylem embolism recovery in *Pinus sylvestris* L. J Exp Bot 251:831–836

Sparks JP, Black RA (1999) Regulation of water loss in populations of *Populus trichocarpa*: the role of stomatal control in preventing xylem cavitation. Tree Physiol 19:453–459

Sperry JS, Ikeda T (1997) Xylem cavitation in roots and stems of Douglas-fir and white fir. Tree Physiol 17:275–280

Sperry JS, Pockman WT (1993) Limitation of transpiration by hydraulic conductance and xylem cavitation in *Betula occidentalis*. Plant Cell Environ 16:279–287

Sperry JS, Saliendra NZ (1994) Intra- and inter-plant variation in xylem cavitation in *Betula occidentalis*. Plant Cell Environ 17:1233–1241

Sperry JS, Tyree MT (1988) Mechanism of water stress-induced xylem embolism. Plant Physiol 88:581–587

Sperry JS, Tyree MT (1990) Water-stress-induced xylem embolism in three species of conifers. Plant Cell Environ 13:427–436

Sperry JS, Donnelly JR, Tyree MT (1988) A method for measuring hydraulic conductivity and embolism in xylem. Plant Cell Environ 11:35–40

Sperry JS, Alder NN, Eastlack SE (1993) The effect of reduced hydraulic conductance on stomatal conductance and xylem cavitation. J Exp Bot 44:1075–1082

Sperry JS, Hacke UG, Oren R, Comstock JP (2002) Water deficits and hydraulic limits to leaf water supply. Plant Cell Environ 25:251–263

Steudle E (2000) Water uptake by roots: effects of water deficit. J Exp Bot 51:1531–1542

Tardieu F, Davies WJ (1993) Integration of hydraulic and chemical signaling in the control of stomatal conductance and water status of droughted plants. Plant Cell Environ 16:341–349

Tsuda M, Tyree MT (1997) Whole-plant hydraulic resistance and vulnerability segmentation in *Acer saccharinum*. Tree Physiol 17:351–357

Tyree MT, Ewers FW (1991) Tansley Review No. 34. The hydraulic architecture of trees and other woody plants. New Phytol 119:345–360

Tyree MT, Ewers FW (1996) Hydraulic architecture of woody tropical plants. In: Mulkey SS, Chazdon RL, Smith AP (eds) Tropical forest plant ecophysiology. Chapman and Hall, London, pp 218–241

Tyree MT, Sperry JS (1988) Do woody plants operate near the point of catastrophic xylem dysfunction caused by dynamic water stress? Plant Physiol 88:574–580

Tyree MT, Yang S (1992) Hydraulic conductivity recovery versus water pressure in xylem of *Acer saccharum*. Plant Physiol 100:669–676

Tyree MT, Cochard H, Cruiziat P, Sinclair B, Ameglio T (1993) Drought-induced leaf shedding in walnut. Evidence for vulnerability segmentation. Plant Cell Environ 16:879–882