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Climate-related variability in carbon and oxygen stable isotopes among populations of Aleppo pine grown in common-garden tests

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Abstract The Aleppo pine (*Pinus halepensis* Mill.) is found in the Mediterranean under a broad range of moisture and thermal conditions. Differences in severity and duration of water stress among native habitats may act as selective forces shaping the populations' genetic make-up in terms of contrasting drought strategies. We hypothesised that these strategies should translate into intraspecific variation in carbon isotope composition ($\delta^{13}C$, surrogate of intrinsic water-use efficiency, WUE_i) of wood holocellulose, and such variation might be linked to changes in oxygen isotope composition ($\delta^{18}O$, proxy of stomatal conductance) and to some climatic features at origin. Thus, we evaluated $\delta^{13}C$, $\delta^{18}O$, growth and survival for 25 Aleppo pine populations covering its geographic range and grown in two common-garden tests. We found intraspecific variability for $\delta^{13}C$ and growth, with high-WUE_i populations (which showed ^{18}O-enriched holocellulose) having low growth. These results suggest stomatal regulation as common control for $\delta^{13}C$ and productivity. We also detected sizeable relationships between $\delta^{13}C$ and climate factors related to the magnitude and timing of drought such as the ratio of summer to annual rainfall. The main climate variable associated with $\delta^{18}O$ was minimum temperature, but only in the coldest trial, suggesting differences in growth rhythms among sources. Overall, slow growing populations from highly-seasonal dry areas of the western Mediterranean exhibited a conservative water-use, as opposed to fast growing sources from the northernmost distribution range. The particular behaviour of the Mediterranean Aleppo pine as compared with other conifers demonstrates different selective roles of climate variables in determining intraspecific fitness.

Keywords Water-use efficiency · Adaptation to drought · Growth · *Pinus halepensis* · Mediterranean conifers

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Introduction

In the Mediterranean region, the combined impact of current aridity with climate change will particularly alter ecosystem processes. The Aleppo pine (*Pinus halepensis* Mill.) is the most common conifer around the Mediterranean basin and can be found across environments encompassing a broad range of moisture and thermal conditions. The presence of geographically related patterns in the distribution of intraspecific genetic differentiation (Schiller et al. 1986; Gómez et al. 2005) points to potentially large differences in drought responses among populations, which are likely related to the climatic characteristics at their locations of origin (Mátyás 1996). Provenance research (i.e. the assessment of intraspecific

variability under common-garden conditions) is the traditional approach to study the relevance of ecological factors, especially of climate, in shaping adaptive strategies of forest trees (Mátyás 1996; Rehfeldt et al. 2002). For example, previous findings on common-garden tests of Aleppo pine confirmed the existence of adaptive variation in the performance of four seed sources under different climate conditions in Israel (Atzmon et al. 2004).

One component of adaptedness that is relevant to plant capacity to thrive in dry areas is intrinsic water-use efficiency (WUE$_i$; the amount of carbon acquired per unit of water lost). The analysis of carbon isotope composition (δ^{13}C) in plant tissues offers the possibility to get an integrated record of the ratio of chloroplastic to atmospheric CO_2 concentration (c_c/c_a) during the period in which the carbon was fixed (Farquhar and Richards 1984; Farquhar et al. 1989). Consequently, and assuming near constancy of c_c–c_i (difference between chloroplastic and intercellular CO_2 concentration) at the intraspecific level, δ^{13}C can be used to assess genetic variability in c_i/c_a, and thus in WUE$_i$ (Farquhar and Richards 1984; Warren and Adams 2006). Indeed, earlier studies have shown intraspecific variation for δ^{13}C in conifers, in some cases related to climate conditions at the site of origin, such as VPD (vapor pressure deficit) for *Pseudotsuga menziesii* (Zhang and Marshall 1995) or rainfall for *P. sylvestris* (Cregg and Zhang 2001). Due to the longer time span covered by tree rings, the potential of δ^{13}C to provide information on long-term variation in WUE$_i$ may be better exploited using measurements on wood as compared to leaf material (Klein et al. 2005; Raddad and Luukkanen 2006). Furthermore, the accuracy of δ^{13}C from tree rings of *P. halepensis* to integrate changes in WUE$_i$ over the growing season has been previously confirmed by Klein et al. (2005).

The use of δ^{13}C to determine genetic variability in WUE$_i$ is nonetheless limited because it does not provide information on the ultimate source of changes in c_c/c_a (i.e. differences in stomatal conductance or assimilation rates) (Farquhar and Richards 1984). Scheidegger et al. (2000) proposed a conceptual model for the use of oxygen isotope composition (δ^{18}O) in plant matter to discriminate between stomatal and assimilation effects on δ^{13}C. Briefly, the δ^{18}O of plant tissues reflects variation in (1) isotope composition of source and atmospheric water, (2) evaporative enrichment of leaf water due to transpiration, and (3) biochemical fractionation during the synthesis of organic matter (Farquhar et al. 1998; Barbour 2007). Considering no differences in source water (e.g. for even-aged trees grown in a common garden), the oxygen isotope composition (δ^{18}O) of plant matter would be inversely related to transpiration rates, and hence to leaf stomatal conductance. Accordingly, when δ^{13}C and δ^{18}O are positively associated

it can be assumed that both δ^{13}C and δ^{18}O variations are mostly driven by changes in stomatal conductance, otherwise differences in δ^{13}C would be related to assimilation rates, not reflected in δ^{18}O (Farquhar et al. 1998; Scheidegger et al. 2000). Following this approach, δ^{18}O has helped to reveal whether δ^{13}C variability is associated with changes in stomatal conductance rather than in photosynthetic capacity, or the opposite, for a number of C_3 species including forest trees (Xu et al. 2000; Prasolova et al. 2001). However, during wood formation some of the oxygen atoms are exchanged with xylem water (see e.g. Saurer et al. 1997). Still, the original transpiration signal from the leaves is well preserved in tree rings of pines, particularly in water-limited environments (Barbour et al. 2002; Ferrio and Voltas 2005; Barnard et al. 2007; Gessler et al. 2008).

As reviewed by Warren et al. (2001), δ^{13}C may be a useful indicator of water availability for conifers in seasonally dry climates. This point was confirmed by Ferrio et al. (2003) for Aleppo pine trees sampled across a rainfall gradient. As a result, water availability may have acted as selective force leading to contrasting genetic profiles for WUE$_i$ among populations. We hypothesised that these differences, if present, would translate into substantial intraspecific variation in δ^{13}C, and that such variation might be linked to changes in δ^{18}O and to some climatic features defining the potential impact of drought at the sites of origin. Accordingly, we aimed to (1) characterise in two common-garden tests variation in carbon and oxygen stable isotopes of tree ring cellulose, together with growth and survival (as additional indicators of fitness), for a set of 25 seed sources of Aleppo pine covering its present range of geographical distribution; and (2) elucidate whether such variation correlates with climatic indicators of native habitats. This information may be important to understand the risk of maladaptation of Mediterranean conifers owing to abrupt global warming.

Materials and methods

Plant material

Aleppo pine (*P. halepensis* Mill.) seeds from 56 native populations spanning the distribution range for this species were collected in 1995 to establish a provenance trial network in Spain. Open-pollinated seeds from 20 to 30 trees, spaced at least 100 m apart, were obtained within each population and subsequently bulked into population seedlots. One-year old seedlings were grown throughout the 1997 growing season under standard container-nursery cultural practices in Spain (Cortina et al. 2006). For each

seedlot, seedlings were randomly allocated to six different experimental sites in 1998.

A representative subset of 25 populations was chosen using a stratified sampling strategy (based on temperature/precipitation gradients). This selection preserved the original range of variation in thermal and precipitation regimes among population origins, avoiding the dependence between both bioclimatic indicators for the complete set and thus reducing their potential confounding effects in the interpretation of adaptive patterns (Table 1). For each source, means of monthly maximum (T_{max}) and minimum (T_{min}) temperature and of total monthly precipitation (P) for the period 1960–1990 were obtained through the WorldClim database (Hijmans et al. 2005) implemented in DIVA-GIS with a spatial resolution of a square kilometre. Mean monthly potential evapotranspirative demand (PET, according to the Hargreaves method; Hargreaves and Samani 1982) and mean monthly vapour pressure deficit (VPD; after Ferrio and Voltas 2005) for the same period were inferred with long-term estimates of temperature and precipitation. Finally, the ratio of precipitation to PET (P/PET) was calculated on a monthly basis.

Study sites

Of the six Aleppo pine provenance trials originally installed in April 1998, we selected two sites located in central (Arganda, Madrid province) and eastern (Altura, Castellón province) Spain, respectively (Table 1). These are characterised by contrasting precipitation and temperature regimes, but similar photoperiod patterns (and also incident solar radiation) owing to comparable latitudinal coordinates. The climate at Arganda is semiarid continental Mediterranean with cold winters and scarce summer rains (about 5% of total precipitation). The trial was planted on a flat area located in a forest nursery and was tilled with a mould board plough before plantation. Soil type was a fertile typic xerofluvent of about 1.25 m depth. The climate at Altura is Mediterranean humid with cool winters and a less prolonged summer drought period (summer rainfall accounts for about 22% of the annual precipitation). The trial was installed on a reforestation site of 5% slope facing southeast and was tilled with a disk ripper before plantation. Soil type was a shallow calcixerollic xerochrept of about 0.5 m depth. Although Altura receives higher precipitation than Arganda, particularly in summer, it is a more drought-prone site due to a much lower soil water retention capacity. For each site, 16 seedlings from each of the 56 native populations were planted at spacings of 2.5 m × 2.5 m (Altura) and 2 m × 3 m (Arganda) in four replicates consisting of four-tree line plots following a row-column design for a total of 896 test seedlings per trial. Tree height and diameter at breast height (DBH) were

measured at age six on both sites from the four trees of each replicate plot in spring 2003. Survival rate (%) per plot was recorded at age six in both sites. Measurements on growth and survival were restricted to the subset of 25 seed sources used in this study.

Tree ring sampling and carbon and oxygen isotope analyses

In June of 2003, two representative trees were selected per replicate plot at every site for the subset of 25 populations. For each tree, a healthy, south-facing branch from the middle part of the crown was chosen and removed at its basal end using telescopic loppers. Care was taken to select 3-year old branches of similar size by whorl back-counting to ensure similar growing conditions. Wood slices of about 5 cm length were then taken from the cut basal end of each branch and were visually inspected to verify that they included three complete tree rings. The eight 3-year old fragments (2000–2002 period) recovered per population and site (i.e. two trees × four replicates) were bark-peeled and current-year spring wood removed with a disk sander. Next, each fragment was reduced to a small sample of fixed dimensions using a sharp blade, and the eight samples were pooled and milled to a fine powder. Wood powder was then purified to holocellulose adapting the method of Leavitt and Danzer (1993) for the removal of extractives and lignin, as detailed in Ferrio and Voltas (2005). Finally, holocellulose samples were oven-dried (60°C, 48 h) and $\delta^{13}C$ (‰) and $\delta^{18}O$ (‰) were determined by mass spectrometry at Iso-Analytical (Sandbach, Cheshire, UK). The accuracy of analyses (standard deviation of working standards) was 0.07‰ ($\delta^{13}C$) and 0.23‰ ($\delta^{18}O$). Mean annual climatic values (and their standard deviation) for the 2000–2002 period were 21.1°C (0.17) (T_{max}), 9.7 (0.29) (T_{min}), 569 mm (161.7) (P), 1,159 mm (41.3) (PET), 0.49 (0.141) (P/PET) and 991 Pa (46.6) (VPD) at Altura, and 21.4°C (0.09) (T_{max}), 7.5 (0.31) (T_{min}), 353 mm (65.8) (P), 1,260 mm (15.5) (PET), 0.28 (0.053) (P/PET) and 1,056 Pa (16.2) (VPD) at Arganda.

Statistical analysis

Growth measurements (height and DBH) were subjected to a mixed model analysis of variance (ANOVA) at the individual-tree level for randomised complete block designs consisting of fixed trial, population and population by trial effects, a random block within trial effect, a pooled intra-block error term and a term accounting for the between-tree within-plot variation. Survival data were expressed as percentage of living trees per plot and subjected to a mixed model ANOVA consisting of fixed trial, population and population by trial effects, a random block

Table 1 Geographic origin and habitat characteristics of 25 Aleppo pine populations and two study sites

Seed source or study site	Code	Country	Latitude	Longitude	Elevation (m.a.s.l.)	$T_{\max_{an}}$ (°C)	$T_{\min_{an}}$ (°C)	P_{an} (mm)	P_s (mm)	P_s/P_{an}	PET_{an} (mm)	P_{an}/PET_{an}	VPD_{an} (Pa)
Cabanellas	1	Spain	42°14′N	2°47′E	210	18.3	10.6	730	161	0.22	905	0.81	729
Tivissa	2	Spain	41°03′N	0°45′E	400	19.5	10.2	587	122	0.21	1,015	0.58	852
Zuera	3	Spain	41°55′N	0°55′W	575	17.1	6.8	474	94	0.20	1,004	0.47	743
Alcantud	4	Spain	40°34′N	2°19′W	950	16.6	5.0	505	87	0.17	1,042	0.48	781
Colmenar de Oreja	5	Spain	40°05′N	3°20′W	750	19.3	8.0	437	52	0.12	1,136	0.38	948
Tuéjar	6	Spain	39°49′N	1°09′W	600	18.4	8.0	424	85	0.20	1,047	0.40	832
Tibi	7	Spain	38°31′N	0°39′W	1,010	18.5	7.7	546	89	0.16	1,075	0.51	905
Bicorp	8	Spain	39°06′N	0°51′W	650	20.0	9.4	454	80	0.18	1,104	0.41	947
Benicàssim	9	Spain	40°05′N	0°01′E	430	19.0	10.3	523	109	0.21	973	0.54	831
Vilajoiosa	10	Spain	38°29′N	0°18′W	70	22.9	12.5	422	65	0.15	1,186	0.36	1,012
Monovar	11	Spain	38°23′N	0°57′W	700	19.6	8.3	467	72	0.15	1,133	0.41	923
Benamaurel	12	Spain	37°42′N	2°44′W	920	19.9	8.4	452	44	0.10	1,181	0.38	1,015
Santiago de la Espada	13	Spain	38°13′N	2°28′W	680	20.1	8.2	432	48	0.11	1,190	0.36	971
Alhama de Murcia	14	Spain	37°51′N	1°33′W	870	20.8	9.3	449	39	0.09	1,214	0.37	1,082
Frigiliana	15	Spain	36°49′N	3°58′W	570	21.0	10.4	456	25	0.05	1,181	0.39	1,010
Palma de Mallorca	16	Spain	39°09′N	2°56′E	30	20.8	12.9	549	74	0.13	1,004	0.55	860
Ses Salines	17	Spain	39°17′N	3°03′E	10	20.8	12.9	568	79	0.14	1,003	0.57	851
Alcudia	18	Spain	39°52′N	3°10′E	100	19.6	11.9	704	103	0.15	955	0.74	783
Alcotx	19	Spain	39°58′N	4°10′E	100	20.1	13.0	613	90	0.15	949	0.65	837
Atalix	20	Spain	39°54′N	4°03′E	50	20.3	13.0	621	91	0.15	956	0.65	830
Amfilohia	21	Greece	38°53′N	21°17′E	25	20.0	9.5	967	82	0.08	1,148	0.84	785
Kassandra	22	Greece	40°05′N	23°52′E	25	18.6	9.6	520	64	0.12	1,018	0.51	743
Litorale Tarantino	23	Italy	40°37′N	17°06′E	10	19.6	10.7	551	89	0.16	1,032	0.53	784
Gargano Marzini	24	Italy	41°32′N	15°51′E	200	20.8	11.4	472	95	0.20	1,080	0.44	926
Gargano Monte Pucci	25	Italy	41°54′N	15°56′E	100	17.4	10.9	527	110	0.21	843	0.63	690
Altura, Castellón province	Al	Spain	39°49′N	0°34′W	640	20.2	9.3	652	121	0.19	1,115	0.58	935
Arganda del Rey, Madrid province	Ar	Spain	40°18′N	3°28′W	600	20.8	7.3	445	58	0.13	1,248	0.36	985

$T_{\max_{an}}$ mean annual maximum temperature, $T_{\min_{an}}$ mean annual minimum temperature, P_{an} annual precipitation, P_s summer precipitation, P_s/P_{an} ratio of summer to annual precipitation, PET_{an} annual potential evapotranspiration, P_{an}/PET_{an} ratio of annual precipitation to annual potential evapotranspiration, VPD_{an} mean annual vapour pressure deficit

within trial effect, and a pooled intra-block error term. Carbon and oxygen isotope compositions were subjected to two-way ANOVA consisting of fixed trial and population effects because a single replicate was available per population-trial combination. For each site, simple correlations involving least square means of growth and survival traits, carbon and oxygen isotope composition measurements and climate data were calculated to assess relationships between variables. Consistency of population performance for WUE$_i$ was estimated using Spearman rank correlations of different relationships involving $\delta^{13}C$ and $\delta^{18}O$ within and across sites. We also performed path analyses (Li 1975) to quantify the relative contributions of direct and indirect effects of climate variables on stable isotopes and height. A path analysis determines simple correlations between independent factors (in this case, climate

variables), and regresses all independent factors on each dependent factor (δ^{13}C, δ^{18}O or height) to obtain direct effects in the form of partial regression coefficients (i.e. path coefficients). Indirect effects are calculated by multiplying the path coefficients by the simple correlations, so the total correlation is obtained by adding the direct and indirect effects. For each trial, a reduced model was identified as reproducing the original correlation matrix nearly as well as the full, saturated model (i.e. a model in which there is a direct path from each variable to each other variable), according to some goodness-of-fit statistics for the model including a Chi-square (or minimum discrepancy index, C_{min}), and the minimum sample discrepancy function (C_{min}/df) (Arbuckle 1997).

Unless otherwise stated, differences among treatments and correlation coefficients were considered statistically significant when $P < 0.05$. All analyses were performed using standard SAS-STAT procedures (SAS Institute Inc., Cary, NC).

Results

Population and trial effects on growth, survival and stable isotopes

The ANOVAs revealed significant trial effects on growth traits. Trees at Arganda averaged 3.29 m (height) and 10.2 cm (DBH), which represents an overall advantage of 29 and 17.5% in height and DBH, respectively over those growing at Altura. In contrast, no significant trial effects could be identified for δ^{13}C, δ^{18}O and survival rate (results not shown). Mean values for these traits across trials were −22.80‰ (δ^{13}C), 31.83‰ (δ^{18}O) and 88.4% (survival). Population performances for growth and survival were similar across sites according to non-significant population × trial interactions. There were significant differences among populations for height, DBH and δ^{13}C, but not for survival rate (results not shown). Despite considerable variation in δ^{18}O among populations (range >5‰ at both sites) we found no differences for this trait, pointing to a large population × trial interaction (the error term in the two-way ANOVA). The ranking of provenances across sites for δ^{13}C was similar as suggested by a significant Spearman rank correlation ($r = +0.59$, $P = 0.002$). Besides, the ranking of provenances for δ^{13}C and δ^{18}O was also linked at each site ($r = +0.40$, $P = 0.04$ for Altura; $r = +0.48$, $P = 0.01$ for Arganda); for example, populations from Alhama and Benamaurel showed high δ^{13}C and δ^{18}O values at both sites, whereas Bicorp, Kassandra and Tivissa exhibited low δ^{13}C and δ^{18}O irrespective of the trial. The association between δ^{13}C and δ^{18}O for population means of both trials is shown in Fig. 1a. Significant
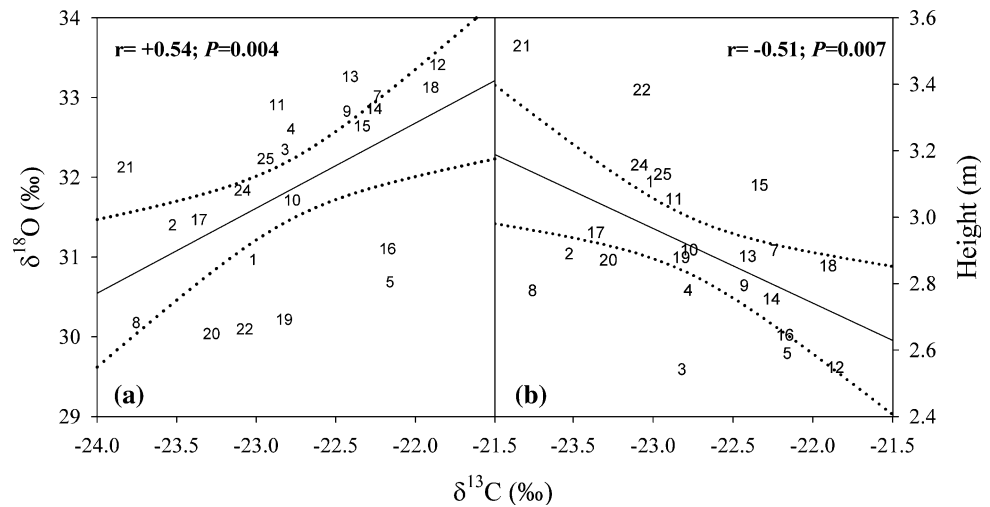
within-trial simple correlations between population means of height and DBH were detected (results not shown). Comparing the two trials, we found significant relationships between population means for height ($r = +0.68$, $P < 0.001$), but not for DBH. Several significant associations between δ^{13}C and growth variables were detected, with high-δ^{13}C populations showing on average lower growth at both trials. Hence, correlation between δ^{13}C and tree height amounted to −0.40 ($P = 0.03$) and −0.38 ($P = 0.04$), and correlations between δ^{13}C and DBH to −0.38 ($P = 0.04$) and −0.13 ($P = 0.39$), for Altura and Arganda, respectively. The association between δ^{13}C and tree height for population means of both trials is shown in Fig. 1b. On the contrary, no significant correlations between δ^{18}O and growth measurements could be detected (results not shown).

Relationships between stable isotopes and climatic characteristics of seed sources
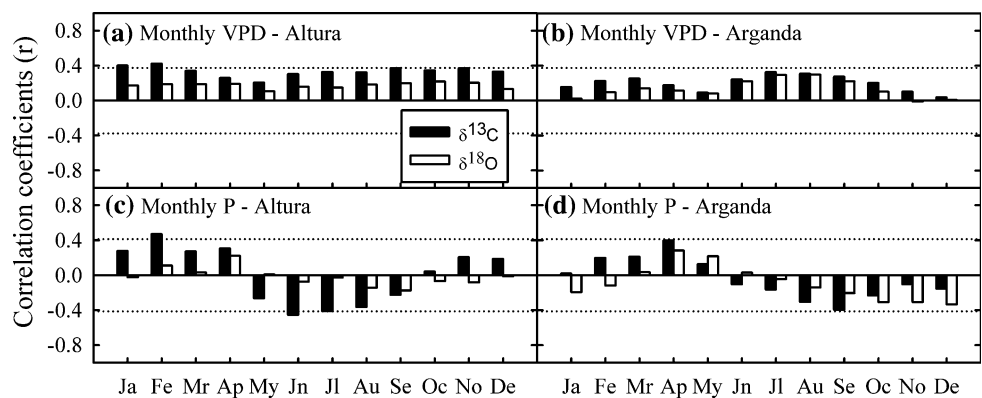
VPD and T_{min} were climatic parameters consistently associated with δ^{13}C across months, showing positive correlation coefficients with VPD and negative with T_{min}. However, the strength of the relationships with δ^{13}C was site-dependent. For VPD, correlations were larger in Altura than in Arganda (Fig. 2a, b), whereas the opposite was true for T_{min} (results not shown). We obtained similar results using yearly values of VPD and T_{min}, with near-significant correlations between δ^{13}C and VPD ($r = +0.37$; $P = 0.06$) for Altura, and between δ^{13}C and T_{min} ($r = −0.34$; $P = 0.09$) for Arganda. Other climatic characteristics such as T_{max}, P, PET or P/PET appeared to be inconsistently or non-significantly related to δ^{13}C, both on a yearly and on a monthly basis. However, scatter plots relating monthly precipitation to δ^{13}C revealed that records from a single seed source (the Greek Amfilohia population) clearly deviated from the overall trends expressed by the remaining populations. After removal of that atypical source, a number of significant correlations between monthly precipitation and δ^{13}C arose at both trials (Fig. 2c, d), without affecting the significance of most other relationships. Overall, correlations with δ^{13}C were positive through the winter and early spring and negative in summer and early autumn. Such contrasting δ^{13}C dependence on changes in monthly rainfall distribution of seed sources led us to test the relationship between δ^{13}C and the ratio of summer (July–September) to annual precipitation (hereafter, P_s/P_{an}) as indicator of rainfall seasonality. This climatic variable was negatively correlated with δ^{13}C for each trial, with populations from climates with highly seasonal precipitation having higher δ^{13}C than those from areas with more evenly distributed rainfall (Fig. 3a, b).

T_{min} was the main climate variable associated (negatively) to $\delta^{18}O$ across months, but only at Arganda, where the correlation coefficients ranged from -0.37 ($P = 0.06$) in March to -0.49 ($P = 0.01$) in May. On a yearly basis, correlations between T_{min} and $\delta^{18}O$ were -0.04 ($P = 0.84$) for Altura and -0.43 ($P = 0.02$) for Arganda (Fig. 3c, d). Correlations between $\delta^{18}O$ and monthly VPD and P showed similar seasonal patterns as those found for $\delta^{13}C$, but they were generally weaker (Fig. 2).

Relationships between growth and climatic characteristics of seed sources

VPD, P and P/PET were significantly correlated with height and, to a lesser extent, DBH of populations through the autumn–winter period at both sites. The strength of these relationships was slightly stronger for VPD and P (as shown in Fig. 4) than for P/PET (results not shown). Sources from climates with wet winter-autumn (e.g. with high P and P/PET, and low VPD) exhibited larger growth than those with drier conditions in their native sites. Other climatic characteristics were unrelated to differences in growth among populations.

Path analysis

The path diagrams in Fig. 5 represent the hypotheses of climate influence on concurrent population variability in $\delta^{13}C$, $\delta^{18}O$ and height. Chi-square for these models was non-significant and the minimum sample discrepancy functions (C_{min}/df) were lower than unity, indicating that they were representative of the original correlation matrix. For the set of 25 populations VPD and P_s/P_{an} exhibited both direct and indirect (through $\delta^{13}C$) effects on height, regardless of the trial (Fig. 5a, b). The direct effects of VPD, P_s/P_{an} and $\delta^{13}C$ on height were of similar magnitude and negative sign. The indirect pathways through $\delta^{13}C$ suggested non-direct effects of VPD (for Altura) and P_s/P_{an} (for Arganda) on height. Variation in $\delta^{13}C$ among populations was partially driven by a direct effect of $\delta^{18}O$, especially in Arganda owing to a large influence of T_{min}. Because these analyses could be greatly influenced by one outlier population, Amfilohia, which grows at humid sites (Table 1), we repeated the path analysis with a reduced dataset, obtaining comparable results (Fig. 5c, d). The main change observed in path coefficients after removing this site was a large increase in indirect effects

Fig. 3 Relationships between holocellulose $\delta^{13}C$ and the ratio of summer precipitation to total annual precipitation (P_s/P_{an}) for Altura (**a**) and Arganda (**b**). Relationships between holocellulose $\delta^{18}O$ and mean annual minimum temperature (T_{min}) for Altura (**c**) and Arganda (**d**). Population 21 was not included in the correlation analyses involving P_s/P_{an}. *Dotted lines* indicate 95% confidence intervals. *Vertical dashed lines* indicate the corresponding climate value for each trial
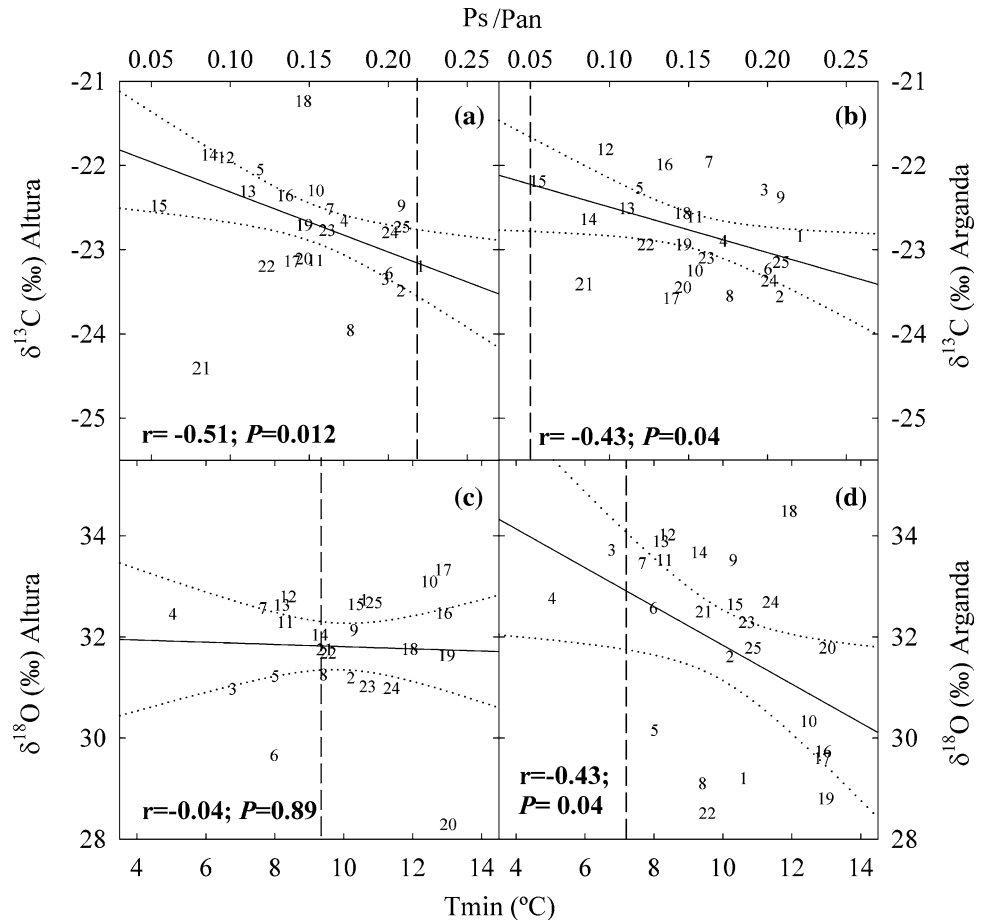
Fig. 4 Correlation coefficients between tree height/DBH and either monthly VPD (**a**, **b**) or monthly precipitation (**c**, **d**) at origin for Altura (**a**, **c**) and Arganda (**b**, **d**). *Dotted lines* indicate the threshold-value for significant correlations ($P < 0.05$, $n = 25$)
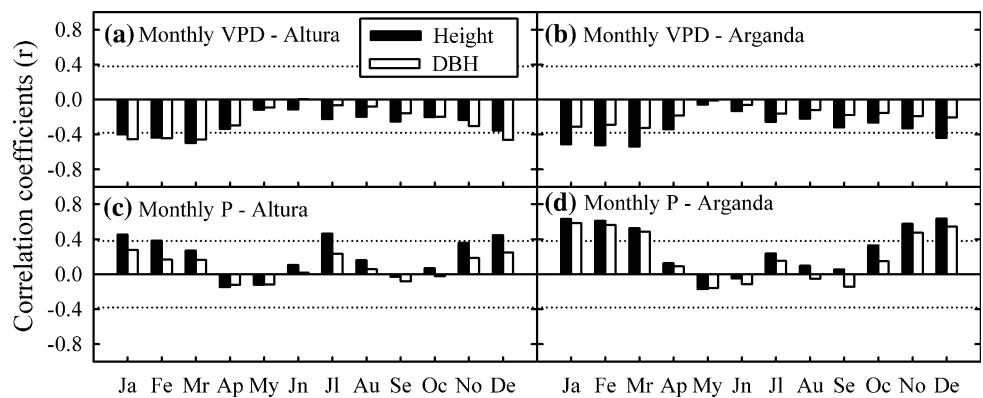
of P_s/P_{an} on height (through $\delta^{13}C$) at the expense of its direct effects.

Discussion

Intraspecific variation in WUE$_i$ and growth performance

According to $\delta^{13}C$, we found at both trials that populations with a prodigal water-use (i.e. lower $\delta^{13}C$, originating mainly from the Mediterranean northern shores) tended to grow faster (either in primary (cf. Fig. 1) or in radial growth) as compared to those conserving water. These results agree with those reported by Cregg and Zhang (2001) for *P. sylvestris*, showing high-$\delta^{13}C$ populations with slower growth rates both under controlled conditions and in a series of range-wide provenance tests. In contrast, Tognetti et al. (1997) observed a strong positive correlation between $\delta^{13}C$ and height across five populations of the Mediterranean *P. pinaster*, but only at one of four field sites in Sardinia, Italy, whereas *P. contorta* populations

Fig. 5 Path analyses of the 25 populations for Altura (**a**) and Arganda (**b**). The same analyses with population Amfilohia removed (**c**, **d**). A *single-headed arrow* between two variables denotes a hypothesis of direct causation, whereas a *double-headed arrow* reflects correlation without necessarily a direct causal relationship. The width of arrows is proportional to the path coefficient values
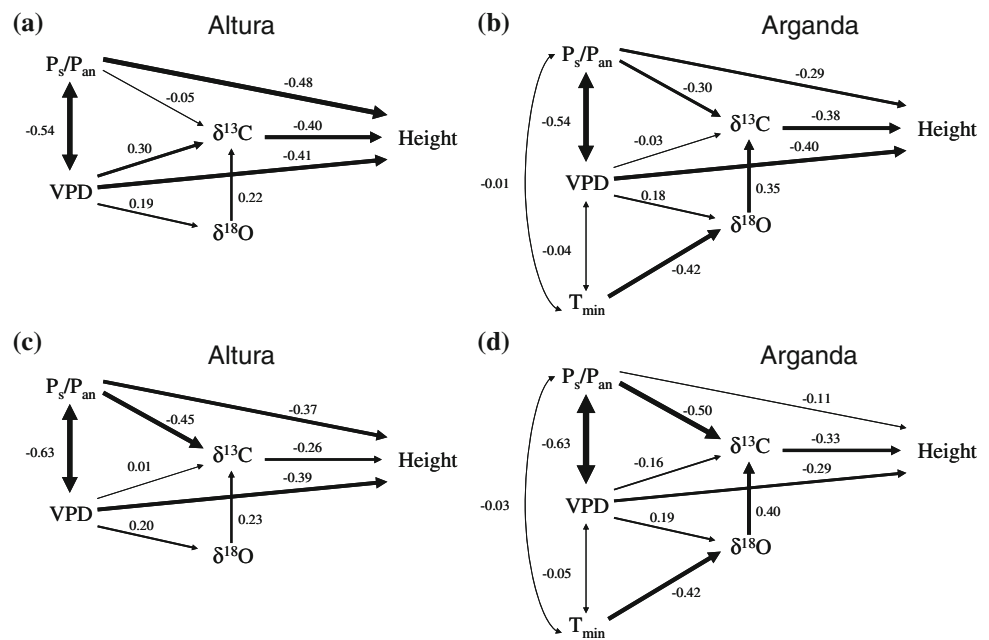
with high cellulose $\delta^{13}C$ showed greater productivity at three sites in British Columbia, Canada (Guy and Holowachuk 2001). Such divergent association patterns between $\delta^{13}C$ and productivity are interpreted as differences in the primary source for genetic differentiation in WUE$_i$ (either photosynthetic capacity or stomatal conductance). Positive trends for the association between $\delta^{13}C$ and $\delta^{18}O$ in our work suggest that variation in both isotopes was mainly related to population differences in stomatal conductance (Farquhar et al. 1998; Scheidegger et al. 2000). This finding agrees with the observed negative correlations between $\delta^{13}C$ and growth, pointing to stomatal regulation, rather than assimilation rate, as the common control for $\delta^{13}C$ and growth for Aleppo pine (Farquhar et al. 1989). In spite of the aforementioned evidence for the existence of intraspecific variation in WUE$_i$ for *P. halepensis*, the potential effect on this trait of factors related to tree age and architecture cannot be discarded. On the one hand, the series of tree rings analyzed for stable isotopes might show a 'juvenile effect' whereby changes in plant CO_2 use, hydraulic conductivity or source-sink relationships could be reflected in $\delta^{13}C$ and $\delta^{18}O$ (McCarroll and Loader 2004). Additionally, divergence among populations in allocational patterns within the tree crown might have produced a bias in stable isotopes provided the south-facing sampled branches were not fully representative of the whole tree. Both factors remain a subject of further study.

P. halepensis is a typically drought-avoiding, water-saving species that shows large phenotypic plasticity for stomatal regulation and, hence, for WUE$_i$ to adjust to environmental conditions (Borghetti et al. 1998; Ferrio et al. 2003). Our results indicate that, in addition to

physiological plasticity, intraspecific variability for WUE$_i$ and growth-related traits is considerable, suggesting the presence of adaptive responses linked to climate of origin. Because WUE$_i$ is proportional to $\delta^{13}C$ (Farquhar and Richards 1984), we estimated a maximum divergence in WUE$_i$ among populations of 26.1% for Altura and of 16.8% for Arganda, which corresponds to a difference of 2.72 and 1.77‰ between extreme $\delta^{13}C$ values, respectively. Population variability in height was also greater at Altura (60.8%) than at Arganda (26.4%). Probably, less favourable growing conditions at Altura (a more drought-prone site due to its shallow soil) enhanced intraspecific differences in their reaction to the environment. Nevertheless, the lack of relationship between $\delta^{13}C$ and survival agrees with previous studies for several *Pinus* species under controlled conditions indicating that greater WUE$_i$ does not necessarily result in increased drought survival (Zhang et al. 1997; Cregg and Zhang 2001).

Associations to climate of origin

Population differences in stable isotopes and growth were related to climate of seed sources, with the most water-use-efficient populations coming from low-latitude, drought-prone habitats (i.e. southeastern Spain) having high VPD and uneven seasonal rainfall. The association of $\delta^{13}C$ and $\delta^{18}O$ with VPD at Altura, a site more affected by water shortage than Arganda, points to transpirative demand as a driving force for genetically-enhanced water conservation in populations from drier areas (Ferrio and Voltas 2005).

Our results indicate that both total precipitation and dry season duration (i.e. rainfall seasonality) are also important

selective factors for water-use efficiency in Aleppo pine. Thus, a conservative water-use would be typical of drought-adapted populations from dry areas exposed to prolonged water stress periods in summer (Li et al. 2000; this work). In contrast, populations with a less efficient water-use and, probably, more rapid consumption of available water would be adapted to more humid sites having a short, mild summer drought (Sandquist and Ehleringer 2003). This behaviour seems genetically determined in Aleppo pine, given that similar population responses have been found in sites with contrasting rainfall seasonality (cf. Fig. 3; Calamassi et al. 2001). The performance of population 21 (Amfilohia) deviated from this overall trend, showing low δ^{13}C values despite the high rainfall seasonality of its native habitat. This population originates from the most mesic area of the climatic gradient (P/PET = 0.84), so it is likely that other influences on δ^{13}C such as light intensity or temperature regime dampened the selective pressure towards water conservation at this site (Warren et al. 2001). Moreover, a later reproductive and vegetative development is characteristic of Greek provenances (Weinstein 1989); this might also lead to a lower δ^{13}C, as tree ring growth would begin later in spring, thus being more exposed to summer drought.

We are unaware of previous works linking δ^{18}O changes to climate characteristics of origin. Recently, Warren et al. (2005) could not associate variation in stable isotope ratios (either ^{13}C/^{12}C or ^{18}O/^{16}O) among *Eucalyptus sideroxylon* subsp. *tricarpa* populations with seed-source rainfall and questioned the assumption that drought-adapted genotypes show high WUE$_i$ at the intraspecific level. For Aleppo pine, however, relationships between δ^{18}O and indicators of aridity such as VPD or rainfall mirrored those involving δ^{13}C, which again suggests genetically-driven differences in stomatal control among populations (Scheidegger et al. 2000). Another climate variable related (negatively) to δ^{18}O (and also to δ^{13}C) was T_{min}, which characterises the intensity of the cold period, but this relationship was significant only at the coldest trial (Arganda). This is probably an indirect effect of differences in growth phenology, which is under strong genetic control in Aleppo pine (Weinstein 1989). *P. halepensis* usually shows two phases with reduced cambial activity: one is defined by the summer drought and the other by low winter temperatures (Liphschitz et al. 1984). Because conifer populations from areas with milder winter tend to have a longer growing period (Jayawickrama et al. 1998), the seasonal pattern in δ^{18}O of precipitation (i.e. source water) might be partly responsible for differences in δ^{18}O among populations, as trees with a prolonged winter growing period will have lower δ^{18}O. Such differences would be enhanced in the colder, continental climate of Central Spain (Arganda),

where changes in precipitation δ^{18}O during the growing season are much higher than along the Mediterranean shores (Altura) (Global Network of isotopes in precipitation; http://www.isohis.iaea.org/). Also, variations in the growth initiation or cessation dates (Weinstein 1989), or in the number of summer growth cycles (as observed for a subset of trees in Altura and reported in Pardos et al. 2003) could have changed the environmental conditions in which tree rings were formed, thus affecting both δ^{18}O and δ^{13}C in cellulose. More research on growth rhythms in relation to water-use patterns is needed to shed light on this issue.

The association between growth and water availability factors (VPD, P and P/PET) demonstrates the adaptive relevance of this trait for Aleppo pine, in agreement with provenance tests involving other *Pinus* species in the Iberian Peninsula (Alía et al. 1997). Central and eastern Mediterranean populations of Aleppo pine, which develop in wetter sites, had consistently higher growth than their Iberian counterparts (e.g. they exhibited a 15.2% overall superiority in height). These results suggest the existence of a trade-off between productivity and drought tolerance for this species whereby high-WUE$_i$ populations from harsher climates exhibit a low growth. This phenomenon is also known to occur in other *Pinus* species, e.g. Scots pine (Cregg and Zhang 2001) or loblolly pine (Boltz et al. 1986). Such a differentiation in adaptive traits in relation to ecological heterogeneity may help understand the potential evolutionary responses of Aleppo pine to climate instability. Because drought and seasonality are becoming more intense in the Mediterranean (IPCC 2007), the direct short-term impact of a warmer climate on contemporary populations should increase the discrepancy between productivity and WUE$_i$ at the intraspecific level for any particular region. For example, trees from a relatively wet niche will show, after sudden warming, an innate potential for higher productivity and lower WUE$_i$ as compared with contemporary populations already growing in drier climates. This behaviour by no means implies an increase in overall productivity for Aleppo pine under a drier scenario, but rather a built-in stimulus towards increased growth and reduced water conservation before long-term evolutionary adjustments occur to restore fitness. In this regard, the short-term impact of climate change on productivity is primarily governed by plastic phenotypic responses (Rehfeldt et al. 2002), which are likely to surpass the influence of divergences in adaptive traits related to growth determination for a very plastic species such as Aleppo pine (Ferrio et al. 2003). Long-term evolutionary adjustments may restore the balance between growth potential and drought tolerance in order to reach equilibrium with climate, but the definition of the exact timing of these adjustments in terms of changes in distribution and

productivity would need the development of quantitative models requiring a larger number of common-garden studies.

Joint influence of climate factors on stable isotopes and growth

The path analysis aided to clarify the importance of direct and indirect (i.e. WUE$_i$-mediated) influences of native climate on growth variation among populations. Two indirect (through δ^{13}C) climate-related sources of variation in height were confirmed: rainfall seasonality and minimum temperature, the latter exclusively for the Arganda site (via its effect on δ^{18}O). A direct causal effect on growth was consistently exerted by differences in VPD among local habitats. In this regard, adaptation of Aleppo pine to drought comprises a number of strategies, in addition to changes in WUE$_i$, that involve characteristics such as biomass allocation (Chambel et al. 2007), hydraulic architecture (Tognetti et al. 1997) and osmotic adjustment (Calamassi et al. 2001). VPD-mediated divergence among populations in such strategies might explain the influence of this climate element on growth. This point could be considered in future research. In most temperate conifers, however, water-related attributes usually have a minor effect on climatic adaptation of trees, with thermoperiod being the most important factor underlying adaptive variation (Mátyás 1996). The contrasting behaviour of the Mediterranean Aleppo pine as compared with such conifers evidences different selective roles of climate variables in determining fitness at the intraspecific level.

Conclusions

Our data emphasize the existence of intraspecific variability for WUE$_i$ and growth of Aleppo pine under rainfed, common garden conditions. High-WUE$_i$ seed sources (i.e. with high holocellulose δ^{13}C) tend to show enriched ^{18}O content coupled with low growth, indicating stomatal regulation, rather than carbon acquisition rate, as common control for δ^{13}C and productivity for this species. The adaptive relevance of water stress in shaping the populations' genetic structure for WUE$_i$ is demonstrated by the association between carbon and oxygen stable isotopes and climate factors related to the magnitude and timing of drought such as VPD and rainfall seasonality. As a result, drought-adapted, slow-growing populations from highly seasonal dry areas of the western Mediterranean basin exhibit a conservative water-use, as opposed to fast growing, less water-use-efficient seed sources from the northernmost distribution range.

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