Sex-specific, age-dependent sensitivity of tree-ring growth to climate in the dioecious tree *Juniperus thurifera*

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Summary

• Tree features may modulate the sensitivity of radial growth to climate, leading to a nonuniform response. Age-related increases in climatic sensitivity have been observed repeatedly. Sex-related climatic sensitivity is also possible because of the long-term differential reproductive cost between the sexes. This study analysed the simultaneous effects of age and sex on the sensitivity of tree-ring growth to climate.
• Ring widths were measured from 50 female and 50 male *Juniperus thurifera* trees, 50–350 yr old, growing under a Mediterranean continental climate. Response functions were calculated based on tree-ring chronologies and monthly climatic records.
• Climatic sensitivity decreased with increasing age. Young trees (50–100 yr) were the most climatically sensitive to June–July precipitation, which affected growth positively. We found a significant interaction between age and sex in the climatic response of *J. thurifera*, with young females the most sensitive to summer water stress.
• Our results suggest that age-dependent climatic sensitivity can be determined by site-specific limiting environmental conditions and species-specific architectural and physiological adjustments during ontogeny. This study supports that the different ontogenetic stages of *J. thurifera* differ in their root structural traits and that sex-related sensitivity to summer drought may be attributable to less efficient water use by females.

Introduction

Compared with other plant life forms, the prominent features typical of trees are large sizes and long lifespans. These features confer on them ecological and physiological benefits, which contribute to their dominance across many terrestrial ecosystems (Rowe & Speck, 2005; Petit & Hampe, 2006). However, size and longevity imply high costs of maintenance in the development of supporting tissues and the production of chemical and structural defences, which allocate photosynthates from other functions such as growth and reproduction (Donaldson *et al.*, 2006). Differences in the resource allocation to growth are traditionally evaluated by changes in annual tree rings (Fritts, 2001). Secondary xylem growth is affected by the carbon supply status of the tree and is therefore extremely sensitive to environmental conditions (Rossi *et al.*, 2008).

Because of the physiological processes that control cambial activity and xylem growth, anatomical and chemical parameters can be used as proxies for the environmental conditions that controlled the availability of free carbohydrates or water during ring ontogeny (García-González & Eckstein, 2003; Fonti & García-González, 2004; Helle & Schleser, 2004). Uniformity in the biological responses to limiting conditions among individuals of different ages is a desirable characteristic in tree species used in dendrochronology, allowing their long-term environmental assessment (Fritts, 2001; Esper *et al.*, 2008). However, studies performed in multi-aged conifer populations have shown that tree-ring growth in younger trees is less sensitive to limiting climatic conditions compared with that in older trees (Szeicz & MacDonald, 1994; Carrer & Urbinati, 2004; Yu *et al.*, 2008). These results are not unexpected because the physiological processes controlling carbon assimilation and growth rate in a tree change according to its size and age (Day *et al.*, 2002; Mencuccini *et al.*, 2005; Ryan *et al.*, 2006).

Age-dependent climatic responses have been attributed to two different physiological mechanisms. Both hypotheses, proposing either increased hydraulic resistance with increasing

length of the hydraulic path (Carrer & Urbinati, 2004) or the delayed basipetal movement of growth hormones in taller trees (Rossi *et al.*, 2008), are more related to increased size than to prolonged lifespan. These hypotheses were derived from studies performed on tall conifers (*Larix decidua*, *Pinus cembra* and *Picea abies*) near the alpine timberline and suggest that, in this temperature-limited environment, age-dependent sensitivity is size-dependent, predicting a lower climatic response in younger/smaller trees than in older/larger individuals.

Sexual dimorphism in the life histories of dioecious plants is a consequence of different requirements for disseminating pollen and producing seeds and fruits. The females of woody dioecious plants usually allocate proportionally more resources to reproduction and less to maintenance and growth than do males (Nicotra, 1999; Obeso, 2002; but see Bond & Maze, 1999; Vamosi & Otto, 2002). Different patterns of resource allocation could potentially induce sex-specific sensitivity of tree-ring growth to climate. Previous studies of the dioecious conifers *Juniperus communis* and *Austrocedrus chilensis* revealed no sex-related differences in the growth sensitivity or climatic response of the tree rings (Marion & Houle, 1996; Rovere *et al.*, 2003; Nuñez *et al.*, 2008). Consequently, a homogeneous climatic response has been assumed and no sex-related corrections are made in the reconstruction of past climatic variations based on tree rings from dioecious conifers (Esper *et al.*, 2002; Touchan *et al.*, 2003; LeQuesne *et al.*, 2006). Nevertheless, the tree rings of male Spanish juniper trees (*Juniperus thurifera*) were more sensitive to precipitation in the previous winter than were those of female trees, as described by Montesinos *et al.* (2006).

There is little information available on the relative importance of age and sex in determining the sensitivity of tree-ring growth to climatic variations in dioecious trees (Rovere *et al.*, 2003; Montesinos *et al.*, 2006). Understanding this variability is basic, not only to our understanding of age- and sex-related processes, but to any evaluation of the extent to which tree-ring series show homogeneous climatic sensitivity, or whether a bias is induced by the proportion of individuals of different ages or sex in a population. Our central hypotheses were that: the tree-ring growth of *J. thurifera* shows an increasing sensitivity to climate according to age; and radial growth of both sexes is identically influenced by limiting climatic factors.

Materials and Methods

Study species

Juniperus thurifera L. is a dioecious long-lived tree, endemic to the western Mediterranean basin, with its most important populations growing under continental and cold climatic conditions in Spain and Morocco. No dendroclimatic studies of *J. thurifera* in central Spain have been published, but a previous study from the French Alps demonstrated that its radial growth is mainly controlled by winter precipitation of snow, by spring temperature, and by summer water stress (Bertaudière *et al.*, 1999).

Study area

The selected study area is a 3300 ha woodland located in the Sierra de Cabrejas, 30 km west of the city of Soria, north-central Spain (41°46′N, 02°49′W; 1100–1300 m altitude). This area constitutes a typical example of juniper woodland, with a composition and structure that are widespread in north-central Spain. The parent rock is Cretaceous limestone and the soils are calcium-rich and mainly shallow, with abundant areas of exposed bedrock covered with lichens and bryophytes. *Juniperus thurifera* forms an open woodland, with a mean density of over 300 trees ha^{-1}, intermingled with some pines (*Pinus sylvestris* and *P. pinaster*), holm oak (*Quercus ilex*), and a variety of evergreen sclerophyllous shrubs. The woodland understorey and open areas are covered by species-rich xeric grassland. The demographic structure is related to past traditional management based on livestock ranging and selective tree logging (Olano *et al.*, 2008; Rozas *et al.*, 2008).

The climate is continental Mediterranean, typical of the supra-Mediterranean belt in central Spain (Rivas-Martínez & Loidi, 1999). The climatic data used in this study for mean monthly temperature and total monthly precipitation were obtained from the Soria meteorological station (41°46′N, 02°28′W; 1082 m altitude), for the period 1944–2004. The coldest month in the study area is January, with a mean minimum temperature of –1.8°C, and the warmest is July with a mean maximum temperature of 28.1°C (Fig. 1a). The mean monthly rainfall ranges from 29.2 mm in August to 58.9 mm in May. We estimated the water balance (WB), calculated as WB = P – PET, where P is precipitation and PET is potential evapotranspiration, estimated as a function of the monthly mean temperatures and geographical latitude (Thornthwaite, 1948). The mean monthly WB ranged between –93 mm in July and 40.5 mm in December, showing a marked tendency to water deficit from June to September (Fig. 1b).

Sampling

The complete woodland was divided on a regular grid of 350 × 350 m. During winter 2004–05, a circular plot of 32 m of diameter was established at every knot of this grid. At 107 plots, a *J. thurifera* tree with diameter at breast height (DBH) > 7.5 cm was felled, after its DBH had been measured and its sex registered. The tree located closest to the central point of the plot was usually selected for felling. At a few plots, the second or third tree nearest to the central point was selected because of severe damage to or hollowing of the more central trees. The total tree height was measured on the felled trees and stem discs were obtained from each felled stem at 30 cm above the ground, to estimate the tree age, and at 130 cm for the analysis of tree-ring series.
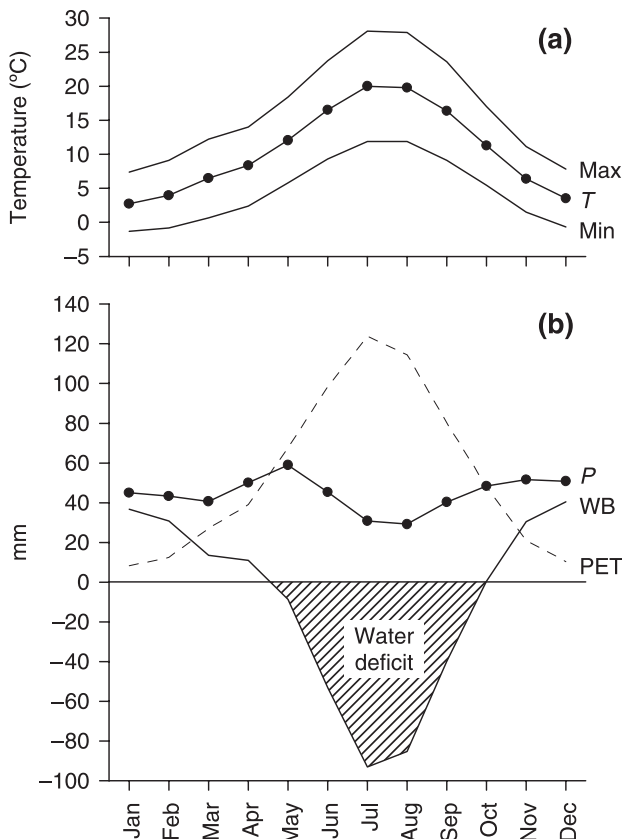
Fig. 1 Climatic diagrams from the Soria meteorological station for the period 1944–2004. (a) Mean monthly maximum (Max), mean (T), and minimum (Min) temperatures. (b) Monthly precipitation (P), potential evapotranspiration (PET), and water balance (WB = P – PET).

Sample processing and tree-ring measurement

The stem discs were mechanically surfaced and then manually polished with a series of successively finer grades of sandpaper until the xylem cellular structure was clearly visible. As in other species of the family Cupressaceae, most of the sampled sections had eccentric and lobate growth, usually associated with strip-bark formation and partial cambial dieback (Kelly *et al.*, 1992; Esper, 2000). Two radii from each stem disc taken at 130 cm were selected along lobes with active growth, where the absent rings were more easily identified and computed in the dating. Two series per disc were dated by assigning calendar years to the rings through the identification of characteristic ring sequences (Yamaguchi, 1991). The total ring widths were measured to the nearest 0.001 mm using a Velmex sliding-stage micrometer interfaced with a computer. The computer program COFECHA (Grissino-Mayer, 2001) was used to quantitatively check the cross-dating errors. Tree age was estimated as the number of rings counted on the basal discs.

Chronology computation and assessment

Of the 107 sampled trees, we selected for this study 50 female and 50 male trees whose ring-width series covered the complete period of analysis, 1945–2004. We discarded seven trees because their sex could not be properly assigned. Each raw ring-width series was standardized with the ARSTAN computer program (Cook & Holmes, 1996). The series were fitted to a spline function with a 50% frequency response of 32 yr, which was flexible enough to reduce the nonclimatic variance by preserving high-frequency climatic information (Cook & Peters, 1981). The residuals obtained were pre-whitened by autoregressive modelling, giving dimensionless indices that represent independent records of annual growth for each measured series. The year-by-year arithmetic mean of the two series of standardized and pre-whitened tree-ring indices was calculated to obtain an individual growth chronology for each tree.

To compute the average tree-ring climatic response for trees stratified by age, we classified the 100 individuals into five age classes (< 101 yr, 101–150 yr, 151–200 yr, 201–250 yr, and > 250 yr) and calculated the tree-ring chronologies for these different classes separately. The indices for individual series were averaged by calculating robust biweight means, and the mean chronologies were subsequently pre-whitened by autoregressive modelling with ARSTAN (Cook & Holmes, 1996). The statistical quality of the chronologies was assessed using standard basic statistics (Briffa & Jones, 1990; Fritts, 2001). These statistics refer to a single series as the first-order auto-correlation (AC) and the mean sensitivity (MS), and to the common signal as the mean between-trees correlation (r_{bt}) and the variance explained by the first principal component (PC1). AC indicates the serial statistical dependence among tree-ring indices, MS describes the mean percentage change from each growth index to the next, r_{bt} is the mean value of all possible correlations between individual series, and PC1 indicates the percentage of year-to-year growth variation shared by trees of the same age class.

Data analysis

The growth-climate relationships for the period 1945–2004 were calculated by taking the monthly temperature and precipitation records as climatic predictors, and the residual index chronologies as the dependent variables. The temporal window for climatic predictors was taken from October of the year before ring formation to September of the year of ring formation. We used response function analysis to estimate the variation in the ring-width indices with multiple regression, after extracting the principal components of the climatic predictors to avoid intercorrelations between them (Fritts, 2001). Calculations were performed with the program PRECON (Fritts *et al.*, 1991), which includes a bootstrap procedure to assess the statistical significance of the regression coefficients.

	Age class				
	< 101 yr	101–150 yr	151–200 yr	201–250 yr	> 250 yr
Number of trees/series	21/42	33/66	19/38	18/36	9/18
Number of females/males	10/11	13/20	12/7	7/11	8/1
Mean diameter ± SD (cm)	11.4 ± 3.1	19.2 ± 7.0	20.9 ± 6.3	25.5 ± 8.1	27.2 ± 9.7
Diameter range (cm)	7.7–18.2	9.5–37.6	9.7–30.0	15.1–41.8	14.0–41.2
Mean height ± SD (m)	4.7 ± 0.8	6.4 ± 1.6	6.4 ± 1.2	6.9 ± 1.3	5.4 ± 1.2
Height range (m)	3.6–6.7	3.4–10.8	4.8–8.7	3.8–8.6	4.2–7.5
AC	0.146	0.316	0.235	0.357	0.321
MS	0.193	0.154	0.155	0.177	0.169
r_{bt}	0.271	0.250	0.193	0.292	0.300
PC1 (%)	32.63	29.69	29.26	36.68	38.39

Table 1 Characteristics of *Juniperus thurifera* trees classified in five age classes and statistics for the derived age-class chronologies

The chronology statistics were calculated for the period 1945–2004. Chronology statistics include first-order autocorrelation before autoregressive modelling (AC), mean sensitivity (MS), mean between-trees correlation (r_{bt}) and the variance explained by the first principal component (PC1).

We performed 10 000 bootstrap iterations (Manly, 1997), and each regression coefficient was considered to be significant ($P < 0.05$) if it was at least twice, in absolute value, its bootstrapped standard deviation ($| r/sd | \geq 2$; Guiot, 1991). Response function analysis was performed on the five age-class chronologies, and also on each of the 100 individual chronologies to assess the individual climatic responses.

To ascertain age- and sex-related effects, we used two-factor analysis of variance (ANOVA; SPSS 13.0 for Windows, SPSS Inc., 2004, Chicago IL, USA) to compare the individual r/sd ratios, the r^2-adjusted coefficients, and the tree heights between age classes, sexes and their interactions. In all cases, the data met the assumptions of normality and homocedasticity. We compared the individual r/sd ratios and the r^2-adjusted coefficients between sexes within a given age class by means of the t-test for paired comparison between means. We used linear regression to explore the dependence of the individual r/sd ratios, the r^2-adjusted coefficients, and the tree heights on tree age for each sex.

Differences between the sexes in mean ring width were tested in the period 1945–2004 using a linear mixed model with a first-order autoregressive covariance structure (SPSS 13.0 for Windows), including tree age as a covariate, which accounted for the autocorrelation of the ring width series considered as repeated measures. We used a t-test to compare the slope of the relationships between summer precipitation and growth indices between sexes for each age class.

Results

### Age-class growth variation and response

Tree size and the proportion of sexes showed great variability within each of the five age classes considered, with a maximum diameter of 41.8 cm and maximum height of 10.8 m (Table 1).

The Pearson's correlation coefficients of the five age-class chronologies varied between 0.503 and 0.860 ($P < 0.001$ in all cases), supporting a consistent shared signal among age classes in the common period 1945–2004. The chronology statistics suggested variable growth behaviour among the different age classes (Table 1). Serial autocorrelation was lowest in trees younger than 101 yr, which indicates that environmental factors more strongly affected their growth. The chronologies of trees younger than 101 yr and older than 200 yr showed the highest values for MS, r_{bt}, and PC1 (Table 1), suggesting great high-frequency variation (MS) and that a large amount of interannual growth variation was shared by the trees (r_{bt} and PC1) in these age classes.

The adjusted coefficients of determination from the response functions revealed an overall age-related decreasing trend in the climatic sensitivity of the five age classes. Climate explained 54.6% of the growth variation in trees younger than 101 yr, 52.2% in trees of 101–150 yr, 46.4% in trees of 151–200 yr, 46.2% in trees of 201–250 yr, and 38.9% in trees older than 250 yr (Fig. 2). The response functions showed that *J. thurifera* growth was mainly influenced by winter precipitation (negative effects in December and March), summer temperatures (negative effects in June) and summer precipitation (positive effects in June and July). A greater sensitivity of trees younger than 101 yr to precipitation in June and July was evident, whereas the oldest trees were more sensitive to summer temperatures (Fig. 2).

Individual growth responses

Two-factor ANOVA performed on individual response functions revealed a significant effect of age on the r/sd ratios for June and July precipitation, the r^2-adjusted coefficient, and tree height (Table 2). Significant differences between female and male trees were observed in the r/sd ratio for July
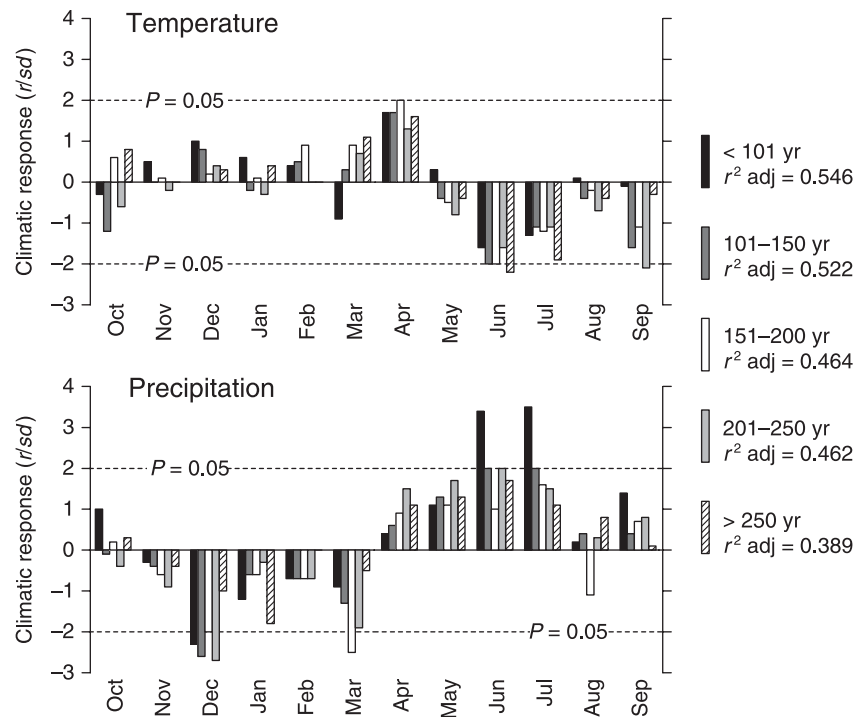
Fig. 2 Response functions expressed as regression coefficients divided by their bootstrapped standard deviations (r/sd) for the five age classes of *Juniperus thurifera* trees in the period 1945–2004. The adjusted coefficients of determination (r^2adj) for the response functions are shown. Dashed lines indicate the significance thresholds ($P = 0.05$) for the r/sd values.

Table 2 Summary of the F-values from two-factor ANOVA of the differences between *Juniperus thurifera* age classes and sexes in r/sd ratios for temperature (T) in April and precipitation (P) in June and July, r^2-adjusted coefficients, derived from individual response functions in the period 1945–2004 and tree height

| Factors	df	r/sd T April	r/sd P June	r/sd P July	r^2-adjusted	Tree height
Age	4	0.775	4.162**	5.413***	3.118*	8.195***
Sex	1	0.146	0.244	6.798*	4.843*	0.639
Age × sex	4	2.497*	2.826*	2.506*	2.798*	0.901
Model	9	1.583	3.339***	4.635***	2.911**	4.289***

Variables not presented showed no significant differences.
*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

precipitation and the r^2-adjusted coefficient. Moreover, a significant age × sex interaction was observed for the r/sd ratios for April temperature, and June and July precipitation, and the r^2-adjusted coefficient, suggesting that the dependency of the climatic response on age was not the same for female and male trees. However, according to the two-factor ANOVA, the r/sd ratios for temperature and precipitation in the other months showed no significant effects of age, sex, or the age × sex interaction ($P > 0.05$ in all cases).

An evident sex-related climatic sensitivity emerged from the analysis of the individual responses to climate, but only for trees younger than 101 yr (Fig. 3). According to the *t*-test for differences between means, the mean r/sd ratios for temperature in April and precipitation in June and July, and also the mean r^2-adjusted coefficients, were significantly greater for female trees than for male trees. Among the mean r/sd ratios, those for precipitation in June and July were only significant

for female trees (mean $r/sd > 2$; $P < 0.05$). However, no differential climatic response between the sexes was observed for trees older than 101 yr (*t*-test for differences between means, $P > 0.05$).

Female trees showed significant negative relationships between age and the r^2-adjusted coefficients (Fig. 4a) and the r/sd ratios for April temperature (Fig. 4b), whereas no significant relationships between these parameters were observed for male trees. The r/sd ratios for precipitation in June and July were only significantly related to tree age for trees younger than 150 yr. Both female and male trees showed negative relationships between the r/sd ratios for June precipitation and tree age (Fig. 4c), whereas only female trees showed a negative relationship with July precipitation (Fig. 4d). Furthermore, both sexes showed positive relationships between tree height and age in trees younger than 150 yr (Fig. 4e). By contrast, no relationships between individual r^2-adjusted coefficients and
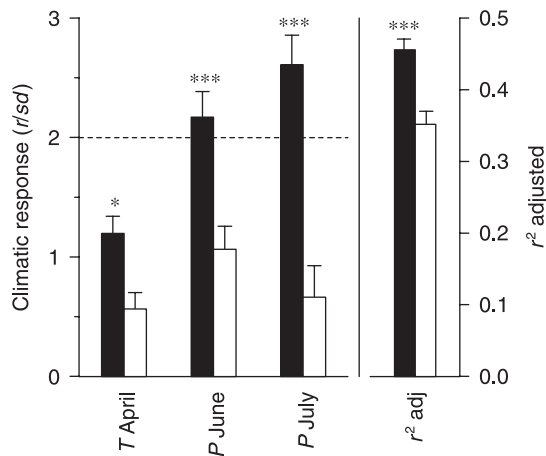
Fig. 3 Mean (+ 1 SE) *r/sd* ratios and r^2-adjusted coefficients calculated for individual response functions between monthly temperature and precipitation and the residual tree-ring series of female (closed columns) and male (open columns) *Juniperus thurifera* trees younger than 101 yr. The dashed line indicates the significance threshold ($P = 0.05$) for *r/sd* values. The significance of the *t*-tests for differences between means is shown: *, $P < 0.05$; ***, $P < 0.001$. Variables not presented (*r/sd* ratios for temperature in October–March and May–September, *r/sd* ratios for precipitation in October–May, August, and September) were not significantly different.

tree height were found (Fig. 4f), suggesting that climatic sensitivity is independent of tree size, in terms of height.

Sex-specific differences in growth and sensitivity

According to the linear mixed models, females younger than 101 yr showed larger ring widths than those of males (mean ring width ± SD = 1.07 ± 0.62 mm for females, 0.81 ± 0.49 mm for males; $F = 5.094$, $P = 0.037$; Fig. 5a), whereas the mean ring widths did not differ significantly between the sexes in age classes older than 101 yr ($P > 0.05$ in all cases; Fig. 5b,c).

In trees younger than 101 yr (Fig. 5d), the sensitivity to summer precipitation differed between females and males (age × sex effect; *t*-value = 2.193, $P = 0.0303$). Females showed a stronger linear relationship between tree-ring indices and June–July precipitation ($r = 0.691, P < 0.0001$) than that of males ($r = 0.394, P = 0.0018$). However, in age classes older than 101 yr, both sexes responded quite similarly to summer precipitation (Fig. 5e,f), with no difference in the regression between the sexes (age × sex effect; 101–150 yr: *t*-value = −0.511, $P = 0.6104$; > 150 yr: *t*-value = 0.296, $P = 0.7676$).

Discussion

#### Responses of *J. thurifera* tree-ring growth to climate

Juniperus species have adaptive architectural features, such as extensive lateral root systems, large root/shoot ratios, and

tracheids with small diameters (Krämer *et al.*, 1996; Martínez-Vilalta *et al.*, 2002; Willson *et al.*, 2008), which confer high resistance to drought-induced xylem embolism and enhance their ability to persist in dry environments. The radial growth of *J. thurifera* in the study area occurs predominantly in June, but the growing period can extend until July when rain occurs during this month (J. J. Camarero, unpublished). Despite their adaptive features to drought, water deficit during the growing period is the main climatic factor constraining the growth of *J. thurifera*, as in other Mediterranean tree species (Cherubini *et al.*, 2003; Touchan *et al.*, 2003; LeQuesne *et al.*, 2006). The radial growth of the studied population was also limited by the intensity of winter snow precipitation, as in other mountain environments (Bertaudière *et al.*, 1999), and relatively high air temperatures in April enhance growth, probably through earlier cambium reactivation (Begum *et al.*, 2008).

Age-dependent sensitivity to climate

Although the existence of an age-related trend in tree growth sensitivity to climate has been refuted by several authors (Colenutt & Luckman, 1991; Fritts, 2001; Esper *et al.*, 2008), there is clear evidence of changes in climatic sensitivity with tree aging (Szeicz & MacDonald, 1994; Carrer & Urbinati, 2004; Yu *et al.*, 2008). These studies established that, in temperature-limited environments, tree-ring growth sensitivity increases with age, with older trees showing a stronger relationship to climate. It has been suggested that this may be the result of more stressful conditions for older trees (Carrer & Urbinati, 2004) and/or a shorter growing period (Rossi *et al.*, 2008). Our results underline the existence of a shift in the biological response of tree-ring growth during tree ontogeny, but, in contrast with previous works, we have shown that climatic sensitivity is higher in earlier life stages. In the previously published literature, higher climatic sensitivity has been interpreted as less efficient resource use, mainly resulting from increasing hydraulic resistance that causes lower transpiration and photosynthetic rates (Esper *et al.*, 2008; Yu *et al.*, 2008). However, higher sensitivity could be also interpreted as a more opportunistic behaviour, probably explained by age-related changes in water-use efficiency. Our data suggest that young trees are able to grow more than old trees under favourable circumstances (i.e. wet summers) and less under unfavourable conditions (i.e. dry summers).

Two different, but not mutually exclusive, physiological mechanisms have been proposed to explain the age-dependent climatic responses. First, the hydraulic limitation hypothesis (Ryan & Yoder, 1997) has been proposed to explain the greater sensitivity of older conifers to climate (Carrer & Urbinati, 2004), attributed to an increase in the hydraulic resistance of the root–leaf continuum as the trees grow taller. The second hypothesis proposes that active xylem growth in older conifers is initiated later and ends earlier than in younger trees because
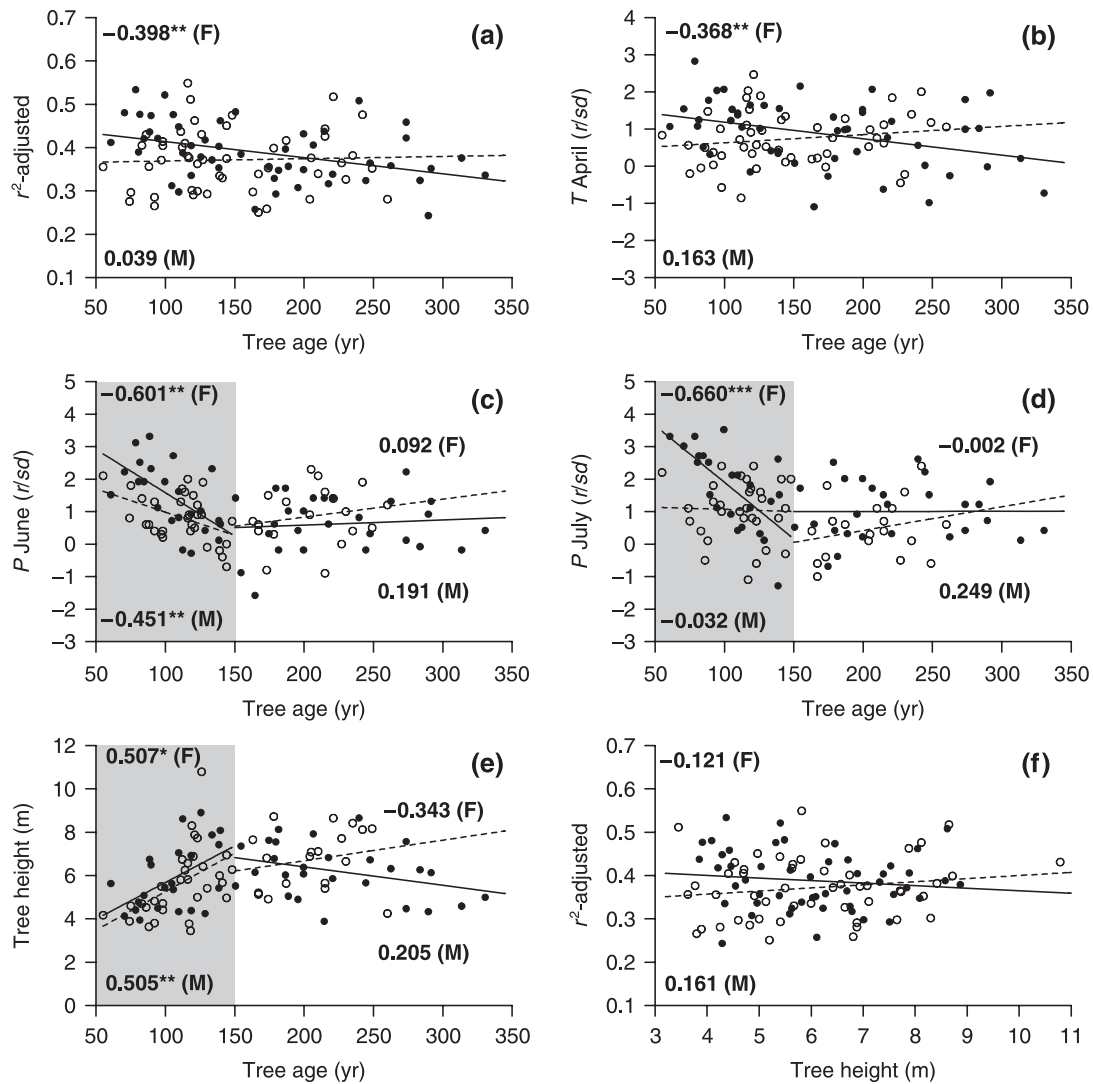
Fig. 4 Tree age relationships to the r^2-adjusted coefficients for individual response functions (a), r/sd ratios for T (temperature) April (b), P (precipitation) June (c) and P July (d), and tree height (e) for female (closed circles, solid line) and male (open circles, dashed line) *Juniperus thurifera* trees. For P June, P July, and tree height, trees younger and older than 150 yr are differentiated. Tree height relationships to the r^2-adjusted coefficients for individual response functions (f). The linear relationships and their correlation coefficients (F for female, M for male) are shown: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

of the delayed basipetal movement in taller trees of the hormones that induce cambium activity (Rossi *et al.*, 2008). According to these interpretations, the climatic sensitivity of tree-ring growth consistently increases during the juvenile phase of exponential height growth, and the maximum sensitivity is reached when the tree reaches its maximum height limit. An experimental approach that distinguished between age- and size-related reductions in growth and assimilation rates concluded that carbon uptake and tree growth are mainly controlled by size, whereas cellular senescence *per se* is not relevant (Mencuccini *et al.*, 2005). However, our results suggest that the climatic sensitivity of *J. thurifera* tree rings is independent of size (Fig. 4f) and is actually age-dependent. The low height of *J. thurifera* trees in comparison to other

conifers would partially explain why its climatic sensitivity is not related to tree size. Our strictly regular sampling design over an extensive woodland guarantees that these results are not biased by possible associations of a given age class with a more favourable environment or topographical position.

Juniperus thurifera trees experience a reduction in their sensitivity to summer water deficit when they are older than 100 yr. This may be partially attributable to several causes, such as: a reduction in height growth rate, which implies a reduction in the demands for resources and water (Carrer & Urbinati, 2004); a reduction in the duration of the xylogenetic period, which reduces the trade-off between vegetative growth and reproduction–defence (Rossi *et al.*, 2008); or the development of a more efficient root system, which allows a
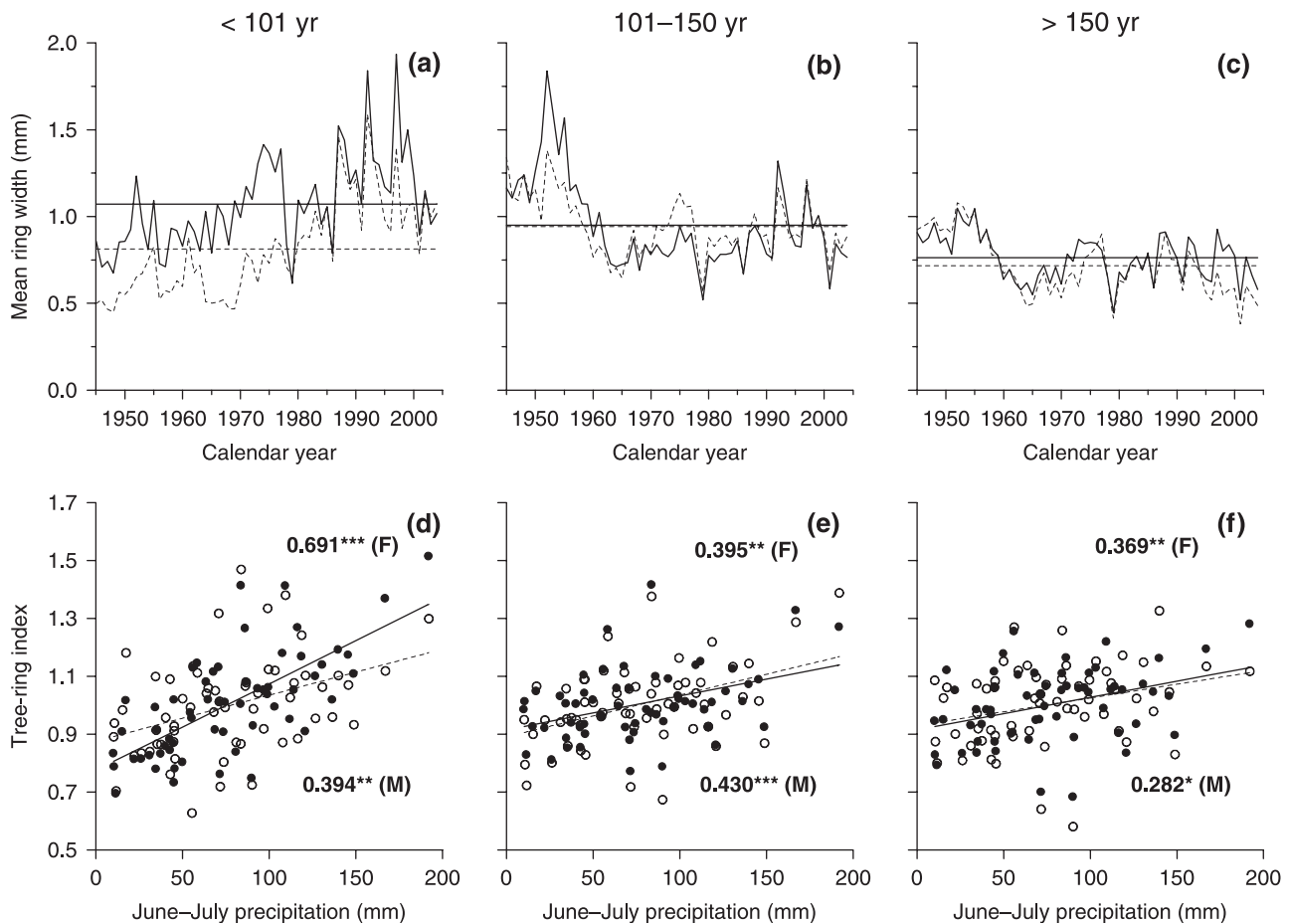
Fig. 5 Temporal variation in the mean ring widths for *Juniperus thurifera* trees classified by age classes (younger than 101 yr (a), between 101 and 150 yr (b), and older than 150 yr (c)) and sex (female, solid line; male, dashed line). The horizontal lines indicate the overall means in the period 1945–2004. Relationships between the tree-ring growth indices and precipitation in June–July for the female (closed circles) and male (open circles) trees younger than 101 yr (d), between 101 and 150 yr (e), and older than 150 yr (f) in the period 1945–2004. The linear relationships and their correlation coefficients (F for female, M for male) are shown: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

high water absorption capacity (Krämer *et al.*, 1996). Relatively strong allometric relationships between above-ground plant size and root system dimensions are attributed to the fact that large plants need more roots to supply a greater leaf area with water (Schenk & Jackson, 2002). In water-limited environments, the lateral root spread of woody plants tends to be strongly related to the above-ground plant size (Magnani *et al.*, 2002), which suggests that the poor development of the lateral roots in young/small trees would exacerbate water stress during the drier season.

When the tree density in the studied woodland is low, the trees are spatially arranged in clumps (L. DeSoto, unpublished data), and competition with neighbours for soil water resources probably plays a significant role in the early ontogenetic stages. Below-ground competition is assumed to be intense in water-limited environments as a result of large lateral root spread (Schenk & Jackson, 2002; Casper *et al.*, 2003). The above-ground size of *Juniperus* is a good predictor of below-ground parameters such as root biomass and lateral root extension

(Krämer *et al.*, 1996), suggesting that the pattern of increasing height and decreasing sensitivity to summer drought (Fig. 4c–e) may result from the progressive development of the root system. The development of intraspecific root grafts is prevalent in nature and implies the transfer of water and minerals through xylem connections and carbohydrates through phloem connections (Fraser *et al.*, 2006). We hypothesize that the growth of the lateral roots and root grafting during tree development might partly explain the age-related reduction in *J. thurifera* sensitivity to summer water deficit.

Sex-related sensitivity to climate

A major finding of this study is the importance of sex to climatic sensitivity, and especially the evident interactions between age and sex (Table 2). In females, age-related variation in the sensitivity of tree-ring growth to different climatic factors was evident from the adjusted coefficients of determination for the response functions, and the response to April temperature

and June–July precipitation. A trade-off between growth and reproduction has been repeatedly predicted to be a consequence of the long-term differential reproductive cost between the sexes (Obeso, 2002). Previous studies of *J. thurifera* demonstrated higher growth rates in males, interpreted as the delayed cost of reproduction in females (Gauquelin *et al.*, 2002; Montesinos *et al.*, 2006). Other studies found no sex-related differences in the growth rates of dioecious conifers (Marion & Houle, 1996; Rovere *et al.*, 2003), even when the reproductive effort of the females was considerably greater than that of the males (Ortiz *et al.*, 2002). Differential sex-specific investment in vegetative growth was observed in our study in trees younger than 101 yr, with higher growth rates in females than in males (Fig. 5a). This result is consistent with the finding that young females had larger growth indices in wet summers than those of males (Fig. 5d). The correspondence between higher growth rates in females and higher sensitivity to summer precipitation suggests that the differential growth between the sexes had a physiological origin but is intensified, at least partly, by climate.

The existence of physiological differences between the sexes has been reported for photosynthetic rates (Correira & Diaz Barradas, 2000; Wheelwright & Logan, 2004), water-use efficiency (Leigh & Nicotra, 2003) and phenology (Espírito-Santo *et al.*, 2003). Different studies describing lower tolerance to drought (Dudley, 2006), higher growth rates, and lower $\delta^{13}C$ isotope ratios (Ward *et al.*, 2002) in females suggest less conservative water use in females than in males. Recently, experiments that compared the physiological responses to water stress in clones of *Salix* and *Populus* showed that male clones were most physiologically plastic, whereas the female clones were more responsive and suffered greater negative effects when grown under drought stress (Dudley & Galen, 2007; Xu *et al.*, 2008). The sex-specific sensitivity to limiting climatic factors demonstrated in this study supports the hypothesis that female and male trees differ in their performance across gradients of abiotic stress (Nuñez *et al.*, 2008), at least for young trees. The females' growth is more strongly affected, both positively and negatively, by water availability in summer. Consequently, in drier environments, higher growth rates might be expected for males (Montesinos *et al.*, 2006; Nuñez *et al.*, 2008).

The higher sensitivity of females may also be related to a diversion of resources during the reproductive period (Leigh & Nicotra, 2003). In fact, studies of the temporal variation in resource allocation to reproduction in dioecious plants usually report greater resource investment by males at the beginning of the reproductive period, whereas females tend to assign greater amounts of resources later in the season to sustain fruit formation (Espírito-Santo *et al.*, 2003). Flowering in *J. thurifera* occurs in February and the growth and ripening of female cones take up to 20 months (Montesinos *et al.*, 2006), but most increase in the size of the cones occurs in the early summer (J. M. Olano, pers. obs.). The investment of resources in cone

growth may partly contribute to the differential sex-specific sensitivity to July water availability (Fig. 4d). We hypothesize that the less conservative water use in females, combined with a certain differentiation in phenology, is important in determining sex-specific sensitivity to summer drought. The development of lateral root systems with a well-balanced root/shoot ratio, and the transference across root grafts of water, minerals, carbohydrates and growth regulators among neighbouring trees, independent of their sex, may partially explain the convergent climatic sensitivity of female and male *J. thurifera* trees when these reach 100 yr of age (Schenk & Jackson, 2002; Fraser *et al.*, 2006). A broad understanding of the architectural and physiological basis of climatic sensitivity differentiaie in dioecious trees is required.

Acknowledgements

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