



Original article

Relictual distribution reaches the top: Elevation constrains fertility and leaf longevity in *Juniperus thurifera*

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ABSTRACT

Juniperus thurifera populations are scattered throughout the western Mediterranean basin and are relictual from its Tertiary distribution due to progressive climatic warming since the last glacial period. To disentangle the factors responsible for its extremely low fertility we combined a microscale experimental design with a macroscale study. At the microscale we experimentally alleviated environmental stress by watering and fertilizing during two years a set of trees in one population. At macroscale we selected 11 populations across a geographical range and sampled them for three years.

Macroscale patterns evidenced that both plant fertility and leaf longevity diminished with increasing elevation. Both microscale and macroscale illustrated the importance of water and nutrient availability on leaf growth and plant fertility: On the microscale experiments, regular supply of water and nutrients increased fruit-set by 300%. Macroscale showed that increases in resource availability (precipitation) resulted in reductions of seed abortion, although paralleled by increases in seed predation.

Altogether, our results indicate that fertility is constrained both by elevation and by resource limitation. Therefore any potential lift in the elevational distribution limits will result in synergistic fertility reductions due to harder physical conditions and lower water and nutrient availability. Both will compromise future regeneration of this relictual species, although population decline might be buffered temporary thanks to longevity of adult trees.

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1. Introduction

Elevational migration of high mountain flora is a common response to environmental warming (Grabherr et al., 1994; Klannerud and Birks, 2003; Walther, 2003). Warming of the environmental conditions increases the elevation of both lower and upper plants distribution limits. Increase of lower distribution limits of a species is not normally due to heat stress but rather to an increase of the upper elevational limit of competitor species with faster growth rates (Loehle, 1998; Walther, 2003; Alward et al., 2006). On the other hand, to increase the elevation of their upper distribution limits plants have to face a set of increasingly harsh environmental factors characteristic of higher elevations, like lower CO₂ and O₂ partial pressure, higher UV, IR and visible radiation, and sometimes shorter growth seasons, stronger winds and more frequent drought events (Barceló et al., 1992; Körner, 2003, 2007; De Lillis et al., 2004).

Ultraviolet radiation from the sun is considered to be particularly intense at high elevations, increasing by 25% from 200 m to 1500 m (Caldwell, 1968). In addition, reduction of stratospheric ozone due to human release of chlorofluorocarbons to the atmosphere has increased UV radiation by 6–14% (UNEP, 2002). Although some plant species have the ability to adapt to high UV levels (Teramura and Sullivan, 1991), UV radiation can be a significant stress factor for leaves (Caldwell, 1968; Robberecht and Caldwell, 1980; Teramura and Sullivan, 1991) resulting in reduced leaf lifespan and leaf growth (Ortiz et al., 2002) and reducing flowering frequency and whole plant fertility (Caldwell, 1968; Koti et al., 2005).

Altogether high mountain conditions can inhibit photosynthetic activity (Kofidis et al., 2003) and reduce net primary productivity (Luo et al., 2004). Under such unfavorable circumstances reproductive allocation tends to decrease with increasing elevation (Ortiz et al., 2002; Obeso, 2002; Sakai et al., 2006) and sometimes plants mitigate their reproductive effort by producing low quality seeds (Houle and Barbeux, 1994). Seed predator species can also migrate to higher elevations due to temperature increases, and elevate their elevational distribution faster than plants do, increasing predation rates of previously unaffected populations and species (Hódar and Zamora, 2004).

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Juniperus thurifera (Cupressaceae) is a dioecious tree inhabiting semiarid cold environments of the Holarctic region and originated during the Tertiary (Suárez Cardona et al., 1991). It attained its maximum distribution during the cold periods of the Pleistocene, since then, progressive warming has reduced its distribution to scattered populations restricted to the high-mountains of the western Mediterranean Basin (Terrab et al., 2008). Therefore its present day habitats represent a compromise between environmental harshness and low presence of competitor species (Gómez-Manzaneque, 1997). Livestock has controlled competition traditionally, but land abandonment has reduced grazing in recent times, leading to increased interspecific competition (DeSoto et al., 2009). Like many junipers, *J. thurifera* presents low seed viability rates (e.g. Roques et al., 1984; García, 1998). A positive relationship between *J. thurifera* vegetative growth and current year precipitation was already known (Montesinos et al., 2006). However, geographical and environmental factors responsible for junipers' low fertility are unclear (García et al., 2000). Juniper seed viability can be limited by environmental harshness (García et al., 2000; Ortiz et al., 2002; Wesche et al., 2005), nutrient and water availability (Stephenson, 1981; García et al., 1999; Drenovsky and Richards, 2005) and pollination failure (Ortiz et al., 2002), although experimental manual pollinations indicate that pollen is not limiting *J. thurifera* fertility (Montesinos unpublished data). Junipers typically present a very high proportion of parthenocarpic seeds (Fuentes, 2000) and moderate to high rates of abortion and predispersal seed predation (Rappaport et al., 1993; García, 1998; El Alaoui El Fels et al., 1999). Explanations for parthenocarpy include frost damage to the ovule, nutrient limitation and defense against seed predators (Traveset, 1993; Verdú and García-Fayos, 1998 and references therein).

In this work we try to elucidate factors responsible for *J. thurifera*'s present day low fertility and to infer the reproductive consequences of an upward migration as a response to a hypothetical rise in temperature. We studied variation in seed fate (viability, abortion, predation and parthenocarpy), leaf longevity and leaf growth at two different scales of environmental variation: at macroscale, by sampling 11 populations along a latitudinal range across the Iberian Peninsula; and at microscale, by experimentally modifying water and nutrient availability on a set of individuals in one population.

2. Material and methods

2.1. Study species

J. thurifera lives in the calcareous high-mountains of the western Mediterranean Basin. It is found in the Alps (France and Italy), across the mountains of the Iberian Peninsula (Spain and French Pyrenees), in the Atlas Mountains (Morocco) and in two small populations in Corsica and Algeria (Gauquelin et al., 1999). Together with two closely related species with similar biology (*Juniperus excelsa* and *Juniperus procera*) they are scattered all along the Mediterranean basin, western Asia and eastern Africa. It forms low-density pure stands or mixed open forests with *Quercus ilex*, *Quercus faginea*, *Pinus nigra*, *Pinus sylvestris* and *Pinus pinaster*. It is often found as a treeline edge species. Trees are 5–10 m high (exceptionally 20 m) and often live for centuries (Bertaudière et al., 1999). Cones take 22 months to develop and ripen and they contain on average 3.5 (1–7) seeds (Montesinos pers. obs. and Adams et al., 2003). Viability and germination of *J. thurifera* seeds is extremely low due to high rates of parthenocarpy, abortion and predation (Ceballos & Ruiz de la Torre, 1979; Melero and García-Fayos, 2001) and seedling recruitment is severely limited by drought and nutrient availability, which can be partially alleviated by sex-biased self-facilitation (Montesinos et al., 2007).

Fleshy cones are attacked by a variety of pests, but main seed predators are the chalcid *Megastigmus bipunctatus* (hereafter *Megastigmus*) and the mite *Trisetacus quadrisetus* (hereafter *Trisetacus*). *Megastigmus* oviposites on unripe seeds the first summer after pollination and larvae develop and grow for one more year before they emerge from the seed leaving a characteristic exit hole (Roques et al., 1984). *Trisetacus* oviposition occurs much earlier, often before pollination occurs. Mites live in colonies and use seeds as growth chambers often leading to the destruction of the totality of the seeds in a cone. Seeds affected by *Trisetacus* colonies present a characteristic brown "fibrous" morphology (Roques et al., 1984).

2.2. Study sites

From French Pyrenees to southern Spain eleven populations (see Fig. 1) were sampled for seeds during three years (2002–2004) and for leaf growth and longevity in 2004. Sampled populations cover a wide range of environmental characteristics (Table 1). In each population, we estimated tree density by counting every individual taller than 2 m in 10 randomly distributed transects each of 100 m long and 10 m wide.

Simultaneously, we randomly selected and permanently marked 20 female trees at population three (Puebla de S. Miguel, Valencia) for the microscale experiment of water and nutrient addition. This population corresponds to a *J. thurifera* forest with intermediate precipitation, temperatures and density in a typical calcareous soil (Table 1). From April 2003 to March 2005, half of the trees were watered and fertilized monthly, except in June and July, the driest months, when they were watered and fertilized fortnightly. This method alleviates resource limitation in the broad sense but does not allow for discrimination of the effect of a particular resource (i.e. the effect of water alone, or N or P alone). On each date every tree was supplied with 100 L of 200 ppm balanced fertilization solution (Universol Blue 18N:11P:18K plus oligoelements, Scotts, USA) according to manufacturer recommended concentrations. Water was distributed on 20 m² around trunks resulting in an approximate increase in precipitation of 50 L m⁻² year⁻¹. This resulted in a net yearly dose of 1000 L of water and 2 kg of fertilizer per tree.

2.3. Seed fate

We collected 10 ripe cones from each of 20 female trees randomly chosen each year across every population. Cones were

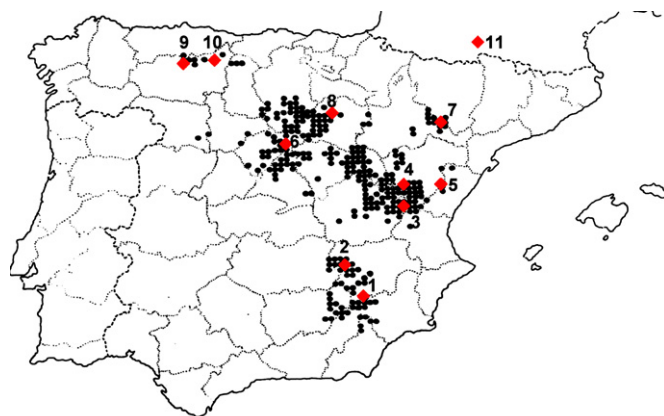


Fig. 1. *J. thurifera* Iberian distribution (dots, based on Gómez Manzaneque et al., 1993) and studied sites (diamonds): 1 – Nerpio; 2 – Ossa de Montiel; 3 – Puebla S. Miguel; 4 – Saldón; 5 – Portell de Morella; 6 – Hornuez; 7 – Purburrell; 8 – Calatañazor; 9 – Barrios de Luna; 10 – Crémenes; 11 – Mt. de Rie.

Table 1
Sampled populations and their environmental characteristics ordered by latitude (Mean \pm SD values).

Population	Latitude (nearest °)	Elevation (m a.s.l.)	Mean annual temperature (°C)	Mean annual precipitation (mm)	Tree density (trees/ha)	Branch growth (g) (2004) (Mean \pm SD)	Leaf longevity (years) (2004) (Mean \pm SD)
1. Nerpio	38°N	1285	14.6	466	690	4.7 \pm 2.29	2.26 \pm 0.42
2. Ossa de Montiel	39°N	1000	12.8	448	905	5.01 \pm 1.60	2.17 \pm 0.42
3. Puebla de S. Miguel	40°N	1500	12.6	535	423	4.62 \pm 1.70	2.03 \pm 0.47
4. Saldón	40°N	1420	10.8	470	596	5.2 \pm 2.63	2.71 \pm 0.23
5. Portell de Morella	41°N	1120	11.2	665	178	2.21 \pm 1.05	2.41 \pm 0.36
6. Hornuez	42°N	1130	11.4	510	350	5.96 \pm 2.39	2.51 \pm 0.50
7. Purburrell	42°N	350	15.2	360	104	6.1 \pm 2.30	3.23 \pm 1.62
8. Calatañazor	42°N	1100	9.8	700	429	3.69 \pm 1.03	2.44 \pm 0.44
9. Barrios de Luna	43°N	1200	9.4	848	267	6.29 \pm 2.01	2.46 \pm 0.39
10. Crémenes	43°N	1150	8.8	1274	327	8.42 \pm 3.75	2.38 \pm 0.48
11. Mt. de Rie	43°N	850	11.0	1050	925	–	–

collected from all around the tree to avoid orientation effects on seed viability and seed set. Ripe cones were collected in October 2002, 2003 and 2004. Seeds were cut to expose the embryo and visually assigned to one of five categories: viable seeds, aborted, parthenocarpic, preyed upon by *Megastigmus* and preyed upon by *Trisetacus*. Previous tests with 300 seeds showed that estimations of seed viability by visual identification and with tetrazolium tests did not differ (*t*-test comparing 3 + 3 sets of 50 seeds each; $t = 0.339$ $df = 4$, $p < 0.751$).

At the experimentally watered set of trees, three individual branches were marked in each marked tree and the total number of flowers was counted in January 2002, before experimental addition of water with fertilizer started. At the end of the experiment, in October 2004, fruit-set was estimated following the same procedure, and seeds collected to study viability.

2.4. Branch growth and leaf longevity

J. thurifera presents a year-long continuous branch growth occasionally limited by high temperatures in summer and low temperatures in winter (D. Montesinos pers. obs.). Natural defoliation due to age and shading from foliage (Montès et al., 2000) results in feather-duster like branches. In October 2004, we collected one branch from each of ten male and ten female trees from each of the 11 populations, and also from the experimental trees at population 3, by cutting them up to the defoliation point. Stems and leaves from the last year (bright green leaves on non-lignified stems) were manually separated from older stems and leaves (dark green leaves on dark lignified stems), dried at 60 °C for 48 h and weighed to the nearest 0.0001 g. Yearly branch growth was compared between populations comparing dry weight of last year growths. Leaf lifespan estimation method was inspired by the principles proposed by Mediavilla and Escudero (2003). The ratio between the dry weight of last year growth and the dry weight of growth from previous years is a convenient approximation to leaf longevity in scale-like leaved plants under the assumption that leaf growth is similar among years. This might be a difficult assumption in Mediterranean environments; however, it can be a useful approximation when used to compare qualitatively between watered and not watered plants.

2.5. Statistical analyses

Several types of analyses were used to suit different types of data at macro, and microscale and depending on the number of years available for each variable.

Linear Mixed Models (LMM) permit the study of time-series accounting for temporal autocorrelations among years; therefore they were used when data from more than one year were available.

LMM with an autoregressive covariance structure of order one and year as repeated measure were fitted to the data to analyze the effect of populations' environmental characteristics (elevation, latitude, mean temperature and precipitation, previous year precipitation and tree density) on each year's seed fate.

Linear Regressions were used to study the effect of different environmental factors (elevation, latitude, precipitation, etc.) on leaf longevity and leaf growth, from which data were obtained only once, at the end of the longevity study. Linear Regressions were also used to study the autocorrelation between the environmental factors.

Finally, for the microscale data, General Linear Models with Gaussian distribution of errors were fitted to the data to determine if seed viability, abortion, parthenocarpic, predation or fruit-set were affected by regular watering with fertilizer.

Statistical values throughout the text show the mean \pm SD. Statistical analyses were performed with SPSS 15.0.

3. Results

3.1. Seed fate

Seed viability averaged 9.5 \pm 8.8% (Mean \pm SD) among populations and presented a negative relationship with elevation. There were no other significant relationships between seed viability and any other environmental or geographical variable (Table 2).

Seed abortion averaged 19 \pm 11% among populations and correlated negatively with previous year precipitation (Table 2 and Fig. 2). Seed predation averaged 13.6 \pm 8.7% among populations. Two seed predators were identified: *Trisetacus* (6.3 \pm 10%) and *Megastigmus* (7.3 \pm 4.6%). The first did not show any relationship with climate while the second correlated positively with previous year precipitation (Table 2 and Fig. 2). The rate of parthenocarpic averaged 58.7 \pm 11.9% and did not correlate with any other variable. There were no significant relationships between mean number of seeds per cone, proportion of filled seeds or seed fate with any other environmental or geographical variable (data not shown).

Regarding the microscale watering and fertilizing experiment at population 3, seed fate rates were unaffected by watering and fertilization (Table 3) although watered and fertilized trees produced three fold more ripe cones per flower (0.28 \pm 0.19 cones/flower) than controls (0.09 \pm 0.05 cones/flower; $F = 6.3$; $df = 1$; $p = 0.028$). It is remarkable that the proportion of viable seeds was similar on both groups.

3.2. Branch growth and leaf longevity

Across the geographical range there were no significant differences between sexes on branch growth ($F = 0.228$; $df = 1$; $p = 0.639$)

Table 2

Macroscale evaluation of seed fate determinants. *F* values for the Linear Mixed Models (df = 1,20). **p* < 0.05; ***p* < 0.01.

	Viability	Abortion	Parthenocarpy	Predation by <i>Trisetacus</i>	Predation by <i>Megastigmus</i>
Elevation	9.00**	0.03	0.03	1.97	2.82
Latitude	1.46	2.65	0.09	0.09	0.07
Mean precipitation	1.47	2.23	0.02	0.06	1.48
Pp. prev. year	0.27	4.16*	1.75	0.15	8.4*
Mean temperature	0.38	1.40	0.22	0.39	1.11
Density	0.42	0.93	0.60	0.30	3.44
Sex ratio	0.06	0.73	1.92	1.08	0.27

or leaf longevity ($F = 0.755$; $df = 1$; $p = 0.396$) (Table 1). Since our study was focused on seed production, subsequent analyses on branch growth and leaf longevity considered only females' leaves. Branch growth did not present any correlation with any environmental or geographical factor; however leaf longevity was negatively correlated to elevation (Table 4).

Microscale experiments of watering and fertilization significantly increased branch growth from 2.8 ± 2.6 g to 4.7 ± 3.9 g ($F_1 = 6.15$; $p = 0.015$) although mean leaf longevity (2 ± 0.3 years) was not affected ($F_1 = 0.33$; $p = 0.57$).

3.3. Independence of factors

Regarding the independence of the environmental and geographical factors, elevation was not correlated with any other variable. However precipitation and temperature were significantly correlated with latitude, with higher precipitations and lower mean temperatures at northern latitudes (none of them affected seed viability). Mean annual precipitation and mean annual temperature were negatively correlated. Finally, tree density was independent of any other factor studied (Table 5).

4. Discussion

Elevation significantly reduced both seed viability and leaf longevity of *J. thurifera* trees, as expected for increasingly harsh environments (Caldwell and Robberecht, 1980; Teramura and Sullivan, 1991; Hemborg and Karlsson, 1998; Kofidis et al., 2003; Körner, 2003; Sakai et al., 2006) including light levels which may induce photoinhibition (Close et al., 2003) and low primary

productivity (Luo et al., 2004). Similarly, the congeneric *Juniperus communis* also experienced reductions in fertility and branch growth across a single-population elevational transect (Ortiz et al., 2002).

On the microscale, branch growth was increased by water and nutrient supply while leaf longevity was not, in concurrence with other studies (e.g. Jonasson et al., 1997) but see Aerts (1995). Combined, the data suggest that *J. thurifera* branch growth might be more affected by nutrient limitation than by water scarcity, while resource availability is not limiting leaf longevity. Differences in chemical composition in leaves and reproductive cones were likely to alleviate reproductive vs. growth trade-offs as previously suggested by Montès et al. (2002), facilitating increases in both branch growth and fruit-set when watered and fertilized.

It is remarkable that in our study, both at the microscale experiment and along the macroscale geographical range, parthenocarpy rate was high and regular ($58.7 \pm 11.9\%$) and was not correlated with any geographical or environmental variable. Trees experimentally supplied with extra water and nutrients maintained both high seed parthenocarpy and low seed viability rates, even though they increased fruit-set more than three fold. Across the macroscale geographical range, precipitation in a given year significantly reduced *J. thurifera* seed abortion of the following seed crop, indicating that water stress can be an important limitation for seed viability. However, this reduction in seed abortion did not result in an increase in seed viability because it was paralleled by a similar increase in seed predation by *M. bipunctatus*. This agrees with evolutionary explanations of parthenocarpy: Seed predators have a long evolutionary history in common with junipers (Roques, 1983; Turgeon et al., 1994; Roux and Roques, 1996) and parthenocarpy might be a by product of seed predation, reducing both pre and postdispersal seed predation because predators spend time and eggs in unviable seeds, thus increasing survival probability of viable seeds (Traveset, 1993; Verdú and García-Fayos, 2000).

Increases in water availability both across the macroscale and experimentally at the microscale did not affect seed viability rates, however at microscale they dramatically increased fruit-set. We therefore can infer that resource availability affects fertility through variation in crop size rather than seed viability. This could be the result of selection for high seed parthenocarpy (Traveset, 1993; Verdú and García-Fayos, 1998), since there seems to be a threshold above which additional viable seeds have low probability of escaping from seed predators.

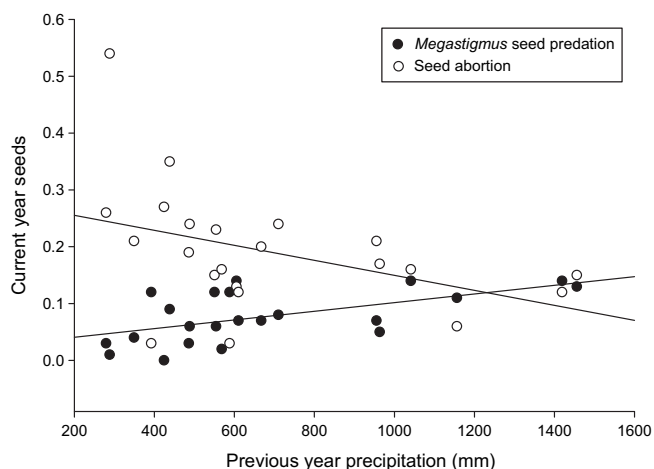


Fig. 2. Precipitation in a given year correlates negatively with following year seed abortion ($p < 0.05$; estimate = $-1.3 \times 10^{-4} \pm 6.5 \times 10^{-5}$) and positively with seed predation ($p < 0.05$; estimate = $7.7 \times 10^{-5} \pm 2.6 \times 10^{-5}$) (LMM statistical values shown on Table 2). Open dots represent seed abortion rates (0–1), filled dots represent seed predation by *Megastigmus*.

Table 3

Microscale watering and fertilization experiments. *F* values for the Linear Mixed Model 2003–2005 (df = 1,13). No significant effects found.

	Watering with fertilization
Viability	0.23
Abortion	0.92
Parthenocarpy	0.46
Predation <i>Trisetacus</i>	0.75
Predation <i>Megastigmus</i>	0.04

Table 4

Macroscale *F* values for Linear regressions (*df* = 9, notice the absence of samples from population 11). ***p* < 0.01.

	Branch growth	Leaf longevity
Elevation	0.21	9.47** (R² = 0.54 ; B = -0.74)
Latitude	2.55	1.19
Mean precipitation	2.43	0.44
Pp. prev. year	0.41	1.19
Temperature	0.37	0.45

Table 5

F values for Linear regressions testing for independence of macroscale factors (*df* = 10). **p* < 0.05; ***p* < 0.01.

	Latitude	Mean precipitation	Mean temperature	Tree density
Elevation	1.03	0.03	1.47	0.26
Latitude	–	9.39* (B = 0.72 ; R² = 0.51)	6.35* (B = -0.64 ; R² = 0.41)	1.10
Mean precipitation	–	–	10.92** (B = -0.74 ; R² = 0.55)	0.01
Mean temperature	–	–	–	0.07

5. Conclusions

High elevation *J. thurifera*'s populations present significant reductions in fertility and leaf longevity compared to that of populations from the lower part of the elevational range. Parthenocarpic seems to be pretty stable among populations and environments and experimental data indicate that variation in fertility comes from variation in fruit-set size as response to variation in available resources.

Plants are expected to perform better when centered on their original elevational range (Yakir et al., 1986; Körner and Diemer, 1987; Angert, 2006; Körner, 2007). Our results permit to hypothesize that *J. thurifera*'s original distribution range could be centered at lower elevations. Phenotypic plasticity might partially buffer the consequences of living in a suboptimal environment, giving room to natural selection to act and favor individuals with characteristics more suitable for harsher habitats. However, rate of adaptation can be extremely slow in long-lived conifers, too long for some species to adapt (Davis et al., 2005 and references therein).

On the other hand, some recent studies indicate that faster-growing species like *Q. ilex* and *P. sylvestris* are extending their distribution limits and transforming nearly monospecific *J. thurifera* forests into mixed stands with high interspecific competition (Olano et al., 2008; DeSoto et al., 2009).

Any additional increase in temperature (e.g. due to global warming) will fasten *J. thurifera* populations into an “environmental clamp”: on one side faster-growing species are likely to push upwards by competition (Loehle, 1998; Walther, 2003; Alward et al., 2006); on the other side a series of environmental factors associated with high elevations (UV irradiance, lower resource availability, etc.) are likely to constrain colonization of higher populations by reproductive downsizing. Besides, previous studies show that seedling recruitment is severely affected by drought and nutrient limitation (Montesinos et al., 2007).

Altogether, this will compromise the species future, although some authors point to persistence through longevity as a survival strategy during interglacial periods (Bennet et al., 1991) and the immediate effect will be attenuated by the long lifespan (>200 years) of junipers (Garcia and Zamora, 2003).

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