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Research paper

Hydraulic traits are associated with the distribution range of two closely related Mediterranean firs, *Abies alba* Mill. and *Abies pinsapo* Boiss.

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Abies alba and *Abies pinsapo* are two closely related fir species that occur in the Iberian Peninsula under very different environmental conditions. *Abies alba* proliferates in the humid European mountains, including the Spanish Pyrenees. In contrast, *A. pinsapo* is a relict species that occurs in some restricted areas of the Mediterranean mountain ranges in Spain and Morocco, which experience intense summer drought periods. To cope with the high atmospheric evaporative demand during summer, *A. pinsapo* may either have a high resistance to xylem cavitation or develop a very efficient conducting system to reduce the soil-to-leaf water potential gradient. To investigate such hypotheses, we measured (i) the xylem vulnerability to cavitation for different populations, and (ii) several anatomical and hydraulic parameters indicating xylem sufficiency for supplying water to the shoot in two contrasting populations of both species. Our results show that the resistance to cavitation was not different between species or populations. However, hydraulic conductivity (K_h), specific hydraulic conductivity (K_s), leaf-specific conductivity (LSC) and whole-shoot hydraulic conductance (K_{shoot}) were higher in *A. pinsapo*, indicating a higher efficiency of water transport, which should contribute to maintaining its xylem tension below the threshold for rapidly increasing cavitation. The higher K_s in *A. pinsapo* was largely a result of its wider tracheids, suggesting that this species may be much more vulnerable to freeze–thaw-induced cavitation than *A. alba*. This is consistent with the absence of *A. pinsapo* in northern mountain ranges with cooler winters. These physiological differences could partly explain the niche segregation and the geographical separation of these two firs.

Keywords: cavitation, summer drought, water potential, winter frost.

Introduction

The concept of 'Mediterranean firs' refers to a group of *Abies* species that occupy disconnected areas around the Mediterranean Basin (Aussenac 2002). Such firs were initially classified into two different sections within the genus according to morphological criteria (Farjon and Rushforth 1989), but more recent studies have shown that they constitute a more strongly supported monophyletic group (Scaltsoyiannes et al. 1999, Suyama et al. 2000, Xiang et al. 2009) with nine species and one natural hybrid (Vidakovic 1991). Among them, silver fir (*Abies alba* Mill.) is the most widespread species, following the distribution of mountain ranges in Europe: the Alps, Carpathians, Apennines and Pyrenees (Pawlaczyk et al. 2005). The current distribution of *A. alba* in the Iberian Peninsula is always associated with the

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humid montane or subalpine altitudinal belts of the Pyrenees (Peguero-Pina et al. 2007). By contrast, the other species within this group have a relict status, living under very specific geographical and ecological conditions (Linares 2011). For instance, *A. pinsapo* is restricted to the Iberian Peninsula, occurring in the south-western Baetic mountain range under a Mediterranean climate with a dry summer season from June to September (Fernández-Cancio et al. 2007). Thus, in contrast to *A. alba*, *A. pinsapo* must cope with a drought period, when precipitation reaches a minimum and evaporative demand reaches a maximum.

If stomata do not restrict transpiration during periods of high vapour pressure deficit (VPD) and low soil water availability, the resulting xylem tension gradients can be steep enough to cause xylem cavitation (Pockman and Sperry 2000) and ultimately lead to catastrophic hydraulic failures (Maherali et al. 2004). Therefore, the survival of plant species in climates with water limitations could be related to the resistance of its xylem to cavitation (Pockman and Sperry 2000). Maherali et al. (2004) reported that the water potential at which plants lose 50% of hydraulic conductivity (PLC_{50}) was six to seven times more negative in species occurring in the driest vegetation types. In conifer species, Brodribb and Hill (1999) noticed that species from wet environments were highly vulnerable to cavitation, while species from the semi-arid zones were extremely resistant to drought-induced cavitation. In contrast, several authors observed that the vulnerability to drought-induced cavitation was unrelated to climate dryness. For instance, Piñol and Sala (2000) showed that a greater resistance to cavitation is not required for survival in more xeric habitats for several conifer species. Similarly, Martínez-Vilalta et al. (2009) did not find differences in the PLC_{50} among several provenances of Scots pine across Europe, although they found an adjustment between hydraulic traits and environmental conditions, resulting in a greater hydraulic capacity at dry sites.

One way for conifers to improve xylem hydraulic capacity is to increase tracheid diameters, thereby decreasing xylem hydraulic resistivity (Pittermann et al. 2006), provided that the tracheid diameter shift does not jeopardize the resistance of the xylem to freeze–thaw embolism (Pittermann and Sperry 2006, Mayr and Sperry 2010).

Based on this assumption, the main objective of this work was to establish a comparison between *A. alba* and *A. pinsapo* in terms of hydraulic parameters, including resistance to cavitation. We hypothesized that xylem vulnerability to tension-induced embolism would be related to differences in the climatic regimes that characterize the habitats of both species. As an alternative, a higher hydraulic conductivity at shoot and branch levels may itself be a mechanism that would allow *A. pinsapo* to cope with a higher evaporative demand during summer.

Materials and methods

Study sites and climatic conditions

We selected several *A. alba* and *A. pinsapo* stands from different locations in the Iberian Peninsula (Table 1). Samples of *A. alba* were collected from three different sites in the Spanish Pyrenees (Gamueta, Ezpela and Oroel). Samples of *A. pinsapo* were collected from its natural distribution area in the south-western Baetic mountain range (Grazalema) (Alba-Sánchez et al. 2010) and from a naturalized reforested population planted in 1913 (Pérez-Soba Díez del Corral 2010) in the southern 'Sistema Ibérico' range (Orcajo). For each site, the climate was described using daily data from nearby meteorological stations for each site. To quantify the aridity of each site, we calculated Martonne's aridity index ($P/(T + 10)$, where P is the annual precipitation in mm and T is the mean annual temperature in °C) and the Gaussen index (the number of months in which $P < 2T$, where P is the monthly precipitation in mm and T is the monthly mean temperature in °C).

Forest microclimate (air temperature and relative humidity) was characterized in two locations selected for the physiological measurements (Gamueta for *A. alba* and Orcajo for *A. pinsapo*) using two Hobo Pro RH/Temp data loggers per site (Onset Computer Bourne, MA, USA). The data logger was located in a solar shield in the lower crown of a tree at 5 m above the soil. Measurements were recorded every 30 min from June 2009 to June 2010. Vapour pressure deficit (kPa) was calculated from the registered values of air temperature and relative humidity, according to Rundel and Jarrell (1989). Additionally, we established correlations for the daily maximum and minimum temperatures between the values provided by the data loggers and those registered in the nearest meteorological station from Gamueta and Orcajo, respectively. These correlations were used to estimate the historical numbers of days with freezing–thawing events in both sites. According to Mayr et al. (2006), a freezing–thawing event was counted when the temperature decreased from >0 °C to <–2 °C, while thawing events were counted when temperatures increased from <–2 °C to >0 °C.

Centrifugation-induced cavitation

The Cavitron technique (Cochard 2002, Cochard et al. 2005, 2010) was used to study xylem vulnerability to cavitation of all the populations. Percentage loss of conductivity (PLC) was measured on 10 healthy branches from 10 different mature well-established trees for each population. Straight south-exposed branches (>0.35 m in length, <0.01 m in diameter) were collected, placed in plastic bags and transported to the laboratory. Once there, after side shoots and all the needles were removed, the main shoots were wrapped with wet paper, put in a plastic bag and stored at 5 °C to prevent dehydration. The technique consists of spinning xylem segments (0.275 m in length) centred on a dedicated rotor to expose them to large negative pressures and then measure the effect on their conductivity (*K*). Both sample ends were inserted in transparent vials containing ultrapure water with 10 mM KCl and 1 mM $CaCl_2$ filtered to 0.2 μm. The solution in the vials was degassed at the start of the measurement but remained in contact with the atmosphere during centrifugation. The water level in the vial where the basal segment end was inserted was adjusted to 1.5 cm before each *K* determination. The water level in the other vial was constant and set to 1 cm. The difference in water levels creates a pressure gradient, and hence a water flow through the sample, which allows *K* to be estimated (Cochard 2002, Cochard et al. 2005). The maximum sample conductivity (K_{max}) was measured at low speed and high pressure (–0.1 or –0.25 MPa). Dividing K_{max} by sample basal wood area yielded sample specific conductivity. Xylem pressure was then lowered stepwise by increasing the rotational velocity, and *K* was determined anew. Sample loss of conductivity [sample percent loss of conductivity (PLC)] was computed as

$$\mathrm{PLC} = 100 \times (1 - K / K_{\mathrm{max}})$$

The water potential (–MPa) at which 12% (Ψ_{PLC12}), 50% (Ψ_{PLC50}) and 88% (Ψ_{PLC88}) loss of conductivity occurs was analysed for each of the studied populations.

Water potential

Shoot water potential (MPa) was periodically measured during 2009 to estimate the most negative seasonal values for winter and summer. Measurements were performed at midday with a Scholander-type pressure chamber on about 10-cm-long terminal segments of twigs from three south-exposed branches per tree, collected from five trees in each studied population (Gamueta for *A. alba* and Orcajo for *A. pinsapo*), following the methodological procedures described by Turner (1988).

Shoot morphology and anatomy

Shoot morphology and anatomy were studied on south-exposed shoots (1, 2 and 3 years old) collected from 10 different trees of each studied population (Gamueta for *A. alba* and Orcajo for *A. pinsapo*). Total supported leaf area, stem diameter, conductive xylem area and tracheid diameter were measured. Total supported leaf area was determined using Ballotini balls (Thompson and Leyton 1971). Stem diameter (m) was measured with a micrometer after removal of the bark. Pith diameter was identified by staining the xylem with phloroglucinol and measured using a micrometer. The conductive xylem area (m^2) was calculated as the difference between the stem and pith area. Afterwards, the middle segment of each shoot was transversely sectioned with a sliding microtome (Microm HM 350 S, Thermo Scientific, Germany). Sections with a thickness of 10–15 μm were stained with safranin and picric acid, dehydrated by means of 96% ethanol, and permanently mounted on slides with Eukitt mounting medium. The cross-sections were studied microscopically (Leica DM LB, Leica Microsystems, Germany) and photographed with a camera (Moticam 2300, Motic, China). The resulting micrographs were analysed using Image-J software (http://rsb.info.nih.gov/nih-image/), to measure in the earlywood the diameter (μm) of about 25 tracheids per year (1, 2 and 3 years old) and shoot (10 shoots per species), yielding about 750 tracheids per species. Moreover, the Huber value (HV) was also calculated, as the ratio between the conductive xylem area and the total leaf area of the shoot. The projected and the total leaf areas of the shoots were determined using Ballotini balls (Thompson and Leyton 1971). Wood density (ρ) was calculated in five wood segments per species (Gamueta for *A. alba* and Orcajo for *A. pinsapo*) as the dry weight per fresh volume, following the methodology of Hacke et al. (2000).

Stem hydraulic conductivity

The hydraulic conductivity (K_h, kg m s^{-1} MPa^{-1}) of stem segments obtained from 10 south-exposed branches (1.5–2 m long) was determined for each population subjected to investigation (Gamueta for *A. alba* and Orcajo for *A. pinsapo*). One branch per tree was collected and the stem segments (1, 2 and 3 years old, 2–6 cm long and up to 0.60 cm in diameter, three segments per branch) were prepared as described in Mayr et al. (2002). The measurement pressure was set to 4 kPa. The flow rate was determined with a PC-connected balance (Sartorius BP221S, 0.1 mg precision, Sartorius AG, Göttingen, Germany) by recording weight every 10 s and fitting linear regressions over 200 s intervals. Conductivity measurements were carried out with distilled, filtered (0.22 μm) and degassed water containing 0.005% (volume/volume) Micropur (Katadyn Products, Wallisellen, Switzerland) to prevent microbial growth (Mayr et al. 2006). The specific hydraulic conductivity (K_s, kg m^{-1} s^{-1} MPa^{-1}) was calculated by dividing K_h by the conductive xylem area. Finally, the leaf-specific conductivity (LSC, kg m^{-1} s^{-1} MPa^{-1}) was calculated by dividing K_h by the leaf area supported by the measured segment.

Shoot hydraulic conductance (K_{shoot})

The whole-shoot hydraulic conductance (K_{shoot}, mmol m^{-2} s^{-1} MPa^{-1}) in *A. alba* (Gamueta) and *A. pinsapo* (Orcajo) was calculated following the methodology described by Brodribb et al. (2005). Six sun-exposed branches from six trees per species were collected at 07:00–08:00 h (solar time), minimizing the possibility of midday K_{shoot} depression (Brodribb and Holbrook 2004), enclosed in sealed plastic bags to prevent water loss, and stored in the dark for a period of at least 1 h, until stomatal closure so that all shoots from the same branch could reach the same water potential. It is assumed that this is the water potential of the shoots prior to rehydration (Ψ_0). Once this value was obtained, one shoot per branch was cut under water to prevent air entry and allowed to absorb water for 120–300 s. The water potential after rehydration was subsequently obtained (Ψ_f). The whole-shoot hydraulic conductance was calculated according to the following equation:

$$K_{\text{shoot}} = C_l \ln[Y_0 / Y_f] / t \qquad (1)$$

where C_l (mol MPa^{-1} m^{-2}) is the leaf capacitance for each species. C_l was calculated as the initial slope of the *P*–*V* relationships, normalized by the leaf area of the shoot (Tyree and Hammel 1972, Brodribb et al. 2005). *P*–*V* relationships for *A. alba* (Gamueta) and *A. pinsapo* (Orcajo) were determined in six shoots per species, following the free-transpiration method described in previous studies (Corcuera et al. 2002, Vilagrosa et al. 2003).

Statistical analysis

Data are expressed as means ± standard error. One-way ANOVA analyses were performed to compare Ψ_{PLC12}, Ψ_{PLC50} and Ψ_{PLC88} among all the studied populations. Multiple comparisons between populations were carried out using the post hoc Tukey's Honestly Significant Difference test. Student's *t*-tests were used to compare the rest of the parameters for *A. alba* (Gamueta) and *A. pinsapo* (Orcajo). The standardized skewness and kurtosis were analysed for the frequency distribution of tracheid diameters. All statistical analyses were carried out using SAS version 8.0 (SAS, Cary, NC, USA).

Results

The total annual and summer precipitations were higher in *A. alba* locations, whereas the mean annual and summer temperatures were higher in *A. pinsapo* locations (Table 1). Martonne's aridity index was much lower in Orcajo and Grazalema, which confirmed the greater Mediterranean influence in *A. pinsapo* populations (Table 1). The Gaussen index was consequently positive in Orcajo and Grazalema (Table 1), which revealed the existence of summer aridity in *A. pinsapo* locations. Microclimatic differences between the locations selected for the physiological measurements confirmed that temperature and VPD were higher in Orcajo (*A. pinsapo*) than in Gamueta (*A. alba*) (Figure 1). On the other hand, minimum winter temperatures were lower in Gamueta than in Orcajo (Table 2), whereas the estimated number of freezing–thawing events (FT) and the maximum number of consecutive days with freezing–thawing events (FT_{max}) were much higher for *A. alba* (Gamueta) than for *A. pinsapo* (Orcajo) (Table 2).

Results indicated that there were no statistically significant differences regarding the vulnerability to drought-induced cavitation because the values of water potential at which 12% (Ψ_{PLC12}), 50% (Ψ_{PLC50}) and 88% (Ψ_{PLC88}) loss of conductivity occurs did not show statistically significant differences between sites and between species. The overall mean values were −3.21 ± 0.03 MPa for Ψ_{PLC12}, −3.80 ± 0.03 MPa for Ψ_{PLC50} and −4.39 ± 0.05 MPa for Ψ_{PLC88}, respectively. It should be noted that the risk of native embolism in Gamueta (*A. alba*) and Orcajo (*A. pinsapo*) could be considered negligible because the lower water potential values registered (−1.84 ± 0.04 MPa and −2.19 ± 0.05 MPa during summer, and −1.89 ± 0.08 MPa and −1.72 ± 0.07 MPa during winter, for Gamueta and Orcajo, respectively) were very far from Ψ_{PLC12}.

Table 3 shows several stem morphological and anatomical parameters for one population of *A. alba* (Gamueta) and one population of *A. pinsapo* (Orcajo). Although *A. pinsapo* showed higher values for all the parameters considered, the differences were not statistically significant at $P < 0.05$, except for tracheid diameter. Furthermore, it should be noted that *A. pinsapo* had a significant number of tracheids between 20 and 25 μm in diameter (~20%), and even >30 μm in diameter (Figure 2). By contrast, *A. alba* had a negligible (<0.1%, Figure 2) number of tracheids with diameter >20 μm. Accordingly, the wood density (ρ, g cm^{-3}) for *A. alba* was higher (0.64 ± 0.02) than for *A. pinsapo* (0.54 ± 0.01), which is consistent with the lower tra-

Table 1. Characteristics of the study sites with populations of *A. alba* (Gamueta, Ezpela and Oroel) and *A. pinsapo* (Orcajo and Grazalema). MAT and MST are, respectively, the mean annual and summer temperature. TAP and TSP are, respectively, the total annual and summer precipitation. MAI is Martonne's aridity index.

Species	*A. alba*			*A. pinsapo*	
Site	Gamueta	Ezpela	Oroel	Orcajo	Grazalema
Latitude (N)	42° 52′	42° 45′	42° 30′	41° 05′	36° 46′
Longitude (W)	0° 47′	0° 51′	0° 25′	1° 30′	5° 25′
Elevation (m a.s.l.)	1350	940	1200	1150	1050
MAT/MST (°C)	7.3/16.0	9.4/17.2	9.4/17.0	10.8/19.1	14.1/22.7
TAP/TSP (mm)	1562/229	1557/203	1129/235	452/92	1110/46
MAI	90	80	58	22	46
Gaussen index	0	0	0	2	4

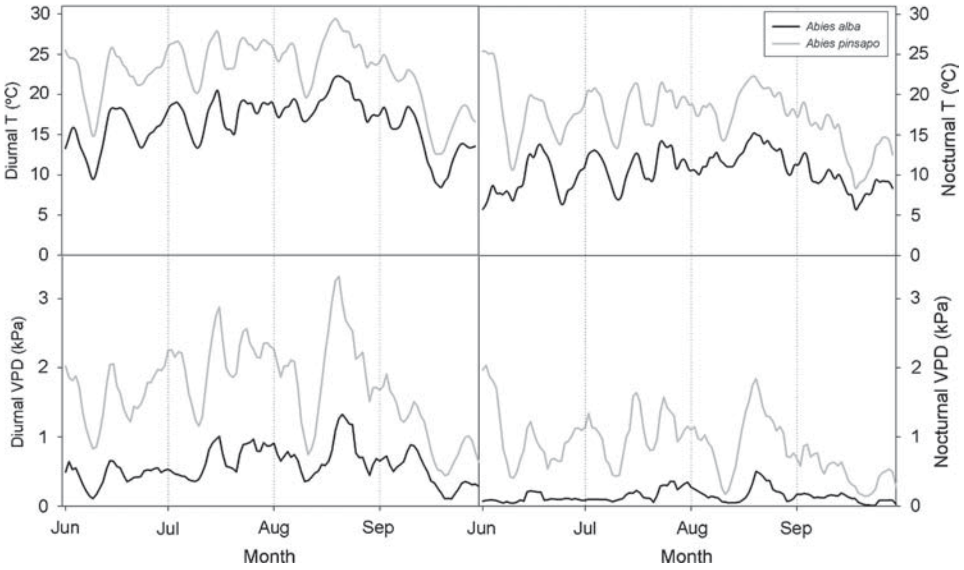

Figure 1. Mean diurnal (from dawn to sunset) and nocturnal (from sunset to dawn) temperature (T, °C) and vapour pressure deficit (VPD, kPa) for Gamueta (*A. alba*) and Orcajo (*A. pinsapo*) during summer 2009.

Table 2. Minimum daily temperature during winter (WMT, °C), estimated number of freezing–thawing events (FT) and maximum number of consecutive days with freezing–thawing events (FT_{max}) per season for *A. alba* (Gamueta) and *A. pinsapo* (Orcajo). Data are mean ± SE. Different letters indicate significant differences at $P < 0.05$ between *A. alba* and *A. pinsapo*.

Species	*mA. alba*	*A. pinsapo*
WMT (°C)	−3.0 ± 0.3 a	0.9 ± 0.2 b
FT	60 ± 4 a	20 ± 2 b
FT_{max}	12 ± 1 a	6 ± 0 b

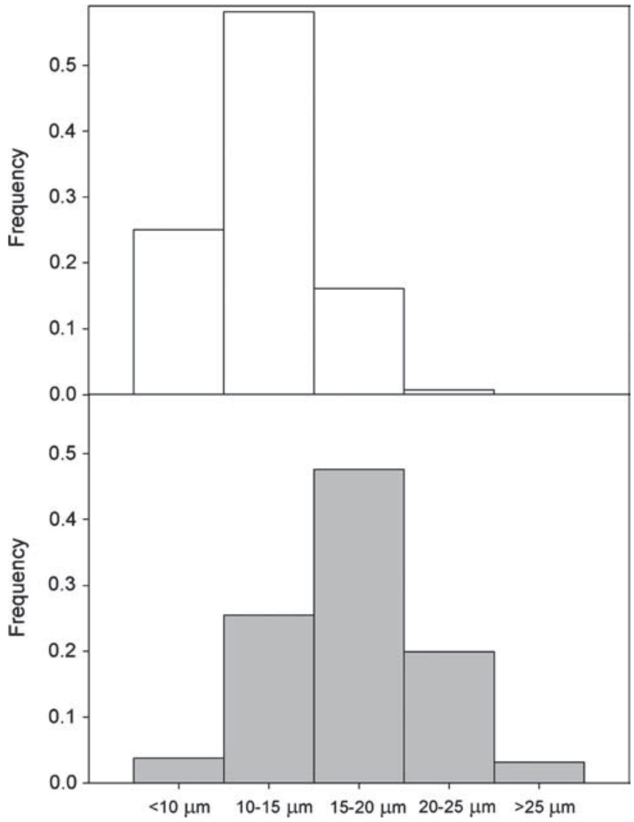

Figure 2. Frequency distribution of tracheid diameters for *A. alba* (white bars) and *A. pinsapo* (grey bars).

cheid diameter found in *A. alba* (Table 3). In addition, regarding the frequency distribution of tracheid diameters, both species had similar positive standardized skewness values although *A. alba* had a higher kurtosis, indicating a distinct peak near the mean with a higher rapidly decline (data not shown). Finally, HV was higher in *A. pinsapo*, although the differences were not statistically significant at $P < 0.05$ (Table 3).

The hydraulic parameters of stem segments for one population of *A. alba* (Gamueta) and one population of *A. pinsapo* (Orcajo) are shown in Table 3. Hydraulic conductivity (K_h, kg m s^{-1} MPa^{-1}) was significantly higher in *A. pinsapo*, especially in 2- and 3-year-old stem segments (Table 3). Specific hydraulic conductivity (K_s, kg m^{-1} s^{-1} MPa^{-1}) was also significantly higher in *A. pinsapo*, although the differences between both species were smaller than those found for K_h (Table 3). This fact can be explained by the higher values of conductive xylem area found in *A. pinsapo* (Table 3), which partially compensated the higher K_h values measured in this species (Table 3). Leaf-specific conductivity (LSC, kg m^{-1} s^{-1} MPa^{-1}) was also significantly higher in *A. pinsapo* than in *A. alba* (Table 3). The differences found between both species for LSC were similar to those found for K_h because both species showed almost the same values of supported leaf area (Table 3). Finally, the values of K_{shoot} showed trends consistent with those described above: the value for *A. pinsapo* (6.33 ± 0.30 mmol m^{-2} s^{-1} MPa^{-1}) was

Table 3. Stem diameter (mm), supported leaf area (m^2), conductive xylem area (cm^2), tracheid diameters (μm), Huber value (HV), hydraulic conductivity (K_h, kg m s^{-1} MPa^{-1}), specific hydraulic conductivity (K_s, kg m^{-1} s^{-1} MPa^{-1}) and leaf-specific conductivity (LSC, kg m^{-1} s^{-1} MPa^{-1}) in 1-, 2- and 3-year-old stem segments for *A. alba* (white bars) and *A. pinsapo* (grey bars). Data are mean ± SE. Different letters indicate significant differences at $P < 0.05$ between *A. alba* and *A. pinsapo*.

	Shoot age (year)	*A. alba*	*A. pinsapo*
Stem diameter (mm)	1	2.12 ± 0.12 a	2.32 ± 0.22 a
	2	3.12 ± 0.19 a	3.69 ± 0.35 a
	3	4.31 ± 0.31 a	4.97 ± 0.47 a
Supported leaf area (m^2)	1	0.019 ± 0.002 a	0.018 ± 0.003 a
	2	0.054 ± 0.006 a	0.060 ± 0.011a
	3	0.108 ± 0.015 a	0.120 ± 0.021 a
Conductive xylem area (cm^2)	1	0.027 ± 0.003 a	0.036 ± 0.008 a
	2	0.070 ± 0.010 a	0.101 ± 0.019 a
	3	0.147 ± 0.022 a	0.203 ± 0.040 a
Tracheid diameters (μm)	1	12.9 ± 0.3 a	16.8 ± 1.0 b
	2	11.9 ± 0.2 a	16.1 ± 0.7 b
	3	11.8 ± 0.2 a	18.7 ± 0.9 b
HV	1	1.39e – 4 ± 3.24e – 5 a	1.97e – 4 ± 7.63e – 5 a
	2	1.28e – 4 ± 3.22e – 5 a	1.67e – 4 ± 6.12e – 5 a
	3	1.36e – 4 ± 3.91e – 5 a	1.69e – 4 ± 6.33e – 5 a
K_h (kg m s^{-1} MPa^{-1})	1	1.21e – 6 ± 1.72e – 7 a	2.00e – 6 ± 2.83e – 7 b
	2	2.82e – 6 ± 3.50e – 7 a	7.14e – 6 ± 1.11e – 6 b
	3	3.49e – 6 ± 3.20e – 7 a	1.08e – 5 ± 1.55e – 6 b
K_s (kg m^{-1} s^{-1} MPa^{-1})	1	0.451 ± 0.031 a	0.650 ± 0.039 b
	2	0.426 ± 0.038 a	0.733 ± 0.029 b
	3	0.263 ± 0.028 a	0.598 ± 0.055 b
LSC (kg m^{-1} s^{-1} MPa^{-1})	1	6.15e – 5 ± 4.33e – 6 a	1.14e – 4 ± 1.57e – 5 b
	2	5.44e – 5 ± 5.89e – 6 a	1.28e – 4 ± 0.98e – 6 b
	3	3.72e – 5 ± 5.61e – 6 a	1.02e – 4 ± 1.09e – 5 b

significantly higher than that for *A. alba* (2.49 ± 0.21 mmol m^{-2} s^{-1} MPa^{-1}).

Discussion

In spite of the genetic proximity between *A. pinsapo* and *A. alba* (Linares 2011), the morphological and physiological traits described in this study are consistent with the differences between niches occupied by *A. alba* and *A. pinsapo* (Alba-Sánchez et al. 2010), suggesting that *A. pinsapo* can be considered a genuine oro-Mediterranean fir according to its environmental requirements (Fernández-Cancio et al. 2007). The presence of this fir in genuine Mediterranean woodlands, which are dominated by *Quercus ilex* L. subsp. *ballota* (Desf.) Samp., *Quercus faginea* Lam. and *Pinus halepensis* Mill., at the lower altitudinal belts in its natural distribution areas, clearly indicates its capacity to withstand a long summer drought (Linares et al. 2009). In fact, palynological evidence has shown that both firs remained geographically isolated throughout their Quaternary history due to an early evolutionary divergence, suggesting a strong ecological segregation (Alba-Sánchez et al. 2010).

The constraints imposed on the vegetation by summer drought in Mediterranean-type climates are consequences of both soil and atmospheric water deficits (Bhaskar et al. 2007). Although the soil water deficit may be buffered by local factors (Corcuera et al. 2006), the high atmospheric VPD requires special morphological or physiological adaptations (Vilagrosa et al. 2003). Under high evaporative demands and when stomata are open, a greater hydraulic sufficiency of stems to supply water to leaves may be required to prevent excessive diurnal and seasonal declines in leaf water potential (Maherali and De Lucía 2001, Addington et al. 2006). In this regard, by comparing species pairs, Bhaskar et al. (2007) showed that a shift in hydraulic traits was a common pattern in species from Mediterranean-type climates, as a strategy to cope with the high atmospheric evaporative demand. The differences between *A. alba* and *A. pinsapo* can be analysed from the same perspective. The higher values of both LSC and K_{shoot} in *A. pinsapo* revealed a higher capacity to supply water to the transpiring needles. From the microclimatic data, we can conclude that the VPD during summer in Orcajo reaches values that are well above those typical for *A. alba* habitats in the Spanish Pyrenees (Figure 1). During the warmest and driest months of the year, the values of VPD in Orcajo are consistent with those registered in a semi-arid Mediterranean-type climate (Martínez-Cob et al. 2008) rather than with those recorded in the humid montane altitudinal belts of the Pyrenees (Peguero-Pina et al. 2007).

An increase in the sapwood-to-leaf area ratio in conifers occupying more arid habitats has been reported as a morphological response to improve the water supply to the crown (Mencuccini and Grace 1995, Addington et al. 2006). This does not seem to be the strategy followed by *A. pinsapo* in order to achieve a higher LSC, because no statistical differences were found when both supported leaf area and conductive xylem area were analysed in branches from the two species (Table 3). However, when the K_s values were compared, a higher efficiency of the xylem in *A. pinsapo* was evident, consistent with larger tracheid diameters in *A. pinsapo* (Sperry et al. 2006).

Efforts to detect a trade-off of xylem efficiency against vulnerability to cavitation have been made since the very early studies in plant hydraulic architecture (Zimmermann and Brown 1977). Martínez-Vilalta et al. (2002) showed empirical evidence for such a trade-off between efficiency (K_s) and safety (PLC_{50}) when comparing several Mediterranean woody species, mainly angiosperms. However, the development of tracheids with a higher protection against cavitation caused by water stress seems to be uncoupled from conduit size and conducting efficiency (Pitterman and Sperry 2006, Sperry et al. 2006). On the other hand, Hacke et al. (2000)observed that wood density was correlated with cavitation resistance through the trend in wall reinforcement to prevent wall collapse as the tension in the xylem becomes higher. The safety factor from implosion by negative pressure (the square of the ratio between conduit wall span and wall thickness) was higher in conifer species with more negative PLC_{50} values. When this value was calculated for the species studied here, it was ~0.19 and 0.12 for *A. alba* and *A. pinsapo*, respectively, which agrees with a denser wood in silver fir.

However, both firs performed similarly in terms of xylem vulnerability to drought-induced cavitation, irrespective of the differences found in climatic conditions among the studied populations. This fact has been previously reported in conifers distributed across a wide range of climate dryness (Piñol and Sala 2000, Martínez-Vilalta et al. 2009), suggesting that the evolution of hydraulic transport efficiency seems to be independent of the evolution of hydraulic resistance to drought-induced cavitation (Bhaskar et al. 2007).

The values for minimum summer water potential registered under natural conditions revealed that both species operate far from risky values in terms of cavitation, assuming PLC_{12} as a good estimate of the xylem water potential at which cavitation begins (Sperry and Tyree 1988). This observation may suggest that the improvement of xylem sufficiency for supplying water to the transpiring needles when stomata are open in *A. pinsapo* may enable this species to cope with the high summer atmospheric evaporative demand characteristic of Mediterranean-type climates.

In spite of these benefits, the higher diameter of the tracheids of *A. pinsapo* would jeopardize its chance for occupying colder environments due to an inherent higher vulnerability to freeze–thaw embolism during winter (Mayr et al. 2003*a*, Pittermann and Sperry 2006, Mayr and Sperry 2010). It should be noted that the occurrence of freeze–thaw embolism in conifers has been associated with the number of freeze–thaw cycles (Sparks et al. 2001). Specifically, Mayr et al. (2003*b*) showed that *Picea abies* lost 50% of hydraulic conductivity after 65 cycles (such as the situation in Gamueta), whereas the loss of conductivity was negligible after 21 cycles (such as the situation in Orcajo). Thus, a hypothetical expansion of *A. pinsapo* in the Iberian Peninsula towards northern sites occupied by *A. alba* might be limited by the frequent occurrence of freeze–thaw events in these areas (Table 2), which could increase the risk of winter embolism in the tracheids of *A. pinsapo*. In this regard, the real extent of this process is a matter that deserves further investigations, taking into account that several studies showed that a recovery of winter embolism through refilling could be possible in conifers (Sparks et al. 2001, Mayr et al. 2003*b*).

In conclusion, the functional differences observed between both fir species could help to explain the niche segregation through the evolution of Mediterranean firs from an Arcto-Tertiary common ancestor (Linares 2011). Whereas *Abies alba* remains associated with the upper belts in nemoral mountain ranges, *A. pinsapo* has been able to occupy the oro-Mediterranean-type environment. In such an adaptive process, the hydraulic traits of *A. pinsapo* may play a key role in coping with the challenge of withstanding a dry atmosphere during the summer period.

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