

## CHAPTER 4

### Hydraulic architecture of *Pinus halepensis*, *P. pinea* and *Tetraclinis articulata* in a dune ecosystem of Eastern Spain

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*submitted*

*Maderas, las maderas humildemente nobles,  
lentamente crecidas, cargadas de pasado,  
nutridas de secretos terrenos y paciencia,  
de primaveras justas, de duración callada,  
de savias sustanciadas, felizmente ascendentes.*

(G. Celaya, *A Andrés Bastera* (1949))

### Resum

En aquest treball s'estudia l'arquitectura hidràulica de *Pinus halepensis*, *P. pinea* i *Tetraclinis articulata* en una zona de dunes de l'est d'Espanya. Les variables mesurades inclouen la vulnerabilitat a l'embolisme, la conductivitat hidràulica i la discriminació isotòpica foliar. Els potencials hídrics de les fulles es van avaluar també durant un període d'eixut extrem. Els resultats van mostrar que el diàmetre de les traqueïdes i la conductivitat hidràulica era sempre major a les arrels que a les branques. Les arrels eren també més vulnerables a l'embolisme i operaven més a prop del seu límit hidràulic (*i.e.*, amb marges de seguretat més estrets). Encara que no la vam quantificar, vam observar una mortalitat extensiva d'arrels en els dos pins. *T. articulata* va resultar molt més resistent a l'embolisme que *P. pinea* i *P. halepensis*. Al mateix temps, *T. articulata* presentava potencials hídrics més baixos. De resultes d'això, els marges de seguretat eren raonablement similars per les tres espècies. L'estudi posa de manifest grans diferències en l'estratègia d'ús de l'aigua entre *T. articulata* i els pins. Mentre l'estratègia de *T. articulata* era marcadament conservativa, basada en la poca vulnerabilitat del seu sistema conductor, els dos pins presentaven mecanismes reguladors a diferents nivells (*i.e.*, embolisme, demografia de rels) que limitaven l'absorció d'aigua quan aquesta esdevenia escassa.

### Summary

The hydraulic architecture of *Pinus pinea*, *P. halepensis* and *Tetraclinis articulata* was studied in a coastal dune area from Eastern Spain. The measured variables include vulnerability to xylem embolism, hydraulic conductivity and carbon isotopic discrimination in leaves. Leaf water potentials were also monitored in the three studied populations during an extremely dry period. Our results showed that roots had always wider tracheids and higher hydraulic conductivity than branches. Roots were also more vulnerable to xylem embolism and operated closer to their hydraulic limit (*i.e.*, with narrower safety margins). Although it was not quantified, extensive root mortality was observed in the two pines. *T. articulata* was much more resistant to embolism than *P. pinea* and *P. halepensis*. At the same time, *T. articulata* experienced lower water potentials. As a result, the safety margins were reasonably similar for the three species. The study outlined very different water-use strategies for *T. articulata* and the pines. Whereas *T. articulata* had a conservative strategy that relied on the low vulnerability of its conducting system, the two pines showed regulatory mechanisms at different levels (*i.e.*, embolism, root demography) that constrained the absorption of water when it became scarce.

## Introduction

Plants tolerance to drought is defined by several components (e.g., Rambal 1993), including rooting extension and depth, the regulation of transpiration, and the water transport properties of the xylem. However, there is increasing evidence that hydraulic constraints within the xylem limit how different plant species cope with drought (Pockman & Sperry 2000, Sperry 2000). Under dry conditions, substantially negative water potentials tend to cause the cavitation of water inside xylem conduits. As a result, these conduits are filled with air from the surrounding tissue (Zimmerman 1983). These embolisms reduce the water transport capacity of the xylem, causing a decrease in leaf water potential which again produces more embolisms. This cycle can become unstable ("runaway embolism", Tyree and Sperry 1988), leading to a breaking of the hydraulic continuum between soil and leaves, and to branch (or plant) dieback (e.g., Rood *et al.* 2000). Xylem embolism is thus an important ecological factor not only because it directly reduces a plant's potential for gas exchange (Sperry *et al.* 1998) but also because of the limit it sets to the minimum water potential that the plant can tolerate.

Species in the genus *Pinus* tend to be more vulnerable to xylem embolism than most conifers, and their stem presents relatively homogeneous vulnerability curves (Cochard 1992, Linton *et al.* 1998, Hacke *et al.* 2000a, Piñol & Sala 2000, Martínez-Vilalta & Piñol 2001). This relatively high vulnerability to embolism is associated with an efficient stomatal control over water use (Linton *et al.* 1998, Rundel & Yoder 1998). In contrast, the

family Cupressaceae, to which *Tetraclinis articulata* (Vahl) Mast. belongs, contains some of the most embolism-resistant species ever measured, like *Juniperus monosperma*, with a water potential causing 50% embolism of approximately -12 MPa (Pockman & Sperry 2000), and *J. ashei*, with a slightly higher value of -11 MPa (W.T. Pockman *et al.*, unpublished results). Within individuals, roots are usually more vulnerable to embolism than branches, both in angiosperms (Sperry & Saliendra, 1994) and conifers (Linton *et al.* 1998).

Coastal dunes pose special problems to plant establishment, growth and survival (Ranwell 1972). In the Mediterranean region, low rainfall and high temperatures combine with the small water-retention capacity of sandy soils to establish the water-limited conditions that prevail in coastal dunes. The effects of soil porosity on plant water use have been discussed by Bristow *et al.* (1984) and Sperry *et al.* (1998). As water in coarse soils is retained by weaker capillary forces because of the larger pore spaces (Hillel 1980), plants can potentially extract water at relatively high water potentials. At the same time, the range of water potential over which water is available is smaller (Hillel 1980), thus critically low rhizosphere hydraulic conductivities are easily reached. As a result, the overall sensitivity of plants to soil water potential needs to be particularly high in sandy substrates for survival to be possible (Hacke *et al.* 2000a).

In this study we characterize the hydraulic architecture of adult individuals of *Pinus pinea* L. (Stone pine), *Pinus halepensis* Mill. (Aleppo pine), and *Tetraclinis articulata*

(Barbary thuja) in an area of coastal dunes during a dry period. The hypotheses we addressed were: (1) within a species, roots live closer to their hydraulic limit than branches of comparable size, (2) among species, pines are more vulnerable to xylem embolism than *T. articulata*, but because of the extremely dry conditions that prevail in the study area, (3) pines need other adaptations (e.g., high hydraulic efficiencies) to compensate for the greater vulnerability of their xylem.

## Material and Methods

### *Study site and plant material*

The populations studied are located at the Guardamar-La Marina area, Alacant, SW Spain (38° 10' N, 0° 38' W). The dune system comprises a surface of 848 ha, and is one of the most important systems of coastal dunes in the Iberian Peninsula (Escarré *et al.* 1989). The climate is Mediterranean arid with a mean temperature of 17.5°C and an annual rainfall of 312 mm (average for the 1961-1990 period). The long-term average of potential evapotranspiration in Guardamar is 870 mm (Pérez 1994). Soils are sandy (sand >99%) with predominance of the 0.05-0.2 mm grain-sizes (Escarré *et al.* 1989).

The study was carried out during the late spring and the summer of 2000, an extremely dry period (only 5 mm of rainfall between May and August). *P. pinea* and *P. halepensis* were sampled from the same mixed plantation (ca. 75 years old; Aldeguer *et al.* 1997), whereas *T. articulata* was sampled from a more recent (ca. 17 years old) plantation within less than 1

km. The substrate of the two sites is almost identical (Jiménez-Ortiz 2001).

### *Water potentials*

Leaf water potentials were measured in July and August of 2000 with a pressure bomb (Model 3005, Soilmoisture Equipment corp., Goleta, CA, USA) (Scholander *et al.* 1965). On each sampling date one shoot tip from 6 different individuals per species was measured at predawn (0230-0400 solar hour) and at midday (1130-1300 solar hour). Predawn water potentials ( $\psi_{pd}$ ) were assumed to be in equilibrium with soil water potentials and were used to compare with the vulnerability curves of roots and establish its minimum safety margins (Hacke *et al.* 2000b; see the "Vulnerability to xylem embolism" section). For branches, the comparison was done with midday water potentials ( $\psi_{md}$ ).

### *Xylem anatomy*

Tracheid diameters were measured on the same stem and root segments that had been used to establish vulnerability curves. Transverse sections (ca. 25  $\mu\text{m}$ ) were cut using a rotary microtome (Reichert, Vienna, Austria). The sections were stained with safranin (0.1%) to improve contrast and mounted in glycerol. The slides were viewed at 100x (branches) or 50x (roots) with a compound microscope (Olympus BH-2, Hamburg, Germany) attached to a monochrome video camera (JVC TK-1270, Yokohama, Japan) and a computer. Two to four representative regions from the outermost rings of each section, situated 90°

apart, were captured in black and white format and analysed with a standard image analysis package. Within each image all open tracheids wider than 2  $\mu\text{m}$  (stems) or 7  $\mu\text{m}$  (roots) were sampled. These values were selected in each case to maximize the agreement between the visually identified tracheids and those selected by the computer. For each selected conduit the program determined the total cross sectional area and the perimeter. At least 500 conduits were measured from each section (the average was 1855 conduits).

Three variables were used to characterize the xylem anatomy of each species: the mean tracheid diameter ( $d$ , in  $\mu\text{m}$ ), the mean hydraulic diameter ( $d_h$ , in  $\mu\text{m}$ ), and the mean lumen area per cross sectional area ( $A_x:A_s$ , in %). The hydraulic diameter was calculated assuming that hydraulic conductivity is proportional to the diameter raised to the fourth power. The following expression was used:  $\sqrt[4]{d_i^4/N}$  (Tyree *et al.* 1994).

### **Hydraulic conductivity**

Hydraulic conductivity was measured following Sperry *et al.* (1988). Segments *ca.* 20 cm long and with a diameter of  $0.7 \pm 0.2$  cm were re-cut underwater from the sampled roots and branches. After removing the bark, their proximal ends were connected to a tubing system. The system was filled with a filtered (0.22  $\mu\text{m}$  pore size) and degassed solution of HCl (pH *ca.* 2). Hydraulic conductivity ( $K_h$ , in  $\text{m}^4 \text{MPa}^{-1} \text{s}^{-1}$ ) was calculated as the ratio between the flow through the segment and the pressure

gradient ( $P = \text{ca. } 6 \text{ kPa}$ ). The flow was measured gravimetrically. To obtain the maximum hydraulic conductivity the measure solution was previously injected at *ca.* 100 kPa for 60 min. to remove all native embolisms from the segment. Specific hydraulic conductivity ( $K_s$ , in  $\text{m}^2 \text{MPa}^{-1} \text{s}^{-1}$ ) was calculated as the ratio between maximum hydraulic conductivity and mean cross sectional area of the segment (without bark); and leaf-specific conductivity ( $K_L$ , in  $\text{m}^2 \text{MPa}^{-1} \text{s}^{-1}$ ), as the quotient between maximum hydraulic conductivity and leaf area. Finally, the ratio between cross sectional area and leaf area ( $A_s:A_L$ , Zimmerman 1983) of each branch segment was also calculated.

### **Vulnerability to xylem embolism**

Vulnerability curves show the relationship between water potential (or pressure) in the xylem and percentage loss of hydraulic conductivity (PLC) due to embolism. The air injection method (Cochard *et al.* 1992, Sperry & Saliendra 1994) was used to establish the curves. This method has been validated for several species, including conifers (Cochard 1992, Sperry & Ikeda 1997). In each run, six segments were put inside a pressure chamber with both ends protruding. Proximal ends were connected to the measuring circuit, and maximum hydraulic conductivity was measured. The pressure inside the chamber was then raised to 0.5 or 1 MPa, and maintained during 10 min. Next, the pressure was lowered to a basal value of *ca.* 10 kPa, and after 15 min. to allow the system to equilibrate, conductivity was measured again. The process was repeated for the following injection pressures: 0.5, 1, 1.5, 2, 3, 5 and 7

MPa (roots); and 1, 2, 3, 4, 6 and 8 MPa (branches).

Vulnerability curves were fitted with the following function (Pammenter & Vander Willigen 1998):

$$PLC = \frac{100}{1 + e^{a(P-b)}} \quad (\text{eq. 1})$$

where PLC is the percentage loss of hydraulic conductivity, P is the applied pressure, b is the pressure (*i.e.*, - ) causing a 50% loss of hydraulic conductivity, and a is related to the slope of the curve. Parameter values were obtained by regression analysis (least squares) after linearization of equation 1.

The fitted curves were used to calculate the safety margins at which each species (and tissue) was operating, in a way similar to Pockman & Sperry (2000; see also Hacke *et al.* 2000b). The safety margins were defined as the difference between the minimum water potential measured in the field (minimum  $\psi_{pd}$  for roots and minimum  $\psi_{md}$  for branches) and the water potential required to cause a 95 PLC, calculated from the fitted equation.

### $\delta^{13}\text{C}$ measurements

Carbon isotope discrimination measured from leaves of the same branches used for hydraulic measurements was used as a proxy of integrated water-use efficiency (WUE, Farquhar & Richards 1984, Ehleringer & Osmond 1989). Since the studied populations were very close, differences in WUE due to microclimatic effects were negligible (Farquhar *et al.* 1989, Marshall & Zhang

1994). After drying, leaves were ground and sub-samples were separated to measure their carbon isotope composition. All analyses were carried out at the Serveis Científico-Tècnics of the Universitat de Barcelona with an elemental analyser Carlo Erba EA1108 (Milano, Italy) attached to a Delta C isotope mass spectrometer, and using a CONFLO II interface (Thermo Finnigan MAT, Bremen, Germany). The accuracy of the measurements was 0.15 ‰. The relationship between carbon stable isotopes was expressed in relation to PDB (Pee-Dee Belemnite standard), and converted to discrimination ( $\delta = \frac{^{13}\text{C}_{\text{air}}}{^{13}\text{C}_{\text{plant}}} - 1$ ), assuming that  $^{13}\text{C}_{\text{air}}$  was -8 ‰.

## Results

### *Water potentials*

Predawn and midday water potentials were much lower in *T. articulata* than in the two pines (Two-way ANOVA with repeated measurements;  $F=70.02$ ,  $p < < 0.001$  for predawn and  $F=113.99$ ,  $p < < 0.001$  for midday values) (Figure 1). Predawn water potentials decreased significantly between July and August for the three species ( $F=51.63$ ,  $p < < 0.001$ ), whereas midday values decreased only for the two pines and, hence, the global effect was not significant ( $F=3.10$ ,  $p=0.098$ ). The difference between midday and predawn water potentials was less in August for the three species ( $F=22.47$ ,  $p < 0.001$ ).

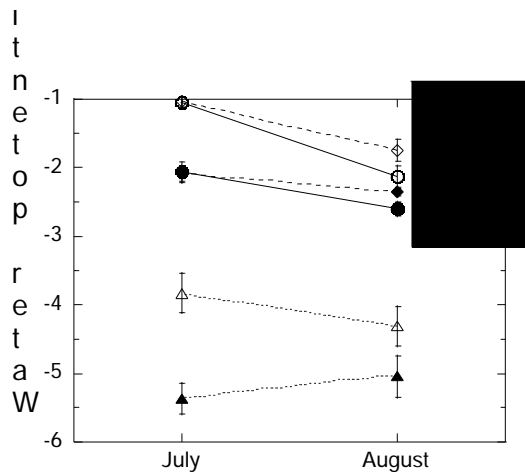


Figure 1. Predawn (p) and midday (m) water potentials measured in *P. halepensis* (Ph), *P. pinea* (Pp) and *T. articulata* (Ta). The error bars represent standard errors (n=6).

### Xylem anatomy

The between-tissue differences in xylem anatomy were always highly significant (Two-way ANOVA (species×tissue),  $p < 0.001$ ). Tracheid diameter (d), hydraulic tracheid diameter ( $d_h$ ) and the  $A_X:A_S$  ratio in roots always exceeded those in branches (Table 1 and Figure 2). The differences were more pronounced in the two pines than in *T. articulata* (see Figure 3 for a *P. pinea* example).

Tracheids were narrower in the roots of *T. articulata* than in the roots of the two pines (Table 1 and Figure 2). The differences were significant for d (One-way ANOVA;  $F=5.73$ ,  $p=0.011$ ) and for  $d_h$  ( $F=7.41$ ,  $p=0.004$ ). In branches the pattern was the same, but the differences were smaller and not significant ( $F=1.99$ ,  $p=0.164$  for d;  $F=1.63$ ,  $p=0.221$  for  $d_h$ ). The percent of conducting area per cross sectional area ( $A_X:A_S$ ) was higher in the two pine species than in *T. articulata* ( $F=13.11$ ,

$p < 0.001$  in roots; and  $F=7.46$ ,  $p=0.004$  in branches).

### Hydraulic measurements and safety margins

Specific hydraulic conductivity ( $K_S$ ) was higher in roots than in branches for the three species (Two-way ANOVA (species×tissue);  $F=10.75$ ,  $p=0.002$ ) (Table 1). *P. pinea* had higher  $K_S$  than the other two species both for roots and branches, although the differences were (marginally) significant only in branches (One-way ANOVA;  $F=3.34$ ,  $p=0.058$ ). The differences in  $A_S:A_L$  were not significant ( $F=2.29$ ,  $p=0.130$ ), but the larger values in *P. pinea* than in *T. articulata* (Table 1) combined with the higher  $K_S$  in *P. pinea* branches caused leaf-specific conductivity ( $K_L$ ) to be significantly greater in *P. pinea* than in the other two species ( $F=5.44$ ,  $p=0.014$ ) (Table 1).

Roots were more vulnerable to embolism than branches (Figure 4). This was reflected in the centrality parameters (b) of the vulnerability curves (Two-way ANOVA (species×tissue);  $F=63.32$ ,  $p < 0.001$ ) and in their slopes (a) ( $F=9.96$ ,  $p < 0.001$ ) (Table 1). *T. articulata* was more resistant to xylem embolism than the two pines (Figure 4). The parameters a and b of the vulnerability curves of roots and branches were significantly higher in *T. articulata*, except for the comparison between the slopes of the curves of *T. articulata* and *P. pinea* roots (One-way ANOVA,  $p=0.320$ ) (Table 1). The vulnerability of the two pines was similar although *P. pinea* were slightly more resistant (Figure 4 and Table 1).

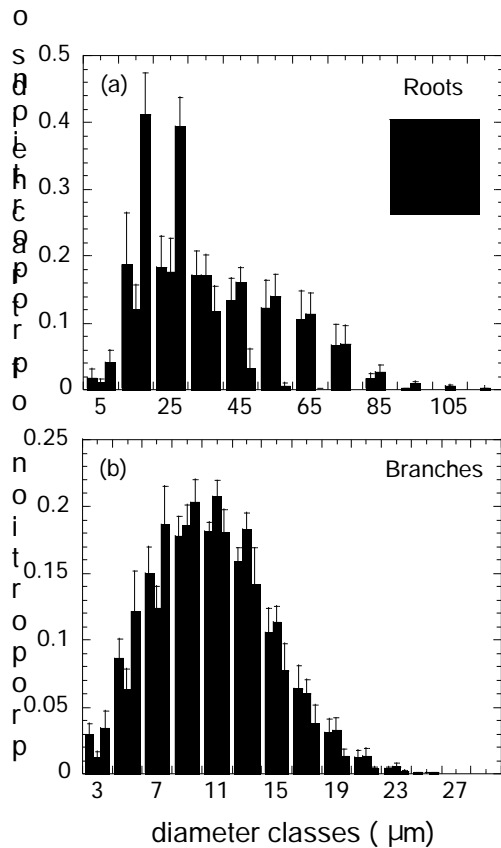


Figure 2. Tracheid-size distributions of roots (a) and branches (b) of the three studied species. Abbreviations as in Figure 1. The error bars represent standard errors ( $n=6-8$ ).

The difference between the water potential causing a 95 PLC and the minimum water potential experienced during the study (safety margin) was always narrower for roots than for branches (Figure 5). The mean safety margin was only 0.4 MPa for roots, whereas it was 5.7 MPa for branches. Among species the differences were not so pronounced: only *T. articulata* branches showed a safety margin (9.6 MPa) clearly wider than that of pines (around 4 MPa).

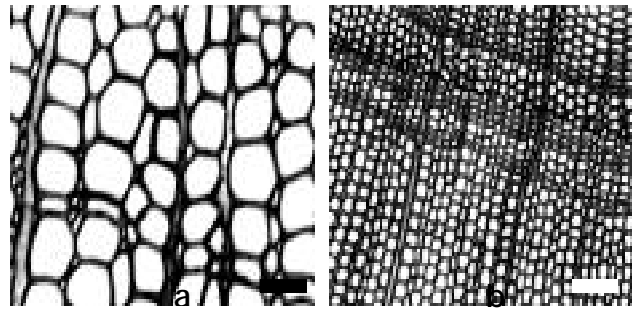


Figure 3. Photographs of representative sections of the xylem of a root (a) and a branch (b) from the same individual of *P. pinea*. The black/white bars have a length of 100  $\mu\text{m}$ .

### $\delta^{13}\text{C}$ measurements

Carbon isotope discrimination ( $\delta^{13}\text{C}$ ) was higher in *P. pinea* than in the other two species (One-way ANOVA;  $F=36.49$ ,  $p<<0.001$ ), among which no difference was found (Table 1). As air temperature and vapour pressure were common for the three species, and for needle-leaved plants leaf temperature is often close to air temperature (Campbell & Norman 1998), vapour pressure deficit was similar at least for the two pine species. Thus, differences in  $\delta^{13}\text{C}$  between the two pines presumably indicate long-term differences in  $C_i/C_a$  and in water use efficiency. The measured difference denotes (Farquhar *et al.* 1989) a 26% smaller water-use efficiency (integrated during needle life) for *P. pinea*.

### Discussion

Large differences in the hydraulic architecture of above- and below-ground tissues have been repeatedly reported in the literature. Roots usually have wider conduits and higher  $K_S$  (Pallardy *et al.* 1995, Ewers *et al.* 1997,



Chapter 3) and are more vulnerable to xylem embolism. In fact, roots are not only more vulnerable but normally live closer to their hydraulic limit (*i.e.*, with narrower safety margins; Hacke *et al.* 2000b, Chapter 3). Our results support all these findings (Figs 2, 3, 4 and 5). It is worth noting the extreme dimorphism between the anatomy of roots and branches that we have found in *P. halepensis* and *P. pinea*. For most species the mean conduit diameter in surface roots tends to be less than double the mean diameter in branches or stems of similar size (Ewers *et al.* 1997, Hacke *et al.* 2000b, Chapter 3). For the two pines in this study xylem conduits were almost four times larger in roots, reaching sizes that are exceptional among tracheid-bearing species (Zimmerman 1983). This might be a general character in pines, because Hacke *et al.* (2000a) found similar diameters in *P. taeda* roots from E USA, with a mean hydraulic diameter of *ca.* 49  $\mu\text{m}$  (although their hydraulic means were calculated in a slightly different way). The fact that Hacke *et al.* (2000a) found no difference in tracheid diameter between loamy and sandy substrates suggests that this singular xylem anatomy is not related to the greater vulnerability to xylem embolism predicted for coarse soils (Sperry *et al.* 1998). Highly efficient roots from an hydraulic point of view have been reported previously for *P. ponderosa* (Rundel & Yoder 1998) and *P. taeda* (Hacke *et al.* 2000a). The extremely porous roots of pines can act as water-storing bodies, as has been proposed for *P. halepensis* (Schiller 2000). Xylem cavitation results in the net withdrawal of water from the affected tissue (Tyree & Yang 1990). This water becomes then available for transpiration and has been proposed as one

of the main components of plant water storage (Zimmermann 1983, Holbrook 1995). The contribution to transpiration of stored water is particularly high in conifers (*e.g.*, Waring *et al.* 1979). The cycles of cavitation-refilling observed by Borghetti *et al.* (1998) in *P. halepensis* are in agreement with that hypothesis.

It is also interesting to note that the roots of the three studied species were very close to (or beyond) their hydraulic limit during the summer drought of 2000. In fact, since predawn water potentials give an integrated measure over the entire "wet" rooting volume and we only sampled surface roots, it is likely that the actual safety margins were even lower than the calculated ones. This suggests that there is a considerable mortality of roots during dry periods, which was corroborated in the case of pine species by our own observations during the sampling. The explanation of this behaviour might be related to the particular morphology and anatomy of the root system of the studied pines: (1) these roots have low wood densities and, hence, their construction cost is probably small; (2) within a dense root system, from which only some roots have access to water, it may be advantageous to lose the roots that are in contact with the drier parts of the soil to avoid losing water through them (Nobel & North 1993) and to protect more valuable organs (Hacke *et al.* 2000a).

As hypothesized, *T. articulata* was more resistant to xylem embolism than the two pines, which were notably similar (Figure 4). The among-species differences in vulnerability to embolism in this study were at least partially associated with differences in

xylem anatomy: *T. articulata*, the more resistant species, also had smaller tracheids and smaller hydraulic conductivities, although the differences were not always significant (Table 1). This agrees with the existence of a trade-off between xylem conductivity and resistance to cavitation (Zimmermann 1983). Extensive reviews with conifers and non-conifers from around the world (Tyree *et al.* 1994, Pockman & Sperry 2000) show that there is a negative relationship between conductivity and resistance to cavitation, but too weak to have predictive value. Our results are also consistent with the existence of a trade-off within species, since hydraulic conductivity and vulnerability to xylem embolism were always greater in roots than in branches.

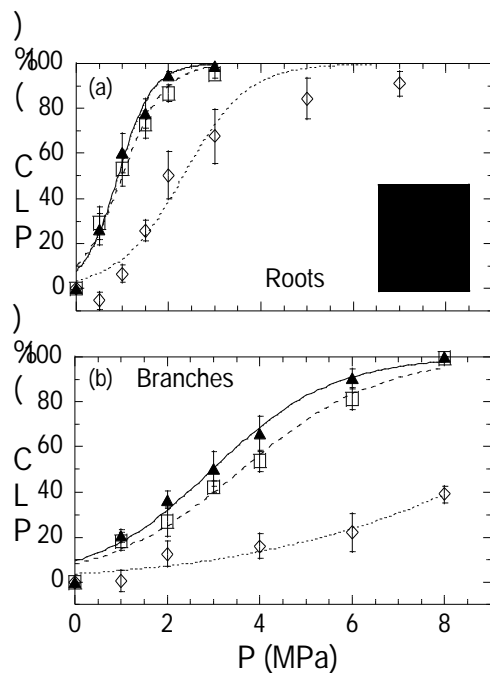


Figure 4. Vulnerability curves of roots (a) and branches (b) of the three studied species. Abbreviations as in Figure 1. The error bars represent standard errors ( $n=5-9$ ).

Despite the large differences in vulnerability to embolism between the pines and *T. articulata*, their safety margins were reasonably similar, particularly in roots (Figure 5). The reason for this are the different strategies that the species used to cope with drought. *T. articulata* has a conservative water-use, in which safety has a central role. The lower  $p_d$  found in this species, and also our own observations during the sampling of root segments, suggest that its root system is less developed than in pines. This character has been related to low vulnerabilities to xylem embolism in the California chaparral (Davis *et al.* 1998). As we have already seen, great resistance to embolism tends to imply low hydraulic efficiencies, although this trade-off is often weak (Tyree *et al.* 1994). Another cost of developing a safe xylem could be the requirement of greater biomass allocation to the construction of that tissue (Hacke *et al.* 2001).

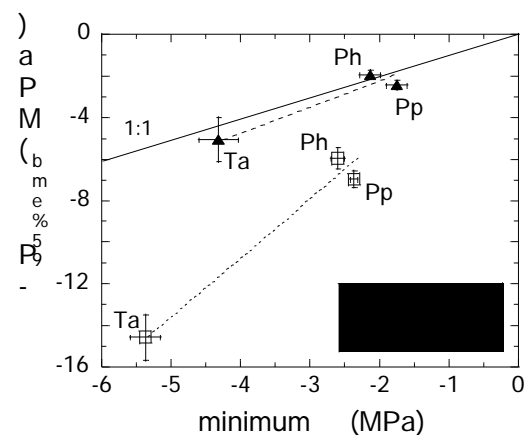


Figure 5. Relationship between the injection pressure that causes a 95 PLC and the minimum water potential measured in the field. The 1:1 relationship and the linear regressions for roots and branches are also depicted. The error bars represent standard errors.

Table 1. Measured variables (means  $\pm$  standard errors) in roots and branches of *Pinus halepensis*, *P. pinea* and *Tetraclinis articulata*. Different letters indicate significant differences among species (lowercase for roots and uppercase for branches).

	<i>P. halepensis</i>		<i>P. pinea</i>		<i>T. articulata</i>	
	Roots	Branches	Roots	Branches	Roots	Branches
d ( $\mu\text{m}$ )	34.0 <sup>a</sup> $\pm$ 5.2	9.4 <sup>A</sup> $\pm$ 0.5	35.2 <sup>a</sup> $\pm$ 3.9	10.1 <sup>A</sup> $\pm$ 0.4	19.8 <sup>b</sup> $\pm$ 1.6	8.6 <sup>A</sup> $\pm$ 0.6
d <sub>h</sub> ( $\mu\text{m}$ )	40.6 <sup>a</sup> $\pm$ 6.0	11.3 <sup>A</sup> $\pm$ 0.6	45.0 <sup>a</sup> $\pm$ 4.7	11.7 <sup>A</sup> $\pm$ 0.5	22.9 <sup>b</sup> $\pm$ 1.9	10.3 <sup>A</sup> $\pm$ 0.7
A <sub>X</sub> :A <sub>S</sub> (%)	30.5 <sup>a</sup> $\pm$ 3.4	16.1 <sup>A</sup> $\pm$ 1.6	32.5 <sup>a</sup> $\pm$ 2.3	17.4 <sup>A</sup> $\pm$ 0.8	16.8 <sup>b</sup> $\pm$ 1.4	10.2 <sup>B</sup> $\pm$ 1.6
A <sub>S</sub> :A <sub>L</sub> ( $\times 10^4$ )	-	7.59 <sup>A</sup> $\pm$ 1.92	-	6.19 <sup>A</sup> $\pm$ 0.53	-	3.64 <sup>A</sup> $\pm$ 0.43
K <sub>S</sub> (m <sup>2</sup> MPa <sup>-1</sup> s <sup>-1</sup> , $\times 10^4$ )	8.93 <sup>a</sup> $\pm$ 3.02	1.52 <sup>A</sup> $\pm$ 0.32	17.0 <sup>a</sup> $\pm$ 5.63	3.51 <sup>A</sup> $\pm$ 0.82	6.58 <sup>a</sup> $\pm$ 2.43	1.63 <sup>A</sup> $\pm$ 0.61
K <sub>L</sub> (m <sup>2</sup> MPa <sup>-1</sup> s <sup>-1</sup> , $\times 10^8$ )	-	9.54 <sup>A</sup> $\pm$ 2.03	-	21.9 <sup>B</sup> $\pm$ 4.81	-	5.78 <sup>A</sup> $\pm$ 2.25
a	-3.80 <sup>a</sup> $\pm$ 0.44	-1.15 <sup>A</sup> $\pm$ 0.13	-2.70 <sup>ab</sup> $\pm$ 0.26	-0.96 <sup>A</sup> $\pm$ 0.07	-1.73 <sup>b</sup> $\pm$ 0.39	-0.51 <sup>B</sup> $\pm$ 0.05
b (MPa)	1.06 <sup>a</sup> $\pm$ 0.09	3.18 <sup>A</sup> $\pm$ 0.23	1.28 <sup>a</sup> $\pm$ 0.12	3.76 <sup>A</sup> $\pm$ 0.21	2.97 <sup>b</sup> $\pm$ 0.56	8.66 <sup>B</sup> $\pm$ 0.85
( )	-	16.1 <sup>A</sup> $\pm$ 0.3	-	18.9 <sup>B</sup> $\pm$ 0.2	-	16.1 <sup>A</sup> $\pm$ 0.2

At the other end of the spectrum, *P. halepensis* and *P. pinea* have a more plastic water use, with high hydraulic efficiencies and vulnerabilities to xylem embolism, and well developed root systems. Pines can extract water quickly when it is plentiful but need also mechanisms to limit water use when it becomes scarce. These control mechanisms exist at different levels, from roots to the conducting system and, probably, stomata. The extremely wide tracheids in surface roots suggest that at least some roots can access a reliable water reservoir. The main candidates are the water table and the subsurface condensation of dew that takes place in dunes as a result of thermal oscillations (De Jong 1979, Barbour *et al.* 1989). In their study on the influence of soil porosity on water use in *P. taeda*, Hacke *et al.* (2000a) predicted that, in sandy soils, there would be a shift to the use of deep water during surface drought. Although *P. pinea* has higher hydraulic efficiencies in relation to the supported leaf area, and tends to have slightly lower vulnerabilities to xylem embolism (Table 1), safety margins are practically identical for both pines (Figure 5). We hypothesize that the hydraulic superiority of the xylem of *P.*

*pinea* allows this species to have a less strict stomatal control than *P. halepensis*. This is consistent with larger values measured in *P. pinea*.

We have shown that from the point of view of the hydraulic architecture, *T. articulata* is more resistant to drought than the two pines. If we consider that most models predict an increase in aridity in the Mediterranean region as a result of climate change (Palutikof *et al.* 1994, Rambal & Hoff 1998), the ability of plants to cope with extreme droughts will be of increasing importance. In this context, the differences that we have found may be critical; not only because of the narrower safety margins in pines, but also because of the nature of their strategy to cope with drought. Their stress-avoider strategy, based on the protection of the vulnerable xylem by stomatal control or biomass allocation (Rundel & Yoder 1998), is presumably less capable of resisting extremely dry conditions than the intrinsically resistant xylem of the stress-tolerant *T. articulata*. In fact, extensive drought-induced mortalities have been already reported in NE Spain for another pine species (*P. sylvestris*), with a hydraulic

architecture similar to *P. halepensis* and *P. pinea* (Martínez-Vilalta & Piñol 2001).

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## CHAPTER 5

### Drought-induced mortality and hydraulic architecture in pine populations of the NE Iberian Peninsula

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*Forest Ecology and Management, in press*

*Si jo fos marxant a Prades  
-A les boires de tardor-  
Per guardar-vos de glaçades  
Us donaria flassades  
I caperons de castor.  
I, per guarnir la pallissa,  
Les figures de terrissa  
On Vós vestiu de Pastor.*

[...] *Però no ho só! Tinc cabana  
I llibres, pertot, en feu;  
Faig el sord a la campana  
-O bé escric a l'altra plana  
de la Llei que Vós dicteu.  
Us duc només l'esperança  
Que, Contrast de la Balança,  
Alceu els ulls i em mireu*

(J.V. Foix, *Si jo fos marxant a Prades* (1958))

### Resum

Els estius de 1994 i, en menor grau, 1998 van ser particularment secs a l'est d'Espanya. Aquest eixut va provocar danys importants en algunes espècies. En aquest treball s'estima la mortalitat associada a l'eixut en diverses poblacions de tres espècies de pins que coexisteixen a la zona d'estudi (*Pinus nigra*, *P. pinaster* i *P. sylvestris*). Per cada població, vam mesurar també la conductivitat hidràulica, la vulnerabilitat a l'embolisme i el gruix dels anells de creixement. Els resultats van mostrar que la mortalitat només va afectar *P. sylvestris*, i que l'afectació va ser diferent en dues poblacions d'aquesta espècie. Encara que les conductivitats hidràuliques màximes i les vulnerabilitats a l'embolisme van resultar molt semblants entre espècies i poblacions, aquestes diferien en altres aspectes de la seva arquitectura hidràulica. En particular, (1) la conductivitat hidràulica per unitat d'àrea foliar era menor en la població de *P. sylvestris* que va resultar més afectada per la sequera. Conductivitats específiques foliars baixes provoquen majors gradients de potencial hídic i, per tant, majors nivells d'embolisme (si les vulnerabilitats són iguals). Suggerim que aquesta diferència va ser la principal causant del patró de mortalitats que vam observar entre poblacions. (2) *P. pinaster* mostrava una major eficiència en l'ús de l'aigua (inferida a partir de la discriminació isotòpica del carboni a les fulles) que les altres dues espècies. Respecte a la resposta a la sequera a nivell de població, la població més afectada de *P. sylvestris* va augmentar lleugerament el creixement després de la sequera de 1994. Aquest resultat el relacionem amb una possible relaxació de la competència entre els individus supervivents. L'elevada taxa de mortalitat observada en aquest estudi suggereix que climes més secs, com els previstos per la majoria de models climàtics, poden posar en perill la supervivència de diverses poblacions de *P. sylvestris* a la conca mediterrània.

The summers of 1994 and, to a lesser extent, 1998 were particularly dry in eastern Spain. As a result, several plant species were severely affected. We estimated drought-induced mortality in populations of three pine species that co-exist in the study area (*Pinus nigra*, *P. pinaster* and *P. sylvestris*). Hydraulic conductivity, vulnerability to xylem embolism, and tree-ring width were also measured for each population. Results showed that mortality only affected *P. sylvestris*, and that there were significant differences between two populations of this species. Although maximum hydraulic conductivity and vulnerability to embolism were almost identical among species and populations, they differed in other aspects of their hydraulic architecture. In particular: (1) hydraulic conductivity per unit of leaf area was lower in the most acutely affected *P. sylvestris* population. Lower leaf-specific conductivity causes higher water potential gradients and, hence, higher levels of embolism (if vulnerabilities are alike). We suggest that this difference was the main cause of the observed mortality pattern. (2) *P. pinaster* showed higher water-use efficiency (inferred from  $^{13}\text{C}$  data) than the other two species. Regarding the response to drought at the population level, the most affected *P. sylvestris* population slightly increased growth after the 1994 drought, which we relate to a relaxation of competition among surviving individuals. The important drought-induced mortality observed in the study area suggests that a drier climate (as predicted by climate change simulations) may endanger several *P. sylvestris* populations in the Mediterranean basin.

Climate models predict that, for the western Mediterranean basin, temperatures will rise 3-4°C during the next century (Rambal & Hoff 1998). These simulations also predict, for the same period, a decrease in annual rainfall of 43-110 mm, 2-56% of this reduction occurring in summer. This trend of increased aridity has been already observed in NE Spain for the XX century by direct analysis of climatic series (Piñol *et al.* 1998). On the whole, an increase in the frequency and intensity of extreme droughts is expected (IPCC 2001).

In the summer of 1994 eastern Spain experienced one of the most severe droughts ever recorded (Figure 1). As a consequence, several plant species experienced an extensive mortality (Lloret & Siscart 1995). In 1998 another, less acute drought affected the same area (Figure 1), again causing the death of some adult trees. These two summers were the driest of the decade in NE Spain and besides tree death they were characterised by being the worse wildfire seasons in this period (Piñol *et al.* 1998, and unpublished results). Among the commonest forest species in NE Spain, *Quercus ilex* L. and *Pinus sylvestris* L. were particularly badly affected. *Q. ilex* was able to recover partially by means of crown-resprouting, but affected individuals of *P. sylvestris* died.

*P. sylvestris* is the most widely distributed of all pine species. Its distribution comprises almost the whole of the Palearctic Region, from 8°W to 141°E and from 37°N to 70°N. Although most populations are in the boreal zone, some occur in moderately arid climates in the Mediterranean Region, from

the Iberian

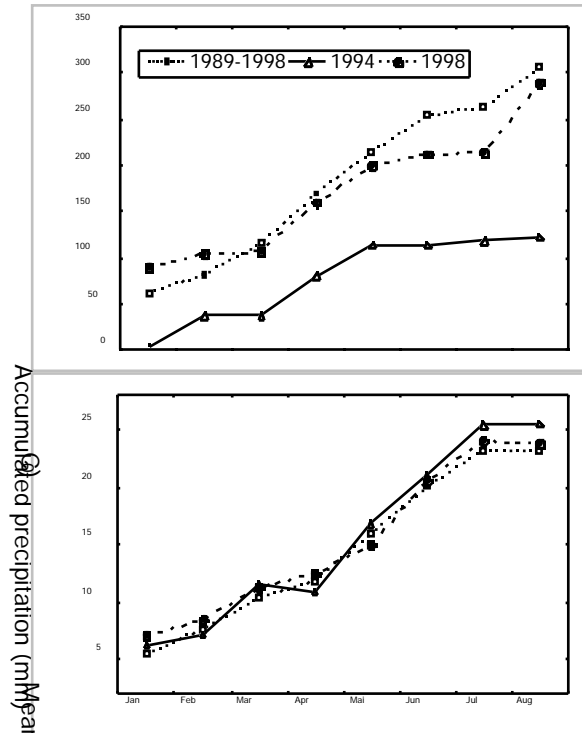


Figure 1. Meteorological data from Poblet station (12 Km from the study area, 500 m asl). Accumulated precipitation and mean temperature, from January to August, are shown.

the Iberian Peninsula to Turkey (Barbéro *et al.* 1998). The south-western limit of the species is in the Iberian Peninsula, with important populations in the Pyrenees and several scattered southern, more arid localities (Castroviejo *et al.* 1986). On the other hand, *P. nigra* Arnold and *P. pinaster* Aiton are typically Mediterranean pines. These two species are distributed over mountainous areas of southern Europe and northern Africa, and although they co-exist with *P. sylvestris* in several regions, they tend to occupy drier areas (Barbéro *et al.* 1998). The drier limits of the distribution of species such as *P. sylvestris*, that live mostly in humid environments are the first places to look for the effects of increased aridity.



The capacity of plants to cope with water stress depends on several factors, in particular: (1) rooting extension and depth; (2) regulation of transpiration; and (3) hydraulic architecture, which establishes the capacity of plants to satisfy water demands with the available resources (Tyree & Ewers 1991). Although traditionally less studied, the last of these factors, in particular vulnerability to xylem embolism, is nowadays receiving increasing attention (Tyree & Sperry 1989, Tyree *et al.* 1994). As a result, evidence supporting the idea that plant species tend to live close to the limit of their hydraulic capacity is increasing. This is especially true for species that live in arid areas, where the different components of hydraulic architecture combine to produce a fairly precise form of 'tuning' between a plant's hydraulic capacity and the range of drought conditions it experiences (*e.g.*, Kolb & Sperry 1999).

This study deals with effects of the 1994 and, to a lesser extent, 1998 droughts on several pine populations at a locality in north-eastern Spain. In this area, *P. sylvestris* has an important but isolated population (nearest population at *ca.* 80 km) that co-exists with two other pine species (*P. nigra* and *P. pinaster*). The underlying hypothesis of the study is that differences in mortality among localities and species are caused by differences in hydraulic architecture. The three main objectives are: (1) to quantify the drought-induced mortality in populations of these three pines, and in different *P. sylvestris* populations; (2) to relate the observed mortality pattern with several hydraulic architecture components; and (3) to

determine the consequences of the droughts for tree growth.

## Material and methods

### *Study area and plant material*

The studied populations are located in the Prades Mountains, NE Spain (41° 13' N, 0° 55' E), between 865 and 1060 m asl. The climate is Mediterranean, with moderate rainfall (annual mean of 537 mm for the 1981-1995 period) and moderately warm temperatures (10.0°C mean at Prades, 1000 m asl). The substrate consists of fractured schist, and soils are *xerochrepts* with clay loam texture. Additional information about the study area can be found in Hereter & Sánchez (1999).

We studied pines located on south-facing slopes of two valleys, Castellfollit and Titllar. These two valleys are approximately 2 km apart. In Castellfollit we studied a mixed forest of *Pinus sylvestris* and *P. nigra*, and a monospecific *P. pinaster* plantation. In Titllar the only pine present was *P. sylvestris*. In this valley, pine mortality was also measured in a north-facing population to check for possible aspect effects. All populations were at least 150 years old (Bosch 1995) except the *P. pinaster* plantation, which was approximately 40 years old. In all cases, pines composed the tree layer almost exclusively.

### *Transects*

We used belt transects to estimate pine mortality that had occurred over the previous decade. We attributed mortality to drought because after periodic visits to the

study area dying trees were detected only during the extreme drought of 1994 and the moderate one of 1998. Although no direct stress measurement was available for the studied species, the monitoring of another affected species (*Quercus ilex*) in the same area during the summer of 1994 confirmed that drought was responsible of the observed tree dieback (Gracia *et al.* 1999). Transects were carried out in April 1999 on the south- and north-facing slopes of the Titllar valley, and on the south-facing slope of the Castellfolit valley. We did not estimate mortality of the *P. pinaster* plantation. In all transects we maintained aspect and progressed in altitude. Inside each plot we counted all live and (recently) dead individuals and measured the DBH of those larger than 15 cm in perimeter. We aggregated the 1994 and 1998 mortalities because in some cases it was impossible to decide whether the tree had died in 1994 or in 1998. Nevertheless, most of mortality (>90%) occurred in 1994.

### *Annual rings*

We estimated growth by measuring the width of annual rings. Four to six trees with DBH larger than 15 cm were sampled in each population. In the Titllar valley we sampled both individuals that died in 1998 and surviving ones. In Castellfolit only live individuals were sampled because too few pines that had died in 1998 were present. It was impossible to measure trees that died in 1994 because their wood was too far decomposed. Cores were extracted at breast height from the south face of each tree, and they were air-dried in the lab. We measured, to a precision of 0.01 mm, the

total width of each annual ring corresponding to the last 10 years using a binocular microscope, a linear table attached to a PC, and the programme CATRAS (Aniol 1983). Ring widths were standardised against the total diameter of the tree (relative ring width).

### *Hydraulic conductivity and vulnerability curves*

During the spring of 1999 we sampled one branch from 10 trees of each studied population (except *P. sylvestris* in Titllar-N). The same branches were used for the hydraulic and isotopic measurements. Branches were transported to the lab in plastic bags. Once in the lab, we cut a segment from the proximal end of each branch and stored them at 4°C until their vulnerability curves were established in less than a week. All leaves distal to the segment were removed and their total area measured (with a Li-Cor 3100 Area Meter).

Hydraulic conductivity was measured following Sperry *et al.* (1988). We cut branch segments of *ca.* 20 cm in length and a diameter of 0.5-1 cm, removed their bark, and connected their proximal ends to a tubing system. The system was filled with a filtered ( $\approx 0.22 \mu\text{m}$ ) and degassed solution of HCl (pH *ca.* 2). We calculated hydraulic conductivity ( $K_h$ , in  $\text{m}^4 \text{MPa}^{-1} \text{s}^{-1}$ ) as the ratio between the flow through the segment and the pressure gradient ( $P = \text{ca. } 6 \text{ kPa}$ ). The flow was measured gravimetrically. In order to obtain the maximum hydraulic conductivity we previously injected the measure solution at high pressure (*ca.* 100 kPa) to remove all native embolisms from

the segment. We also calculated specific hydraulic conductivity ( $K_s$ , in  $\text{m}^2 \text{MPa}^{-1} \text{s}^{-1}$ ), as the ratio between maximum hydraulic conductivity and cross sectional area of the segment (without bark); and leaf specific conductivity ( $K_L$ , in  $\text{m}^2 \text{MPa}^{-1} \text{s}^{-1}$ ), as the quotient between maximum hydraulic conductivity and leaf area. Finally, we calculated the ratio between cross sectional area and leaf area ( $A_s:A_l$ , Zimmerman 1983) of each branch segment.

Vulnerability curves show the relationship between water potential (or pressure) in the xylem and percentage loss of hydraulic conductivity (PLC) due to embolism. We used the air injection method (Cochard *et al.* 1992, Sperry & Saliendra 1994) to establish the curves. This method has been validated for several species, including conifers (Sperry & Ikeda 1997). Briefly, we put segments (four each time) inside a pressure chamber with both ends protruding. Proximal ends were connected to the measuring circuit, and maximum hydraulic conductivity was measured. Next, we raised the pressure inside the chamber to 1 MPa and maintained it during 10 min, lowered the pressure to a basal value of *ca.* 10 kPa, waited 15 min to allow the system to equilibrate, and repeated the conductivity measurement. We repeated this process, raising the injection pressure by 1 MPa each time, until the actual conductivity of the segment was less than 5% of the initial, or when we reached 7 MPa.

Vulnerability curves were fitted with the following function (Pammenter & Vander Willigen 1998):

$$PLC = \frac{100}{1 + e^{a(P-b)}} \quad (\text{eq. 1})$$

In this equation PLC is the percentage loss of hydraulic conductivity,  $P$  is the applied pressure,  $b$  is the pressure (*i.e.*, - ) causing a 50% loss of hydraulic conductivity, and  $a$  is related to the slope of the curve. The advantage of this function is that both parameters have a physiological meaning if we accept Zimmermann's (1983) air seeding hypothesis. Parameter  $b$  would be related with the mean of maximum (per conduit) pit-pore size, and  $a$  with the variance of this maximum size. The value of the parameters was obtained by regression analysis (least squares) after linearization of equation 1.

### $\delta^{13}\text{C}$ and $\text{N}$ content measurements

We used carbon isotope discrimination in leaves as a measure of integrated water-use efficiency (WUE, Ehleringer & Osmond 1989, Farquhar & Richards 1984). The fact that all studied populations were very close minimised differences in WUE due to microclimatic effects (Farquhar *et al.* 1989, Marshall & Zhang 1994). After drying, leaves were ground (40 mesh) and sub-samples were separated to measure their carbon isotope composition and their N content. All analyses were carried out at the Stable Isotope Ratio Facility for Environmental Research (SIRFER, University of Utah, Salt Lake City, Utah, USA). The relationship between carbon stable isotopes was expressed in relation to PBD (Pee-Dee Belemnite standard).

### *Additional monitoring*

During the spring and summer of 2000, additional measurements were carried out in the most affected Titllar population (*P. sylvestris*) to further characterise its response to drought. Leaf water potentials were monitored monthly from May to August in 14 individuals from the population. Each sampling date, predawn and midday water potentials were measured with a pressure bomb (Scholander *et al.* 1965). In July two branches from each of the same individuals were sampled and its hydraulic properties were measured as above. The average of the two measurements was used in all subsequent analyses.

### *Statistical analyses*

All analyses were carried out with STATISTICA (v. 5.0, Statsoft Inc., Tulsa, OK, USA). For the frequency data we used log-linear analyses (and the G-test; Sokal & Rohlf 1995) to test the existence of associations between variables. Two continuous variables from transects were converted to discrete variables to analyse them jointly with the other transect variables: tree diameter (6 levels) and altitude (3 levels). If not otherwise stated, only trees with a DBH greater than 5 cm were used in the analyses, because we considered that 1994 mortality estimates were not totally reliable for smaller individuals. For continuous variables we used one-way ANOVA (and the HSD test

when needed) to compare populations. To test for the existence of a change in growth pattern after the 1994 drought, we analysed growth data using a one-way ANOVA with repeated measures. The levels of the repeated measures factor were mean growth before and after 1994. Repeated measures ANOVA was also used to compare water potentials through the 2000 study period.

## Results

### *Quantification of mortality*

Mortality was higher in *P. sylvestris* populations than in *P. nigra* (Table 1;  $G=5.54$ ,  $p=0.019$ ). This association between species and mortality disappeared if only the Castellfollit valley (the only one where the two species co-exist) was considered ( $G=1.84$ ,  $p=0.175$ ). However, no dead *P. nigra* was found (inside or outside transects), suggesting that this species was unaffected by the 1994 and 1998 droughts. Though unmeasured, no mortality was visually detected in the *P. pinaster* plantation during the summers of 1994 or 1998. *P. sylvestris* mortality was higher in the Titllar valley than in Castellfollit (Figure 2 and Table 1;  $G=8.14$ ,  $p=0.004$ ). In Titllar pine density was slightly lower ( $F=4.32$ ,  $p=0.092$ ), and trees were larger (Figure 3 and Table 1). No difference in mortality was found in the Titllar valley between north- and south-facing slopes. The three-way interaction between valley, diameter and mortality was not significant ( $G=5.82$ ,  $p=0.213$ ). However, the presence of two-way interactions indicated that there was both an intrinsic effect of the valley on

mortality, and an indirect effect through different diameter distributions. Mortality in smaller trees (DBH < 5 cm) was much higher in the Titllar valley (Figure 2;  $G=46.26$ ,  $p < 0.001$ ). No association was found between altitude and mortality ( $G=5.47$ ,  $p=0.141$ ).

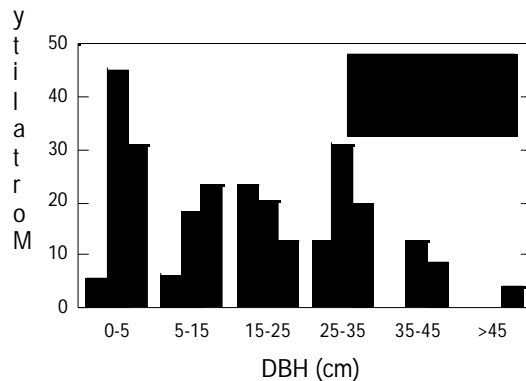


Figure 2. Distribution of mortality among diameter classes of the three studied *P. sylvestris* populations. Abbreviations as in Table 1.

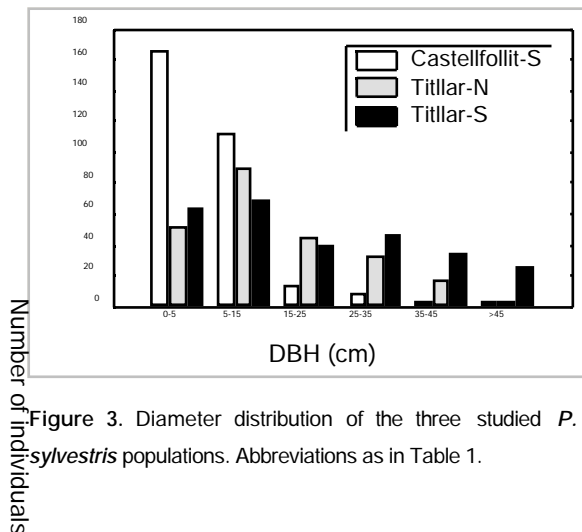


Figure 3. Diameter distribution of the three studied *P. sylvestris* populations. Abbreviations as in Table 1.

### Tree growth

Tree growth increased slightly in the Titllar valley after the 1994 drought ( $F=3.69$ ,  $p=0.087$ ) (Figure 4). No differences in growth were found in this valley between the individuals that died during summer 1998 and the ones that survived, so we

grouped them and compared them to Castellfollit pines. Relative growth was higher at Castellfollit ( $F=23.87$ ,  $p < 0.001$ ) and, within this valley, higher in *P. sylvestris* than in *P. nigra* ( $F=9.06$ ,  $p=0.024$ ). No change in growth pattern was found in Castellfollit pines after the 1994 drought (Figure 4).

### Hydraulic properties

No differences in specific hydraulic conductivity were found between species or populations, but there were significant differences in leaf specific conductivity between the two *P. sylvestris* populations (Table 2;  $F=3.16$ ,  $p=0.036$ ).  $K_L$  was 113% higher in Castellfollit than in Titllar, as a consequence of a higher  $A_s:A_l$  in the former (Table 2;  $F=3.13$ ,  $p=0.037$  for the global ANOVA).  $K_L$  values were uncorrelated with tree-size in *P. sylvestris*, so the observed differences in  $K_L$  between populations are unrelated with its different mean tree-diameter. Vulnerability curves were very similar for the four studied populations (Figure 5), with pressures of *ca.* 3.2 MPa causing a 50 PLC. No differences were found between populations for parameters a and b of equation 1 (Table 2).

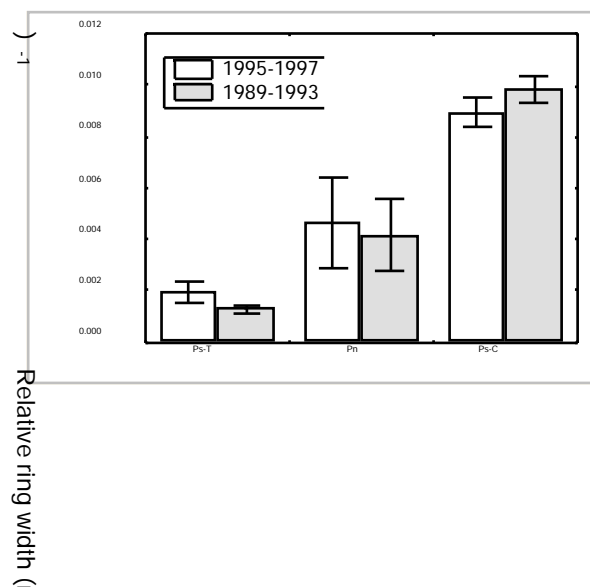


Figure 4. Differences in growth patterns after and before the 1994 drought in three of the studied populations. Relative tree-ring width is used as a measure of growth. Pn: *P. nigra*;

Ps-C: *P. sylvestris* from Castellfollit; Ps-T: *P. sylvestris* from Titllar. Error bars show standard errors.

Table 1. Results from transects. S: south-facing slope; N: north-facing slope. Standard errors are in brackets.

	<i>P. nigra</i> Castellf-S	<i>P. sylvestris</i> Castellf-S	<i>P. sylvestris</i> Titllar-N	<i>P. sylvestris</i> Titllar-S
Number of trees	34	303	236	278
Mean diameter <sup>1</sup> (cm)	22.3 (15.1)	15.6 (12.7)	26.5 (14.2)	33.6 (17.8)
Total density (trees/ha)	95 (42)	838 (270)	351 (77)	370 (27)
Basal area (m <sup>2</sup> /ha)	3.7	16.0	19.3	32.6
Mortality (% individuals) <sup>1</sup>	0.0	7.6	20.2	16.3
Mortality (% basal area) <sup>1</sup>	0.0	12.1	18.2	10.8

<sup>1</sup> Considering only individuals with DBH > 5 cm.

Carbon isotope discrimination was lower in *P. pinaster* than in the other populations, among which no differences were found ( $F=15.48$ ,  $p < 0.001$  for the global ANOVA; Table 2). This lower isotope discrimination (higher <sup>13</sup>C) denotes a 24.5% higher water-use efficiency (integrated during needle life) for *P. pinaster* in relation to the mean of the other three populations, and supposing common abiotic conditions. We found differences in N content only between *P. nigra* and *P. sylvestris* from Castellfollit, values being higher in the latter ( $F=3.03$ ,  $p=0.041$  for the global ANOVA).

showing percentage loss of conductivity (PLC) as a function of applied pressure. Equation 1 (see the 'Material and methods' section of the text) was used to fit the curves. Abbreviations as in Figure 4; Pp: *P. pinaster*. Error bars show standard errors.

### Additional monitoring

$K_L$  ( $2.06E-07 \pm 3.01E-08 \text{ m}^2 \text{ MPa}^{-1} \text{ s}^{-1}$ ) and  $\omega_{50\%}$  ( $2.95 \pm 0.11 \text{ MPa}$ ) measured the year 2000 were not statistically different to those measured the previous year at the Titllar *P. sylvestris* population (Table 2). During the spring-summer of 2000, predawn water potentials at this population decreased from  $-0.6 \text{ MPa}$  in June to  $-1.9$  in August, and midday water potentials from  $-0.9 \text{ MPa}$  in June to  $-2.3 \text{ MPa}$  in August (Figure 6). These minimum water potentials correspond to a mean of 34% (range 20-60%) loss of conductivity due to xylem embolism (Figures 6 and 5). The difference between predawn and midday water potentials increased significantly from  $0.3 \text{ MPa}$  in June to  $0.7 \text{ MPa}$  in July, but decreased significantly ( $p < 0.001$ ) in August to  $0.3 \text{ MPa}$ .

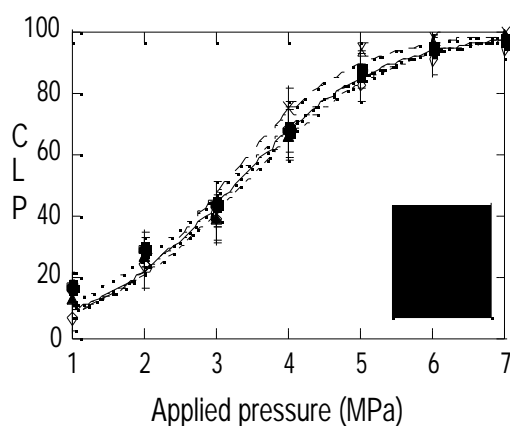


Figure 5. Vulnerability curves of the studied populations,

At the individual level, there was no

relationship between  $K_L$  or  $\Delta A_5:A_1$  and water potentials (correlation analysis). However, the decrease in the difference between predawn and midday water potentials from July to August affected preferentially the

individuals with lower  $K_L$ . We divided the 14 sampled trees in two groups, one with the 7 trees with the highest  $K_L$  (mean  $K_L=2.89 \cdot 10^{-7} \text{ m}^4 \text{ MPa}^{-1}$

Table 2. Some measured parameters from the studied populations (year 1999). The meaning of abbreviations is explained in the 'Material and methods' section of the text. Standard errors are in brackets. Different lowercase letters in upperindices indicate differences significant at the 0.05 level.

	<i>P. nigra</i> Castellf.	<i>P. pinaster</i> Castellf.	<i>P. sylvestris</i> Castellf.	<i>P. sylvestris</i> Titllar
$A_5:A_1 (\times 10^4)$	7.86 (0.88) <sup>ab</sup>	9.87 (0.81) <sup>ab</sup>	11.0 (2.5) <sup>a</sup>	5.80 (0.67) <sup>b</sup>
$K_s (\text{m}^2 \text{ MPa}^{-1} \text{ s}^{-1}, \times 10^4)$	4.07 (0.36) <sup>a</sup>	3.52 (0.44) <sup>a</sup>	4.97 (0.46) <sup>a</sup>	4.03 (0.39) <sup>a</sup>
$K_L (\text{m}^2 \text{ MPa}^{-1} \text{ s}^{-1}, \times 10^7)$	3.24 (0.52) <sup>ab</sup>	3.43 (0.53) <sup>ab</sup>	4.98 (0.92) <sup>a</sup>	2.34 (0.43) <sup>b</sup>
a	-1.25 (0.06) <sup>a</sup>	-1.29 (0.04) <sup>a</sup>	-1.20 (0.09) <sup>a</sup>	-1.23 (0.06) <sup>a</sup>
b	3.20 (0.16) <sup>a</sup>	3.01 (0.08) <sup>a</sup>	3.57 (0.32) <sup>a</sup>	3.20 (0.32) <sup>a</sup>
$^{13}\text{C} (\text{‰})$	-26.55 (0.27) <sup>a</sup>	-24.67 (0.36) <sup>b</sup>	-27.00 (0.31) <sup>a</sup>	-27.43 (0.29) <sup>a</sup>
N (%)	0.86 (0.09) <sup>a</sup>	1.03 (0.06) <sup>ab</sup>	1.16 (0.05) <sup>b</sup>	1.00 (0.05) <sup>ab</sup>

$\text{s}^{-1}$ ) and another with those trees with the lowest  $K_L$  (mean  $K_L=1.22 \cdot 10^{-7} \text{ m}^4 \text{ MPa}^{-1} \text{ s}^{-1}$ ). The difference between predawn and midday water potential was almost the same in July and August in the first group of trees (t-test,  $p=0.866$ ), whereas it decreased by 50% in the second group (t-test,  $p=0.003$ ).

## Discussion

Our results showed a higher mortality of *P. sylvestris* at Titllar valley than at Castellfollit, and in *P. sylvestris* than in *P. nigra* and *P. pinaster* (Table 1). Several factors, biotic and abiotic, can explain differences in drought resistance. In the present study climate regime and lithology are common among populations, so biotic factors seem to be the most important in explaining observed differences in drought response. The studied populations differ both in ecophysiological characteristics (at the individual level) and in population attributes. The interpretation of these differences is

not straightforward because most measurements were only made on survivors, and not on trees that died. Nevertheless some results suggest that in our populations main ecophysiological parameters did not change significantly as a result of drought events. In particular, (1) there was no difference in growth between individuals that died as a result of 1998 drought and those that survived; (2) the higher mortality in Titllar than in Castellfollit detected in 1994 was again observed in 1998 (personal observation); and (3)  $K_L$  and vulnerability to xylem embolism were very similar in the Titllar population in 1999 and 2000, despite important differences in climate (precipitation between January and August was 27% higher in 2000). In addition, there is experimental evidence which shows that  $A_5:A_1$  ratios remain constant after drastic modifications of population density (Margolis *et al.* 1995). Taking into account all the above considerations, we will assume in the following discussion that measured parameters were representative of pre-1994

conditions.

Our results showed no differences among species or populations in maximum water transport capacity of branches ( $K_s$ , Table 2) or in their response to water stress (vulnerability to embolism; Figure 5). This homogeneity in hydraulic capacity is quite general in pines (*e.g.*, Jackson *et al.* 1995). Regarding the two *P. sylvestris* populations, the important difference in their hydraulic capacity per unit

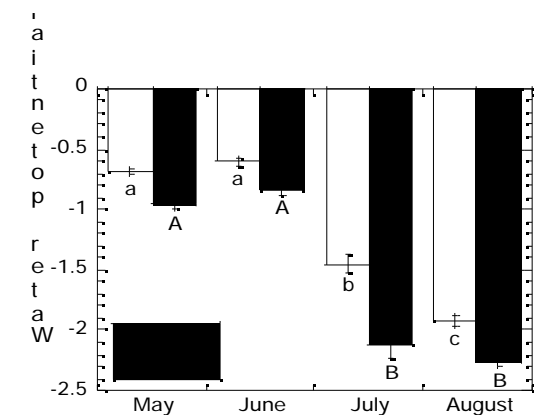


Figure 6. Leaf water potentials (pwp=predawn, and mwp=midday) during the spring-summer 2000 at the Titllar *P. sylvestris* population. Error bars show standard errors. Different letters indicate significant differences ( $p < 0.05$ ) between sampling dates.

of leaf area (Table 2) is probably related with the observed mortality pattern. The increment of water potential through plants can be expressed as follows:

$$= E \frac{L}{K_L} = g_w \quad w \frac{L}{K_L} \quad (\text{eq. 2})$$

2)

where  $\Delta \psi$  is the water potential increment between two positions within the transpiration stream (in MPa),  $E$  is the transpiration rate (in  $\text{m}^3 \text{m}^{-2} \text{s}^{-1}$ ),  $L$  is the

distance between the two positions (in m),  $K_L$  is the leaf specific conductivity,  $g_w$  is the total conductance to water vapour of the pathway between the evaporating sites and the bulk air (in  $\text{m}^3 \text{m}^{-2} \text{s}^{-1}$ ), and  $w$  is the difference in water vapour partial pressures between the sites and bulk air (in  $\text{Pa Pa}^{-1}$ ). Similar  $w$  between the two populations is suggested by their proximity and by the fact they have the same orientation and altitude. Similar  $g_w$  is suggested by carbon isotope results. Lower leaf specific conductivity in the Titllar valley means then that the water potential gradient through terminal branches has to be higher in this valley (to support the same transpiration rates). Steeper water potential gradients cause lower leaf water potentials and, hence, higher losses of hydraulic conductivity (Figure 5), which result in even lower leaf water potentials. This cycle can become unstable (*sensu* Tyree & Sperry 1988) and lead to branch or plant dieback. Although we have only measured terminal branches, they are specially relevant because the most important water potential drop in the above-ground parts of trees occurs in terminal branches (Zimmerman 1983). Large differences in rooting depth (in relation to supported leaf area, from which no data is available) between populations could partially offset  $K_L$  differences. However the fact that substrates are very similar and that the response to drought was so different between populations makes this hypothesis highly unrealistic.

The monitoring of the Titllar population during the year 2000 supports the idea that hydraulic properties of pines play an important role in the studied *P. sylvestris* populations during summer drought. Even



in the mild 2000 summer (precipitation was 404 mm between January and August, 30% higher than average), leaf water potentials reached values low enough to cause considerable amounts of xylem embolism (Figures 6 and 5). The reduction in the difference between predawn and midday water potentials that occurred between July and August (Figure 6) strongly suggests that pines closed stomata when conditions become dry enough (August). Moreover, stomatal closure only affected the individuals with lower capacity to transport water to leaves (lower  $K_L$ ). It is reasonable to suppose that during extremely dry summers (like the 1994 one) stomatal closure does not suffice to prevent the development of dangerous levels of xylem embolism. Under this situation, pines with lower  $K_L$  (like the Titllar ones in relation to Castellfollit) tend to be more affected.

It is more difficult to interpret the responses to drought of the other two species. In the case of *P. pinaster* its high water-use efficiency inferred from  $^{13}\text{C}$  (Table 2) could be related to the observed lack of mortality. We found almost no difference in hydraulic architecture between the *P. nigra* and *P. sylvestris* populations that coexist at Castellfollit. Lower growth and foliar N concentrations (with similar  $^{13}\text{C}$ ) in *P. nigra* suggest lower transpiration rates and water potential gradients (Equation 2) and, hence, less embolism. Although this result could help to explain the observed pattern, other unmeasured factors could also be important (e.g., differences in the root system or in other aspects not related to hydraulic architecture).

The increase in growth we observed in the

most affected *P. sylvestris* population is probably related with a relaxation of competition for resources. It is interesting to note that we did not find the memory effect in growth observed by Irvine *et al.* (1998), probably because autumn 1994 was exceptionally wet and this would have favoured a quick recovery of surviving trees (Gutiérrez 1989). This is probably one of the reasons why the Titllar population was again more affected by drought in 1998.

The high mortality we found in the Titllar valley indicates that *P. sylvestris* is at the limit of its capacity to cope with drought stress. Besides, low density and high mortality in younger individuals at Titllar also endanger the regeneration of the population. If we consider that: (1) climate models predict an increase in the frequency and intensity of extreme drought events, and (2) despite its Eurosiberian origin *P. sylvestris* is distributed over several dry areas in the Mediterranean basin (Barbéro *et al.* 1998); it is reasonable to think that important changes can take place in the distribution of this species. In the study area, there are other species (e.g., more resistant pines, sclerophyllous trees) that can potentially replace *P. sylvestris*.

The vulnerability to embolism and hydraulic conductivity values we found for the three studied species agree with previous studies in that pines tend to be more vulnerable to embolism and more efficient in terms of conductivity than other conifers (Linton *et al.* 1998, Piñol & Sala 2000, Richardson 1998). Besides, pines seem to be very homogeneous in their vulnerability to embolism (Martinez-Vilalta & Piñol 1999) suggesting that, as has been found in the

present study, other mechanisms play a major role in adaptation and acclimatization to drought stress (Borghetti *et al.* 1998, Irvine *et al.* 1998, Mencuccini & Grace 1995). We hypothesise that this high vulnerability and homogeneity of the conducting system may compromise the ability of some pine species to resist the expected increase of aridity in the western Mediterranean. Several studies have stressed the importance of xylem constraints in conifer distribution (*e.g.*, Brodribb & Hill 1999). Important drought-induced mortality in pine species has already been reported in other regions. In a recent study, for example, Allen & Breshears (1998) found that mortality of *Pinus ponderosa* in response to a severe drought caused a very rapid landscape shift between pine forest and piñon-juniper woodland in New Mexico (USA).

In conclusion, studied pine populations differed in their response to 1994 and 1998 extreme droughts. Although hydraulic properties of the conducting system were very similar among species and populations, the different responses to drought can be explained by other components of their hydraulic architecture (*e.g.*,  $A_s:A_l$  and  $K_L$ ). This result provides additional evidence that plants tend to live close to the limit of their hydraulic capacity and that apparently small changes in one of the components of hydraulic architecture can have major implications even at the demographic level. The important drought-induced mortality observed in the study area suggests that several *P. sylvestris* populations in the Mediterranean basin will be at risk if the climate gets drier.

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